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Late-Instar European Corn Borer (Lepidoptera: Crambidae) Tunneling and Survival in Transgenic Corn Hybrids

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ABSTRACT Field studies were conducted in 1996 and 1997 to determine injury by and survival of late-instar European corn borer, *Ostrinia nubilalis* (Hübner), on genetically altered *Bacillus thuringiensis* Berliner corn, *Zea mays* L. Cry1Ab events 176, Bt11, MON810, and MON802; Cry1Ac event DBT418; and Cry9C event CBH351 were evaluated. Plants of each corn hybrid were manually infested with two third-, fourth-, or fifth-instar *O. nubilalis.* Larvae were held in proximity to the internode of the plant above the ear with a mesh sleeve. Larvae were put on the plants during corn developmental stages V8, V16, R1, R3, R4, R5, and R6. This study shows that not all *B. thuringiensis* hybrids provide the same protection against *O. nubilalis* injury. Hybrids with *B. thuringiensis* events Bt11, MON810, MON802, and CHB351 effectively protected the corn against tunneling by late-instar *O. nubilalis.* Event 176 was effective in controlling late-instar *O. nubilalis* during V12 and V16 corn developmental stages; however, significant tunneling occurred by fourth instars during R3 and R5. Event DBT418 was not effective in controlling late-instar *O. nubilalis* during corn vegetative or reproductive stages of development. Whether the *B. thuringiensis* hybrids satisfied high- and ultra-high-dose requirements is discussed.

KEY WORDS *Ostrinia nubilalis, Bacillus thuringiensis,* resistance management, high-dose strategy

EUROPEAN CORN BORER, *Ostrinia nubilalis* (Hübner), is a serious pest of field and sweet corn, *Zea mays* L. An estimate of $100–250 million in crop losses per year is caused by *O. nubilalis* larvae in Iowa (Bergman et al. 1985). When current pest control methods are used, an average of 5% crop yield is lost in Iowa as a result of *O. nubilalis* feeding and tunneling, and when no insecticide is applied a 12% loss is realized (Bergman et al. 1985). There are many traditional *O. nubilalis* management strategies used by farmers, including chemical and biological [*Bacillus thuringiensis* (Berliner)] insecticide applications, cultural practices (early harvesting, tillage practices), and resistant corn varieties (Mason et al. 1996).

A new alternative for managing *O. nubilalis* involves the use of transgenic corn hybrids. First-generation transgenic corn hybrids express *cry1Ab* (events 176, Bt11, MON810, and MON802), *cry1Ac* (event DBT418), or *cry9C* (event CBH351) genes from subspecies of the soil bacterium *B. thuringiensis.* Cry1Ab and Cry1Ac proteins are from subspecies *kurstaki* and Cry9C is from subspecies *tolworthi.* Event 176 contains a maize pollen-specific promoter and maize phosphenalpyruvate carboxylase promoter; toxin expression is in the pollen grains and green photosynthetic tissues (Koziel et al. 1993). The other five *B. thuringiensis* events contain the cauliflower mosaic virus 35S promoter and express toxin in all plant tissues (Koziel et al. 1993). These Cry proteins are specific to larval lepidopterans and are environmentally safe because they are not toxic to mammals, birds, or aquatic organisms (Bauer 1995).

Dramatic control of *O. nubilalis* by *B. thuringiensis* corn has many scientists concerned with high selection pressure from transgenic toxins and the subsequent adaptation by *O. nubilalis* to these toxins. Resistance to *B. thuringiensis* toxins has been observed with other insects (Gould 1988, Tabashnik 1994, Federici 1998). Several strategies of managing insect resistance to transgenic plants have been proposed (Gould 1998, Roush 1998). Currently, the Environmental Protection Agency (EPA) and many scientists recommend a strategy with two components: high-toxin dose and refuge (Fishhoff 1996, EPA 1998).

A proposed definition of high dose is "25 times the toxin concentration needed to kill susceptible larvae" (EPA 1998). This definition infers that 100% of *O. nubilalis* that carry one gene for resistance (i.e., individuals that are heterozygous for a resistance gene) will be killed. Heterozygous individuals carry the majority of resistance genes and modelers suggest that survival of insects with one copy of a resistance gene could greatly accelerate selection for resistant populations (EPA 1998).

Refuge refers to nontransgenic corn or other suitable *O. nubilalis* hosts that are planted adjacent to or

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Table 1. Mean ± SE tunnel length (cm), pupation percentage, and control-adjusted pupation percentage for hybrid, instar, and corn stage for fourth- and fifth-instar *O. nubilalis* in 1996

Treatment	n^a	Tunnel length[b]	n	Pupation, %[c]	n	Adjusted pupation, %[d]
			Hybrid			
Control	24	3.10 ± 0.161a	24	45.8 ± 2.3a	24	100.0 ± 4.6a
176	24	0.71 ± 0.161b	24	25.4 ± 2.3b	24	41.8 ± 4.6b
MON802	24	0.47 ± 0.161b	24	22.9 ± 2.3b	24	36.8 ± 4.6b
BT11	24	0.34 ± 0.161b	24	21.3 ± 2.3b	24	34.1 ± 4.6b
MON810	24	0.52 ± 0.161b	24	31.3 ± 2.3b	24	36.3 ± 4.6b
			Instar			
4	60	1.12 ± 0.102a	60	6.3 ± 1.5b	60	22.2 ± 2.9b
5	60	0.94 ± 0.102a	60	48.3 ± 1.5a	60	77.5 ± 2.9a
			Corn stage			
V8	60	0.80 ± 0.102b	60	27.0 ± 1.5a	60	52.4 ± 2.9a
V12	60	1.26 ± 0.102a	60	27.7 ± 1.5a	60	47.2 ± 2.9a

Means followed by the same letter are not significantly different (Fisher protected LSD, $P < 0.05$).

[a] Number of plots sampled.

[b] Instar ($F = 1.51$; df = 1, 95; $P = 0.22$), corn stage ($F = 10.15$; df = 1, 95; $P < 0.01$), instar × corn-stage interaction ($F = 0.65$; df = 1, 95; $P = 0.42$), hybrid ($F = 51.97$; df = 4, 95; $P < 0.0001$), instar × hybrid interaction ($F = 15.41$; df = 4, 95; $P < 0.0001$), corn-stage × hybrid interaction ($F = 1.37$; df = 4, 95; $P = 0.25$), instar × corn-stage × hybrid interaction ($F = 5.09$; df = 4, 95; $P < 0.001$).

[c] Instar ($F = 408.51$; df = 1, 95; $P < 0.0001$), corn stage ($F = 0.10$; df = 1, 95; $P = 0.75$), instar × corn-stage interaction ($F = 0.10$; df = 1, 95; $P = 0.75$), hybrid ($F = 20.35$; df = 4, 95; $P < 0.0001$), instar × hybrid interaction ($F = 1.73$; df = 4, 95; $P = 0.15$), corn-stage × hybrid interaction ($F = 0.94$; df = 4, 95; $P = 0.44$), instar × corn-stage × hybrid interaction ($F = 0.26$; df = 4, 95; $P = 0.90$).

[d] Instar ($F = 179.09$; df = 1, 95; $P < 0.0001$), corn stage ($F = 1.62$; df = 1, 95; $P = 0.21$), instar × corn-stage interaction ($F = 0.05$; df = 1, 95; $P = 0.82$), hybrid ($F = 37.31$; df = 4, 95; $P < 0.0001$), instar × hybrid interaction ($F = 11.78$; df = 4, 95; $P < 0.0001$), corn-stage × hybrid interaction ($F = 0.20$; df = 4, 95; $P = 0.94$), instar × corn-stage × hybrid interaction ($F = 0.23$; df = 4, 95; $P = 0.92$).

within a transgenic cornfield. The intention is that susceptible *O. nubilalis* adults emerging from the refuge will mate with rare resistant adults. These matings lessen selection pressure by diluting resistance genes in the population. Pesticide resistance studies have shown that high-dose toxin with refuge reduces selection pressure and reduces resistance alleles in the population (Tabashnik and Croft 1982).

Any biological factors that allow heterozygous individuals to survive could compromise the high-dose strategy. This study investigates two interrelated threats to the high-dose strategy. First, late instars could move to transgenic plants from nontransgenic plants and survive. Second, *B. thuringiensis* toxin levels could fall below the high-dose concentration as plants senesce. Differential survival of late instars, declining *B. thuringiensis* toxin levels, or a combination, could accelerate the development of resistance. These threats were investigated by putting third-, fourth-, and fifth-instar *O. nubilalis* on *B. thuringiensis* corn at various stages (vegetative through maturity) of development and by measuring larval tunneling and survival.

Materials and Methods

1996 Field Research. The study was conducted 2 km south of Ames, IA, at the Iowa State University Johnson Research Farm. Corn-hybrid treatments were Maximizer 454 (event 176, Ciba Seeds, Ciba, Research Triangle, NC), NK7070Bt (event BT11, Northrup King, Stanton, MN), experimental *B. thuringiensis* hybrid (event MON810, Monsanto, St. Louis, MO), experimental *B. thuringiensis* hybrid (event MON802, Monsanto), and a nontransgenic control (public, B73 × MO17). Instar treatments included third, fourth, and fifth instars. Corn developmental stages tested were vegetative stages V8 (8 leaf stage), V12, and V16, and reproductive stages R1 (silking), R3 (milk), and R5 (dent) (Ritchie et al. 1997). Corn treatments were tested during each corn developmental stage by putting two third, fourth, or fifth instars on five plants each. Larvae were confined to the plant with a mesh sleeve (described below).

Corn treatments were randomly assigned to six complete blocks. In each block, alternating rows (4.6 m in length) of treatment corn and nontransgenic corn (Garst experimental) were planted 4 June 1996. Row spacings were 0.76 m and plant densities were ≈50,000 plants per hectare. Fields were prepared with conventional tillage, 140–200 kg/ha N, and preemergence herbicides. The alternating rows of nontransgenic corn and guard rows of nontransgenic corn around each block helped minimize disruption of the microhabitat when treatment corn was removed for evaluation. Each corn developmental stage was randomly assigned to a row for each corn treatment. In all assigned rows, five plants were selected to receive fourth instars, and five plants were selected to receive third or fifth instars.

Larval Containment. Larvae used in the experiments were from laboratory-reared populations (6–8th generation) that were established the previous summers from feral *O. nubilalis* in Iowa. *O. nubilalis* was reared using the methods described by Guthrie (1987). Larvae were collected from rearing dishes and put into plastic 100-μl centrifuge vials (two larvae per vial). The V8 and V12 plants were prepared for receiving larvae by excising the uppermost leaf with a fully exposed collar. The V16 and the reproductive plants were prepared by excising the leaf above the primary ear. For all plants, 3 cm of the leaf-sheath collar was

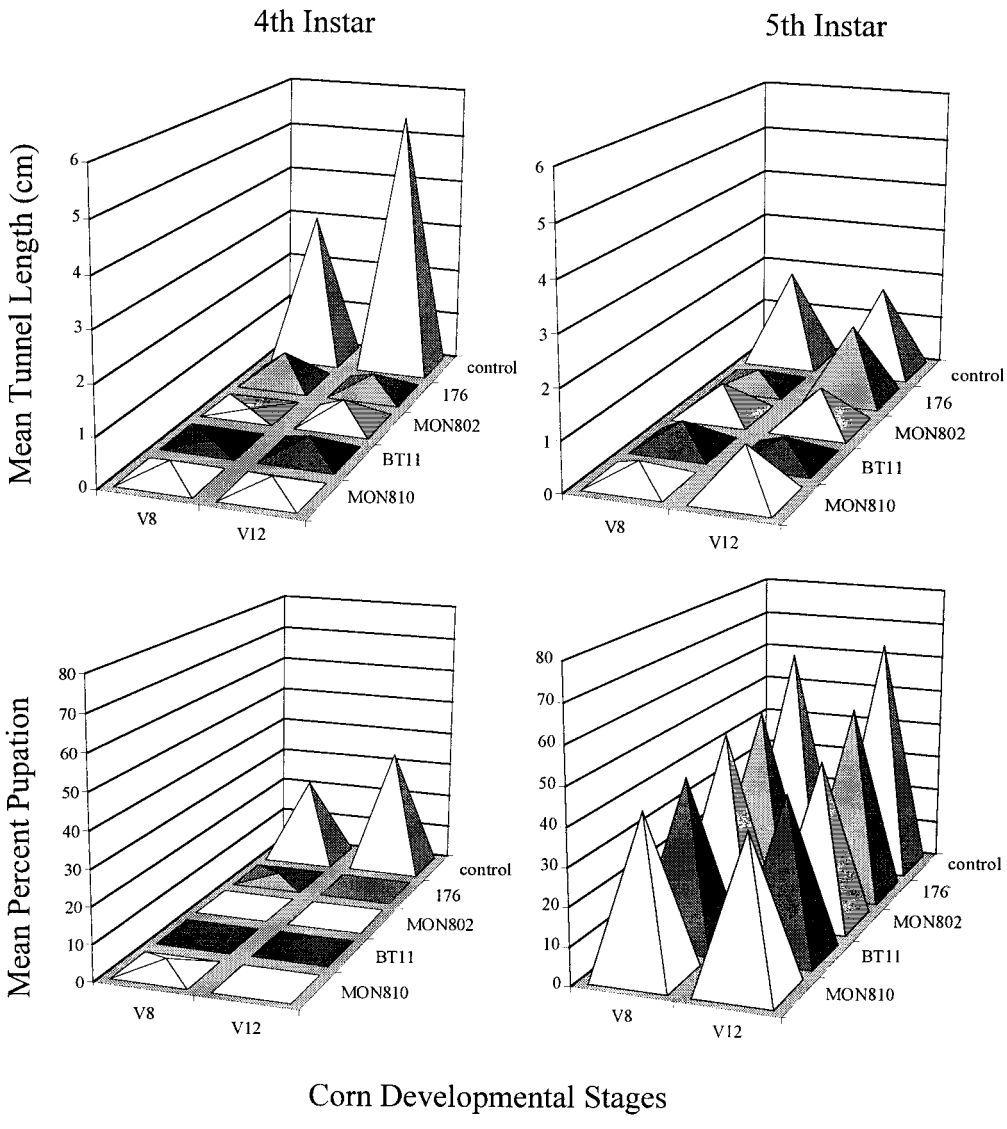

Fig. 1. Mean tunnel length (cm) (top) and mean percentage pupation (bottom) for fourth (left) and fifth (right) instars on four Bt hybrids and a non-Bt control at corn developmental stages V8 and V12 from 1996 sleeve experiment.

left to shelter larvae. A sleeve (20 by 15 cm) made from Lumite (52 × 52 mesh; Division of Synthetic Industries, Gainesville, GA) and Velcro was put around the prepared node and loosely shut with the Velcro. The bottom of the sleeve was closed with a rubber band. A plastic vial with two larvae was put into the sleeve. Both ends of the vial were opened to facilitate larval departure. The top of the sleeve was then closed with a rubber band. Tape was wrapped around the rubber bands to reduce band degradation.

Data Collection and Analyses. Three weeks after larval infestations, all the treated plants from a developmental stage were brought into the laboratory. The sleeves were opened, and the plastic vial, sleeve, and plant were inspected for *O. nubilalis.* Any stage of *O. nubilalis,* dead or alive, was recorded. The plant was inspected for holes in the leaf tissue, leaf collar, and rind. If holes were present, the stalk was split to determine the length of tunnels. Tunnel lengths were averaged for the five plants from each instar for each corn stage, hybrid, and block. A randomized complete block design with a factorial treatment arrangement was used to analyze tunnel lengths; instar, corn stage, and hybrid were treatment groups. Percentage of pupation and percentage of survivors (fifth instars, pupae, and adults) were analyzed similarly. These data also were adjusted to account for control mortality (Abbott 1925). When control survival was <20% the adjustment was not made. The Fisher least significant difference (LSD) procedure was used to separate means for instar, hybrid, and corn-stage treatments at the level of $P < 0.05$ (SAS Institute 1990).

Table 2. Mean ± SE tunnel length (cm), survivor percentage, and control-adjusted survivor percentage for hybrid, instar, and corn stage for third- and fourth-instar *O. nubilalis* in 1996

Treatment	n^a	Tunnel length[b]	n	Survivor, %[c]	n	Adjusted survivor, %[d]
			Hybrid			
Control	48	2.87 ± 0.095a	48	35.4 ± 1.2a	48	100.0 ± 4.3a
176	48	0.46 ± 0.095b	48	5.8 ± 1.2b	48	17.6 ± 4.3b
MON802	48	0.31 ± 0.095bc	48	1.0 ± 1.2c	48	2.8 ± 4.3c
BT11	48	0.14 ± 0.095c	48	0.4 ± 1.2c	48	0.7 ± 4.3c
MON810	48	0.15 ± 0.095c	48	0.2 ± 1.2c	48	1.4 ± 4.3c
			Instar			
3	120	0.63 ± 0.060b	120	7.1 ± 0.8b	120	20.7 ± 2.7b
4	120	0.94 ± 0.060a	120	10.1 ± 0.8a	120	28.3 ± 2.7a
			Corn-stage			
V16	60	0.50 ± 0.085b	60	4.2 ± 1.1c	60	20.8 ± 3.8b
R1	60	1.00 ± 0.085a	60	10.3 ± 1.1ab	60	21.0 ± 3.8b
R3	60	0.80 ± 0.085a	60	11.5 ± 1.1a	60	24.2 ± 3.8ab
R5	60	0.84 ± 0.085a	60	8.3 ± 1.1b	60	31.9 ± 3.8a

Means followed by the same letter are not significantly different (Fisher protected LSD, $P < 0.05$).

[a] Number of plots sampled.

[b] Instar ($F = 13.61$; df = 1, 195; $P < 0.001$), corn-stage ($F = 6.18$; df = 3, 195; $P < 0.001$), instar × corn-stage interaction (F = 3.85; df = 3, 195; $P < 0.02$), hybrid ($F = 154.02$; df = 4, 195; $P < 0.0001$), instar × hybrid interaction ($F = 1.85$; df = 4, 195; $P = 0.12$), corn-stage × hybrid interaction ($F = 4.61$; df = 12, 195; $P < 0.0001$), instar × corn-stage × hybrid interaction ($F = 1.38$; df = 1, 195; $P = 0.18$).

[c] Instar ($F = 7.75$; df = 1, 195; $P < 0.01$), corn stage ($F = 8.94$; df = 3, 195; $P < 0.0001$), instar × corn-stage interaction ($F = 2.28$; df = 3, 195; $P = 0.08$), hybrid ($F = 158.73$; df = 4, 195; $P < 0.0001$), instar × hybrid interaction ($F = 3.43$; df = 4, 195; $P < 0.01$), corn-stage × hybrid interaction ($F = 9.72$; df = 12, 195; $P < 0.0001$), instar × corn-stage × hybrid interaction ($F = 1.47$; df = 12, 195; $P = 0.14$).

[d] Instar ($F = 3.91$; df = 1, 195; $P < 0.05$), corn stage ($F = 1.84$; df = 3, 195; $P = 0.14$), instar × corn-stage interaction ($F = 1.60$; df = 3, 195; $P = 0.19$), hybrid ($F = 99.89$; df = 4, 195; $P < 0.0001$), instar × hybrid interaction ($F = 2.45$; df = 4, 195; $P < 0.05$), corn-stage × hybrid interaction ($F = 1.55$; df = 12, 195; $P < 0.11$), instar × corn-stage × hybrid interaction ($F = 1.11$; df = 12, 195; $P = 0.35$).

1997 Field Research. Field research was conducted at the Iowa State University's Burkey farm located 7 km southwest of Ames. Corn-hybrid treatments were Maximizer 454 (event 176, Ciba Seeds), experimental *B. thuringiensis* hybrid (event MON810, Monsanto), experimental *B. thuringiensis* hybrid (event MON802, Monsanto), DK580Bt (event DBT418, DEKALB Genetics, DeKalb, IL), experimental *B. thuringiensis* hybrid (event CBH351, AgrEvo, Wilmington, DE), and a nontransgenic control (public, B73 × MO17). Plant developmental stages tested were vegetative stages V8 and V16 and reproductive stages R1, R3, R4 (dough), R5, and R6 (maturity) (Ritchie et al. 1997). Corn treatments were randomly assigned to nine complete blocks and planted 20 May 1997. Experimental arrangement, data collection, and analyses were similar to those of 1996 with three exceptions: 12 rows of each treatment were planted for each block, fill corn and guard corn were a *B. thuringiensis* hybrid (Pioneer Hi-Bred 34R06), and three plants were evaluated from each plot.

Results and Discussion

1996 Fourth and Fifth Instars. Tunnels in the control (B73 × MO17) plants were significantly longer compared with those in the four *B. thuringiensis* hybrids (Table 1). Tunnel lengths among the four *B. thuringiensis* hybrids ranged from 0.34 to 0.71 cm, and lengths were not significantly different from each other. The V12 corn had significantly longer *O. nubilalis* tunnels compared with those in V8 corn. There were no tunnel-length differences between fourth and fifth instars, but the mean-tunnel-length graphs for fourth and fifth instars (Fig. 1) suggest that the instar × hybrid interaction was important (Table 1). Fourth instars tunneled more than fifth instars on control plants, but the opposite occurred on *B. thuringiensis* hybrids.

Larvae feeding on control corn had a higher pupation percentage compared with larvae feeding on the four *B. thuringiensis* hybrids (Table 1). Among the four *B. thuringiensis* hybrids, the pupation percentages were not significantly different from each other. Larvae tunneling in V8 and V12 corn had similar pupation percentages. Fifth instars had a higher pupation percentage compared with fourth instars (Table 1). This result was the same when the pupation percentages were control adjusted (Table 1). Fourth instars pupated less than fifth instars when they were on control plants, and nearly none of the fourth instars on *B. thuringiensis* hybrids pupated (Fig. 1). This finding contrasts with a high pupation percentage for fifth instars that were put on *B. thuringiensis* hybrids.

Most of the fourth instars on *B. thuringiensis* corn died after tunneling <1 cm. On *B. thuringiensis* corn, fifth instars tunneled more and had a higher pupation percentage compared with fourth instars (Fig. 1). These results suggest that fifth instars had either a higher tolerance for *B. thuringiensis* or they did not ingest *B. thuringiensis* corn tissue. There might be a fitness cost associated with fifth instars pupating when tunneling in *B. thuringiensis* corn; however, this possibility was not addressed in our study. After preview-

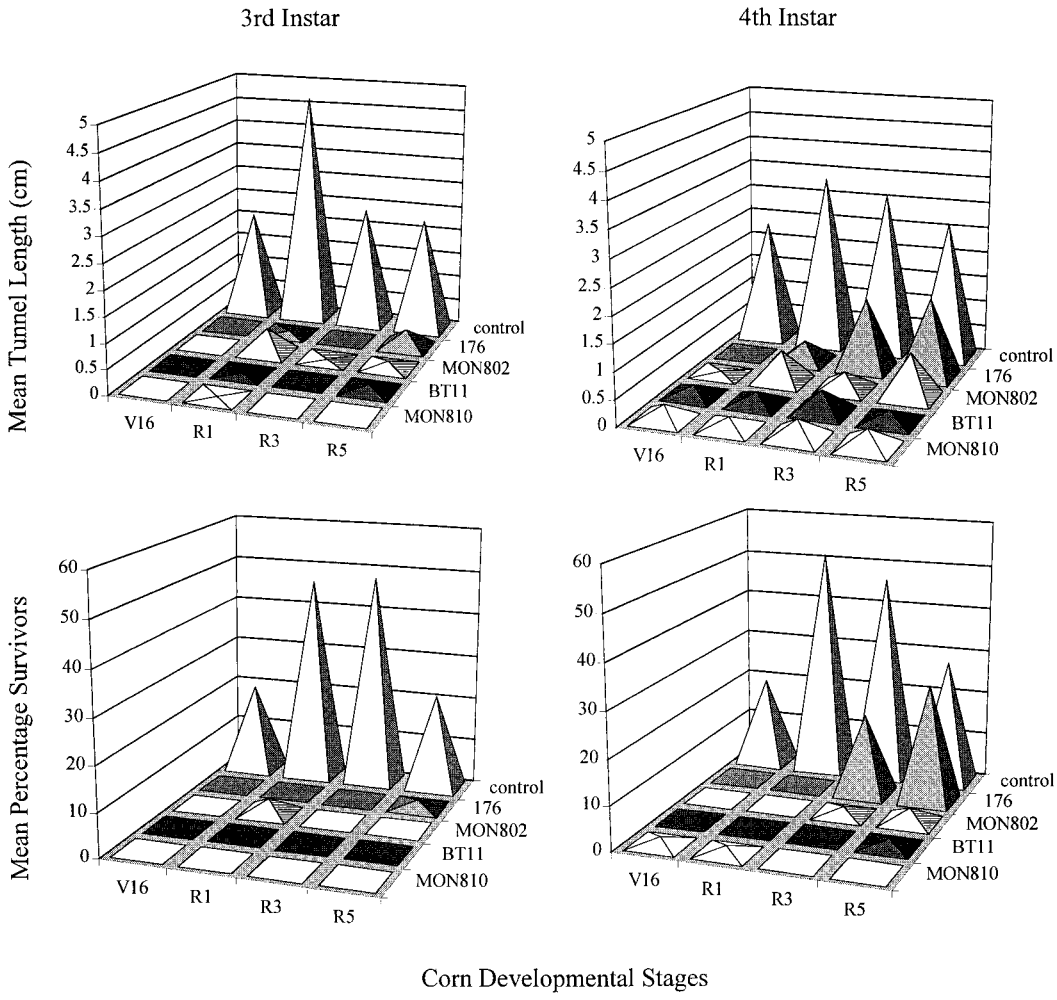

Fig. 2. Mean tunnel length (cm) (top) and mean percentage survivors (bottom) for third (left) and fourth (right) instars on four Bt hybrids and a non-Bt control at corn developmental stages V16, R1, R3, and R5 from 1996 sleeve experiment.

ing these results, third instars instead of fifth instars were used to evaluate corn hybrids in stages V16–R5.

1996 Third and Fourth Instars. Tunnels were significantly longer in the control plants compared with those in the four *B. thuringiensis* hybrids (Table 2). Tunnels in the event 176 plants were significantly longer than tunnels in event BT11 and MON810 plants. There were no significant differences in tunnel lengths between event MON802 hybrids and any of the other *B. thuringiensis* hybrids. Plants infested with fourth instars had longer tunnels than plants infested with third instars. The R1, R3, and R5 corn had significantly longer *O. nubilalis* tunnels compared with V16 corn. The mean-tunnel-length graphs for third and fourth instars (Fig. 2) suggest that the instar × corn-stage, and corn-stage × hybrid interactions (Table 2) were important. All *B. thuringiensis* hybrids deterred tunneling by third instars, but tunneling by fourth instars varied depending on hybrid and corn developmental stage. Event MON810 and BT11 hybrids had mean tunnel lengths <0.5 cm per plant for R3 and R5 corn stages, but mean-tunnel lengths in event 176 hybrids were 1.38 and 1.52 cm for R3 and R5 corn, respectively. Event MON802 hybrids had <0.5 cm tunneling for R3 corn and 0.85 cm tunneling for R5 corn.

Survival of larvae feeding on control corn was higher compared with larvae feeding on the four *B. thuringiensis* hybrids (Table 2). *O. nubilalis* on event 176 hybrids had a significantly higher percentage of survivorship compared with larvae on the other three *B. thuringiensis* hybrids. Larval survival varied over the corn developmental stages, and lowest survival occurred during V16. Fourth-instar survival was higher compared with third instars when the percentages were not adjusted for control morality, and when the percentages were adjusted (Table 1). The instar × hybrid and corn-stage × hybrid interactions were important (Table 2). Third instars did not develop on any of the *B. thuringiensis* hybrids, but fourth-instar development depended on the *B. thuringiensis* hybrid and corn developmental stage (Fig. 2). Survival per-

Table 3. Mean ± SE tunnel length (cm), survivor percentage, and control-adjusted survivor percentage for hybrid, instar, and corn stage for third- and fourth-instar *O. nubilalis* in 1997

Treatment	n^a	Tunnel length[b]	n	Survivor, %[c]	n	Adjusted survivor, %[d]
			Hybrid			
Control	126	1.08 ± 0.033a	126	27.0 ± 0.9a	72	100.0 ± 3.7a
DBT418	126	0.77 ± 0.033b	126	11.6 ± 0.9b	72	50.9 ± 3.7b
176	126	0.37 ± 0.033c	126	5.8 ± 0.9c	72	12.6 ± 3.7c
MON802	126	0.14 ± 0.033d	126	0.4 ± 0.9d	72	1.7 ± 3.7d
CBH351	126	0.13 ± 0.033d	126	0.1 ± 0.9d	72	1.0 ± 3.7d
MON810	126	0.14 ± 0.033d	126	0.1 ± 0.9d	72	0.7 ± 3.7d
			Instar			
3	378	0.35 ± 0.019b	378	5.6 ± 0.5b	216	27.3 ± 2.1a
4	378	0.53 ± 0.019a	378	9.5 ± 0.5a	216	28.3 ± 2.1a
			Corn-stage			
V8	108	0.63 ± 0.036a	108	16.8 ± 0.9a	108	31.7 ± 3.0a
V16	108	0.56 ± 0.036ab	108	9.9 ± 0.9bc	108	36.0 ± 3.0a
R1	108	0.51 ± 0.036bc	108	12.3 ± 0.9b	108	21.8 ± 3.0b
R3	108	0.41 ± 0.036d	108	7.4 ± 0.9c	108	21.7 ± 3.0b
R4	108	0.33 ± 0.036d	108	1.7 ± 0.9d		
R5	108	0.43 ± 0.036cd	108	3.2 ± 0.9d		
R6	108	0.22 ± 0.036e	108	1.2 ± 0.9d		

Means followed by the same letter are not significantly different (Fisher protected LSD, $P < 0.05$).

[a] Number of plots sampled.

[b] Instar ($F = 43.98$; df = 1, 664; $P < 0.0001$), corn stage ($F = 15.11$; df = 6, 664; $P < 0.0001$), instar × corn-stage interaction ($F = 3.40$; df = 6, 664; $P < 0.01$), hybrid ($F = 143.79$; df = 5, 664; $P < 0.0001$), instar × hybrid interaction ($F = 0.52$; df = 5, 664; $P = 0.076$), corn-stage × hybrid interaction ($F = 4.17$; df = 30, 664; $P < 0.0001$), instar × corn-stage × hybrid interaction ($F = 1.07$; df = 30, 664; $P = 0.37$).

[c] Instar ($F = 31.38$; df = 1, 664; $P < 0.0001$), corn stage ($F = 40.19$; df = 6, 664; $P < 0.0001$), instar × corn-stage interaction ($F = 3.12$; df = 6, 664; $P < 0.01$), hybrid ($F = 151.78$; df = 5, 664; $P < 0.0001$), instar × hybrid interaction ($F = 8.74$; df = 5, 664; $P < 0.001$), corn-stage × hybrid interaction ($F = 18.68$; df = 30, 664; $P < 0.0001$), instar × corn-stage × hybrid interaction ($F = 0.82$; df = 30, 664; $P = 0.75$).

[d] Instar ($F = 0.10$; df = 1, 376; $P = 0.75$), corn stage ($F = 5.81$; df = 3, 376; $P < 0.001$), instar × corn-stage interaction ($F = 0.59$; df = 3, 376; $P = 0.62$), hybrid ($F = 120.95$; df = 5, 376; $P < 0.0001$), instar × hybrid interaction ($F = 0.46$; df = 5, 376; $P = 0.81$), corn-stage × hybrid interaction ($F = 5.86$; df = 15, 376; $P < 0.0001$), instar × corn-stage × hybrid interaction ($F = 0.72$; df = 15, 376; $P = 0.76$); corn stages R4, R5 and R6 were not adjusted because control treatments had < 20% survivorship.

centages for fourth instars on event 176 hybrids were 18.3 and 26.7 for R3 and R5 stages, respectively. This finding corresponds with that of other reports indicating second-generation *O. nubilalis* survival on event 176 corn in late-stage corn (Dornbos 1996, Witkowski 1996).

1997 Third and Fourth Instars. Tunnels were significantly longer in the control plants compared with those in the five *B. thuringiensis* hybrids (Table 3). Event DBT418 hybrids had significantly longer tunnels than did event 176, MON802, CBH351, and MON810 hybrids; and event 176 hybrids had significantly longer tunnels than did event MON802, CBH351, and MON810 hybrids. Plants infested with fourth instars had longer tunnels than plants infested with third instars. There was a general trend for *O. nubilalis* tunneling to decrease when the plants were infested at the later growth stages (Table 3). The instar × corn-stage and corn-stage × hybrid interactions were important (Table 3). *B. thuringiensis* hybrids MON810, CBH351 and MON802 deterred third instars from tunneling during all corn developmental stages (Fig. 3). Comparatively, third-instar larvae tunneled in events 176 and DBT418 hybrids during all stages. Event DBT418 hybrids, particularly, received substantial tunneling during V8 and V16 with declining amounts from R1 through R6 (Fig. 3). When infested with the fourth instars, mean-tunnel lengths were higher for most of the hybrid and corn-stage combinations compared with plants infested with third instars (Fig. 3). Event MON810, CBH351, and MON802 hybrids effectively controlled *O. nubilalis.* Event DBT418 hybrids sustained substantial injury during corn stages V8 through R5, and event 176 hybrids experienced substantial injury during V8 and R5 (Fig. 3). For hybrids with the fourth-instar treatment, including the controls, there was a general trend for mean-tunnel lengths to decrease when late stage plants were infested. This trend might be attributed to plant senescence, decline in plant moisture, or increase in plant lignin.

The relatively high tunneling by fourth instars for event 176 hybrids during V8 was unexpected because such injury did not occur in 1996. The differences between years could be caused by environmental or biological factors that influenced either the plants or the larvae. Perhaps *O. nubilalis* tunneling in event 176 hybrids was high during V8 because fourth instars were able to tunnel through the green rind to the nonexpressing pith. The high amount of tunneling by fourth instars in event 176 plants during V8 probably is not an important factor in the central Corn Belt because fourth instars do not usually occur at this stage of corn development. Yet, this might be a factor in other corn-growing regions, particularly in the tropics,

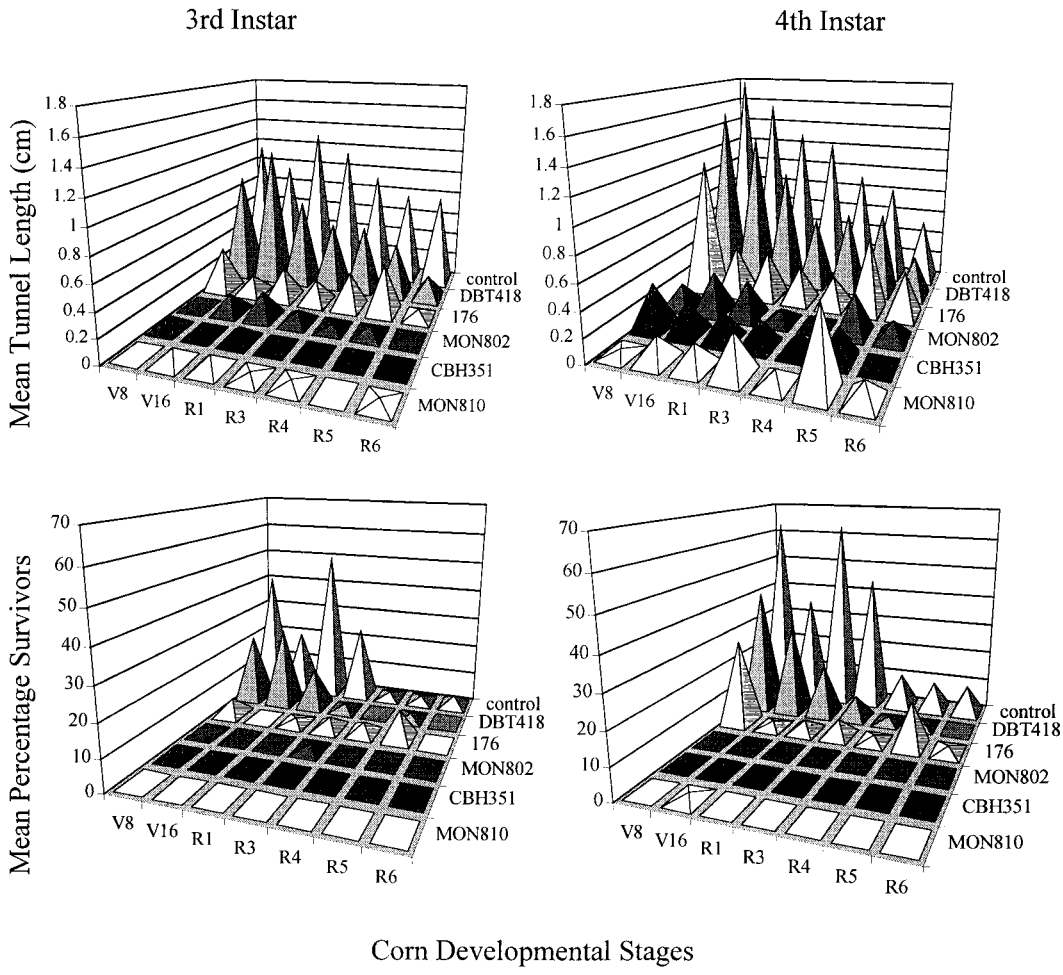

Fig. 3. Mean tunnel length (cm) (top) and mean percentage survivors (bottom) for third (left) and fourth (right) instars on five Bt hybrids and a non-Bt control at corn developmental stages V8, V16, R1, R3, R4, R5, and R6 from 1997 sleeve experiment.

where late instars of other Lepidoptera might encounter V8 corn.

Survival results are similar to the tunnel results. Larval survival was highest in the control hybrid, lower in event DBT418 and 176 hybrids, and lowest in event MON802, CBH351, and MON810 hybrids (Table 3). Fourth instars had a higher survival percentage than third instars, but this difference did not occur when the percentages were control adjusted (Table 3). There was a general trend for *O. nubilalis* survivorship to decrease when the plants were infested at the later growth stages (Table 3). The instar, hybrid, corn-stage interactions were important (Fig. 3; Table 3). Third and fourth instars did not survive on event MON810, CBH351, and MON802 hybrids. Survivorship of third instars on event 176 hybrids varied depending on the corn developmental stage and was highest during R5. Survivorship of third instars on event DBT418 hybrids was high during the early corn stages and low after R3. Survivorship of fourth instars on event 176 hybrids was high during V8, then fluctuated during R1 through R5. Fourth-instar survival on event DBT418 hybrids generally declined from V8 to R4, a trend similar to that in control plants.

We selected corn stages V16 and R3 from 1997 experiments to consider if *B. thuringiensis* hybrids satisfied high-dose requirements. Third and fourth instars commonly occur on corn during these developmental stages in Iowa cornfields and throughout most of the Corn Belt. For the purposes of this evaluation, we use the EPA (1998) definition of high dose (toxin 25 times the concentration needed to kill susceptible larvae). Bioassays with Dipel (commercial formulation of *B. thuringiensis* endotoxins) suggest that third instars are >25 times more tolerant than neonates (Huang et al. 1999). Thus, if *B. thuringiensis* hybrids effectively control third instars, they would satisfy the EPA definition of high dose. An ultrahigh-dose strategy that would be high enough to kill any projected resistant heterozygotes, i.e., any heterozygous instars, has been recommended as an alternative to the high-dose strategy (Denholm and Rowland 1992, Huang et

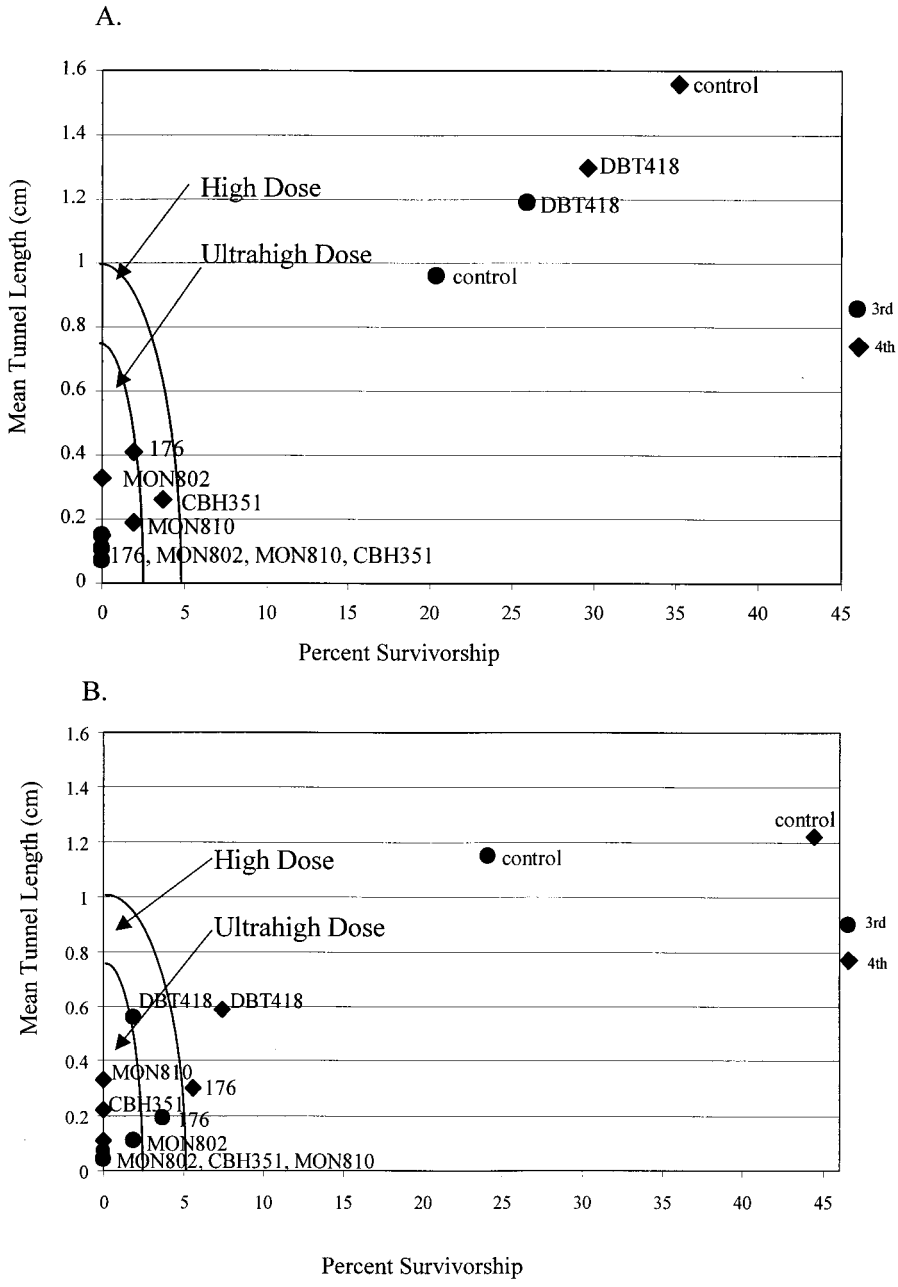

Fig. 4. Mean tunnel length (cm) (y-axis) plotted against mean percentage survivorship (x-axis) for third (circles) and fourth (diamonds) instars for V16 (A) and R3 (B) stage corn from 1997 sleeve experiment.

al. 1999). Thus, for this evaluation we assume that an event satisfies criteria for ultrahigh dose if both third and fourth instars are effectively controlled.

Instar and hybrid differences are apparent when tunnel length is plotted against percentage survivorship for these corn stages (Fig. 4). The V16 graph suggests that all *B. thuringiensis* hybrids, except event DBT418, controlled the third instars. There was some survival of fourth instars during V16; however, it was not determined whether these few individuals would have developed into normal adults. Some fourth instars developed into moribund fifth instars that probably would not have pupated. Event DBT418 hybrids did not effectively control the fourth instars. Based on these results, event MON810, CBH351, MON802, and 176 satisfy high-dose requirements and are close to satisfying ultrahigh-dose requirements during this corn stage. Event DBT418 hybrids do not satisfy high-

dose requirements. Lines on the V16 graph designate possible high-dose and ultrahigh-dose areas (Fig. 4).

The R3 graph (Fig. 4) suggests that all the *B. thuringiensis* hybrids, including event DBT418, effectively controlled third instars. Event DBT418 and 176 hybrids, however, had a relatively high percentage of fourth instars that survived. These results suggest that events MON810, CBH351, and perhaps MON802 satisfy ultrahigh-dose requirements and that DBT418 and 176, because they controlled third instars, satisfy high-dose requirements for this corn stage. Lines designate possible high-dose and ultrahigh-dose areas (Fig. 4). Huang et al. (1999) suggest that ultrahigh dose and high dose should be defined in terms of larval stage. The results from this study suggest that hybrids with a *B. thuringiensis* dose sufficient to kill third-instar *O. nubilalis* could be considered high-dose plants, and hybrids with a *B. thuringiensis* dose sufficient to kill fourth-instar *O. nubilalis* could be considered ultrahigh-dose plants. Fifth-instar *O. nubilalis* were not a reliable test of dosage because they tended to pupate when encountering *B. thuringiensis* toxin. Researchers should consider using the penultimate instar of other holometaboluous insects to test for an ultrahigh dose of toxin.

There are unresolved issues that should be addressed. First, the Dipel bioassays may not be appropriate to determine instar tolerances. In this study, *O. nubilalis* responses were evaluated to single gene products; whereas in Huang et al. (1997, 1999) *O. nubilalis* responses were evaluated to a spore or crystal powder containing multigene products [Cry1A(a), Cry1A(b), Cry1A(c), Cry2A, and Cry2B endotoxins]. The *cry* gene specific assays would be better. Second, sublethal effects and fitness costs of *B. thuringiensis* toxin on diapause, pupation rates, adult emergence, and adult fecundity should be quantified. Third, this study focused on the stalk, but other studies focusing on the ear should be conducted, particularly for the event 176 hybrids where *B. thuringiensis* toxin is expressed in some tissues of the ear and not others.

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