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Short communication

Bt toxin is released in root exudates from 12 transgenic corn hybrids representing three transformation events

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Abstract

The anti-lepidopteran toxin (Cry1Ab protein) encoded by truncated genes from *Bacillus thuringiensis* was released in the root exudates from all hybrids of Bt corn studied and which represented three transformation events (Bt11, MON810, and 176). In vitro and in situ studies indicated that the toxin released in root exudates accumulates in soil, as it adsorbs and binds rapidly on surface-active particles (e.g. clays and humic substances), and retains insecticidal activity for at least 180 d, the longest time studied. The results indicated that the release of the Cry1Ab protein by roots is a common phenomenon with transgenic Bt corn and is not restricted to only the one Bt corn hybrid (NK4640Bt) and tranformation event (Bt11) studied initially.

Keywords: *Bacillus thuringiensis*; Bt corn; Root exudates; Cry1Ab toxin; Clay; Humic acids; European corn borer

The incorporation into plants of genes from *Bacillus thuringiensis* that code for the production of insecticidal toxins reduces many problems associated with the use of chemical pesticides, as the toxins are produced continuously within these plants. However, there is concern that genetically engineered crops may pose risks to natural and agricultural ecosystems (e.g. Rissler and Mellon, 1996; Conway, 2000; Hails, 2000; Stotzky, 2000). Bt corn is maize (*Zea mays* L.) that has been genetically modified to express the *cry1Ab* gene from *B. thuringiensis*. The modified plant produces a larvicidal toxin that kills lepidopteran pests, especially the European corn borer (*Ostrinia nubilalis*), a major pest in Europe and North America that can reduce yields of corn by 3–7% borer^{-1} plant^{-1} (Lynch, 1980). The Cry1Ab protein was released in root exudates from one hybrid of transgenic Bt corn grown in sterile hydroponic culture, in sterile and non-sterile soil in a plant-growth room, and in a natural soil in the field (Saxena et al., 1999; Saxena and Stotzky, 2000). These in vitro and in situ studies indicated that the toxin released in root exudates, as well as from the biomass of Bt corn, adsorbs and binds rapidly on surface-active particles (e.g. clays and humic substances) in soil and remains larvicidal for at least 180 d, the longest time studied (Saxena and Stotzky, 2001a). The toxin was also present in the rhizosphere soil of field-grown Bt corn plants throughout their growth and several months after their death and subsequent frost (Saxena and Stotzky, 2000). If production and release to soil of the toxin exceed consumption and inactivation by insect larvae, degradation by the microbiota, and abiotic inactivation, the toxin could accumulate in the environment to concentrations that may enhance pest control, constitute a hazard to non-target organisms, and result in the selection and enrichment of toxin-resistant target insects (e.g. McGaughey and Whalon, 1992; Addison, 1993; James et al., 1993; Tabashnik, 1994; Johnson et al., 1995; Hilbeck et al., 1999). Accumulation and persistence appear to be enhanced when the toxin is bound on surface-active particles and, thereby, is rendered less accessible for microbial degradation but still retains its toxicity (see Crecchio and Stotzky, 2001).

To determine whether the release of the Cry1Ab protein is a common phenomenon with transgenic Bt corn and is not restricted to only the one corn hybrid (NK4640Bt) studied initially (Saxena et al., 1999; Saxena and Stotzky, 2000), the release of the protein in the exudates of 12 additional Bt hybrids, representing three different transformation events, and of their isogenic non-transgenic counterparts was studied with plants grown in a plant-growth room and in the field. In addition, the persistence in rhizosphere soil of the protein released from all hybrids was evaluated.

Seeds of 12 different hybrids of Bt corn and of their isogenic strains without the *cry1Ab* gene were planted in

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Table 1
Presence of the Cry1Ab toxin in rhizosphere soil of different hybrids of corn with (Bt +) and without (Bt −) the *cry1Ab* gene and grown in a plant-growth room

Company	Bt +					Bt −			
	Hybrid	Transformation event[a]	Immuno. test[b]	Percentage mortality[c]	Larval weight (mg)[c]	Hybrid	Immuno. test	Percentage mortality	Larval weight (mg)[c]
Novartis	N7590Bt	Bt11	+	81 ± 6.3	33 ± 11	N7590	−	0	840 ± 32
Novartis	N67-T4	Bt11	+	81 + 6.3	56 ± 21	N67-H6	−	0	850 ± 21
Novartis	N3030Bt	Bt11	+	38 ± 7.2	153 ± 23	N3030	−	6.3 ± 6.25	830 ± 33
Novartis	NC4990Bt	Bt11	+	81 ± 6.3	114 ± 91	NC4880	−	0	930 ± 43
Novartis	NK4640Bt	Bt11	+	100 ± 0.0	−	NK4640	−	0	1150 ± 51
Novartis	Maximizer[d]	176	+	43 ± 11.9	84 ± 25	–	−	–	–
Pioneer	P32P76	MON810	+	81 ± 6.3	73 ± 53	P32P75	−	0	810 ± 25
Pioneer	P33B51	MON810	+	93 ± 6.3	21 ± 13	P33B50	−	0	910 ± 65
Pioneer	P31B13	MON810	+	50 ± 10.2	75 ± 22	P3223	−	0	930 ± 43
DeKalb	DK647Bty	MON810	+	56 ± 11.9	106 ± 72	DK647	−	6.3 ± 6.25	940 ± 45
DeKalb	DK679Bty	MON810	+	68 ± 18.8	142 ± 24	DK679	−	6.3 ± 6.25	850 ± 21
DeKalb	DK626Bty	MON810	+	68 ± 6.3	84 ± 81	DK626	−	0	750 ± 92

[a] Insertion of the *cry1Ab* gene by transformation.
[b] Determined with Lateral Flow Quickstix; − = no toxin detected; + = toxin detected.
[c] Determined with the larvae of the tobacco hornworm (*Manduca sexta*); at least 16 larvae per assay; expressed as % mortality and mean weight, in mg, of a single larva ± standard error of the mean. No mortality with soils without plants (weight of a single larva: 740–1220 ± 20 mg).
[d] Seeds of Bt − Maximizer were not available.

Table 2
Presence of the Cry1Ab toxin in rhizosphere soil of different hybrids of corn with (Bt +) and without (Bt −) the *cry1Ab* gene and grown in the field

Company	Bt +					Bt −			
	Hybrid	Transformation event[a]	Immuno. test[b]	Percentage mortality[c]	Larval weight (mg)[c]	Hybrid	Immuno. test	Percentage mortality	Larval weight (mg)[c]
Novartis	N7590Bt	Bt11	+	75 ± 10.2	56 ± 22	N7590	−	6.3 ± 6.25	930 ± 62
Novartis	N67-T4	Bt11	+	68 ± 11.9	23 ± 11	N67-H6	−	0	1260 ± 81
Novartis	N3030Bt	Bt11	+	37 ± 7.2	93 ± 43	N3030	−	0	940 ± 31
Novartis	NC4990Bt	Bt11	+	62 ± 12.5	72 ± 24	NC4880	−	6.3 ± 6.25	1140 ± 70
Novartis	NK4640Bt	Bt11	+	81 ± 6.25	91 ± 12	NK4640	−	0	1130 ± 93
Novartis	0966 (Supersweet)	Bt11	+	63 ± 6.5	91 ± 51	PrimePlus (Supersweet)	−	6.3 ± 6.25	850 ± 22
Novartis	Maximizer[d]	176	+	37 ± 7.2	184 ± 35	–	−	–	–
Pioneer	P32P76	MON810	+	68 ± 15.7	83 ± 24	P32P75	−	0	930 ± 60
Pioneer	P33B51	MON810	+	68 ± 15.7	91 ± 14	P33B50	−	0	940 ± 45
Pioneer	P31B13	MON810	+	43 ± 6.5	62 ± 36	P3223	−	6.3 ± 6.25	920 ± 55
DeKalb	DK647Bty	MON810	+	37 ± 12.5	86 ± 47	DK647	−	0	1250 ± 80
DeKalb	DK679Bty	MON810	+	56 ± 11.9	42 ± 13	DK679	−	12.5 ± 6.25	850 ± 31
DeKalb	DK626Bty	MON810	+	50 ± 10.2	91 ± 31	DK626	−	6.3 ± 6.25	930 ± 12

[a] Insertion of the *cry1Ab* gene by transformation.
[b] Determined with Lateral Flow Quickstix; − = no toxin detected; + = toxin detected.
[c] Determined with the larvae of the tobacco hornworm (*Manduca sexta*); at least 16 larvae per assay; expressed as % mortality and mean weight, in mg, of a single larva ± standard error of the mean. No mortality with soils without plants (weight of a single larva: 840–1240 ± 40 mg).
[d] Seeds of Bt − Maximizer were not available.

test tubes containing 15 g of a non-sterile sandy loam soil that naturally contains predominantly kaolinite. The soil was collected at the Kitchawan Research Laboratory of the Brooklyn Botanical Garden, Ossining, NY, and amended to 6% (vol vol^{-1}) with montmorillonite. This soil, with or without clay amendments, has been used extensively in this laboratory for a spectrum of studies, and its physicochemical properties (e.g. pH 5.8; 2.8% organic matter; 0.13% nitrogen; 52, 36, and 12% sand, silt, and clay) have been published (e.g. Tapp and Stotzky, 1998). After 40 d of growth in a plant-growth room (26 ± 2°C, 12 h light–dark cycle) with watering as required, the plants were gently removed from randomly selected tubes (two tubes of each Bt corn hybrid and of its isogenic non-Bt counterpart), and rhizosphere soil was collected by gently shaking the roots to dislodge adhering small clumps of soil. The soil was analyzed immunologically for the toxin with Lateral Flow Quickstix (EnviroLogix; detection limit <10 parts 10^{-9}), which are essentially Western blot assays (Saxena et al., 1999; Saxena and Stotzky, 2000), and by larvicidal assays of soil suspensions using *Manduca sexta* (tobacco hornworm) (Saxena et al., 1999; Saxena and Stotzky, 2000).

Seeds of the 12 Bt hybrids and of their non-Bt isogenic counterparts were also planted in a sandy soil in East Marion, Long Island, NY. Some physicochemical characteristics of this soil are: pH 7.1; 5.24 and 0.25% carbon and nitrogen; 94, 5, and 1% sand, silt, and clay. Rhizosphere soil from the field-grown plants was similarly analyzed after the production of ears of corn (two plants of each Bt hybrid and each isogenic non-Bt corn). The rhizosphere samples from sweet Bt corn variety 0966 and its isoline (PrimePlus), grown in Minnesota, were kindly provided by Professor David Andow.

All samples of rhizosphere soil from the 12 hybrids of Bt corn grown in the plant-growth room were positive 40 d after germination for the presence of the toxin when assayed immunologically with the Quickstix test, and all samples were toxic to the larvae of *M. sexta*, with mortality ranging from 38 to 100% (Table 1). No toxin was detected immunologically or by larvicidal assay in any soil in which plants of non-Bt corn or no plants had been grown. In addition, the size and weight of surviving larvae exposed to soils from Bt corn were significantly lower (ca. 80–98% lower) than those exposed to soils from non-Bt corn or without plants (Table 1), and these larvae usually died after an additional 2–3 d.

The immunological and larvicidal assays of soil from the rhizosphere of all Bt hybrids grown in the field were also positive, whereas they were negative for all non-Bt corn isolines (Table 2). Although the larval mortality in rhizosphere soil from some plants of field-grown Bt corn was only 37%, the size and weight of the surviving larvae were reduced by 85–98% when compared with soil from non-Bt corn or without plants (Table 2), and most of these larvae died after a few more days. There were no discernable or consistent differences in exudation of the toxin (as evaluated by mortality, weight of surviving larvae, or immunologically) between plants derived from different transformation events, regardless of whether they were grown in the plant-growth room or in the field.

Based on these results and those of Saxena et al. (1999) and Saxena and Stotzky (2000), the toxin released in the root exudates of all studied hybrids of Bt corn, representing three different transformation events, accumulates in soil and retains anti-lepidopteran activity, probably as the result of binding on surface-active particles in soil, which renders the toxin resistant to degradation by microorganisms. Although some toxin was probably released from sloughed and damaged root cells, the major portion was derived from root exudates, as there was no discernable root debris when plants were grown in hydroponic culture (Saxena et al., 1999).

In addition to the large amount of toxin that will be introduced to soil in plant biomass after harvest of a Bt corn crop and some that might be introduced from pollen released during tasseling (Losey et al., 1999), the toxin will also be released to soil in root exudates during the entire growth of hybrids of Bt corn. The presence of the toxin in soil could improve the control of insect pests, or the persistence of the toxin in soil could enhance the selection of toxin-resistant target insects and constitute a hazard to non-target organisms. However, the toxin released in root exudates and from biomass of Bt corn had no apparent effects on earthworms, nematodes, protozoa, bacteria, and fungi in soil (Saxena and Stotzky, 2001b). Nevertheless, as about 8.1 million hectares of Bt corn (26% of the total corn crop) were planted in the United States alone in 1999 (US Environmental Protection Agency, 2000), the continued large-scale planting of Bt crops should probably be evaluated for potential ecological effects.

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