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Is the Cry1Ab Protein from *Bacillus thuringiensis* (*Bt*) Taken Up by Plants from Soils Previously Planted with *Bt* Corn and by Carrot from Hydroponic Culture?

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Abstract The uptake of the insecticidal Cry1Ab protein from *Bacillus thuringiensis* (*Bt*) by various crops from soils on which *Bt* corn had previously grown was determined. In 2005, the Cry1Ab protein was detected by Western blot in tissues (leaves plus stems) of basil, carrot, kale, lettuce, okra, parsnip, radish, snap bean, and soybean but not in tissues of beet and spinach and was estimated by enzyme-linked immunosorbent assay (ELISA) to be 0.05 ± 0.003 ng g^{-1} of fresh plant tissue in basil, 0.02 ± 0.014 ng g^{-1} in okra, and 0.34 ± 0.176 ng g^{-1} in snap bean. However, the protein was not detected by ELISA in carrot, kale, lettuce, parsnip, radish, and soybean or in the soils by Western blot. In 2006, the Cry1Ab protein was detected by Western blot in tissues of basil, carrot, kale, radish, snap bean, and soybean from soils on which *Bt* corn had been grown the previous year and was estimated by ELISA to be 0.02 ± 0.014 ng g^{-1} of fresh plant tissue in basil, 0.19 ± 0.060 ng g^{-1} in carrot, 0.05 ± 0.018 ng g^{-1} in kale, 0.04 ± 0.022 ng g^{-1} in radish, 0.53 ± 0.170 ng g^{-1} in snap bean, and 0.15 ± 0.071 ng g^{-1} in soybean. The Cry1Ab protein was also detected by Western blot in tissues of basil, carrot, kale, radish, and snap bean but not of soybean grown in soil on which *Bt* corn had not been grown since 2002; the concentration was estimated by ELISA to be 0.03 ± 0.021 ng g^{-1} in basil, 0.02 ± 0.008 ng g^{-1} in carrot, 0.04 ± 0.017 ng g^{-1} in kale, 0.02 ± 0.012 ng g^{-1} in radish, 0.05 ± 0.004 ng g^{-1} in snap bean, and 0.09 ± 0.015 ng g^{-1} in soybean. The protein was detected by Western blot in 2006 in most soils on which *Bt* corn had or had not been grown since 2002. The Cry1Ab protein was detected by Western blot in leaves plus stems and in roots of carrot after 56 days of growth in sterile hydroponic culture to which purified Cry1Ab protein had been added and was estimated by ELISA to be 0.08 ± 0.021 and 0.60 ± 0.148 ng g^{-1} of fresh leaves plus stems and roots, respectively. No Cry1Ab protein was detected in the tissues of carrot grown in hydroponic culture to which no Cry1Ab protein had been added. Because of the different results obtained with different commercial Western blot (i.e., from Envirologix and Agdia) and ELISA kits (i.e., from Envirologix, Agdia, and Abraxis), it is not clear whether the presence of the Cry1Ab protein in the tissues of some plants under field condition and in carrot in sterile hydroponic culture was the result of the uptake of the protein by the plants or of the accuracy and sensitivity of the different commercial kits used. More detailed studies with additional techniques are obviously needed to confirm the uptake of Cry proteins from soil by plants subsequently planted after a *Bt* crop.

Keywords Cry1Ab protein · *Bacillus thuringiensis* · Plant uptake · Carrot

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Genetic engineering offers the opportunity to develop plants with a variety of new traits that may be useful in, for example, pest control, improvement of crop yields, frost prevention, drought resistance, increased nutritional value in food products, sources of medical and industrial biomolecules, and degradation of a variety of persistent

organic molecules, thereby facilitating bioremediation (e.g., Ghosal et al. 1985; Pontiroli et al. 2007). However, the fate and effects of the novel genes encoding these traits in plants and the potential effects of their products on ecosystems continue to be of concern. The release of DNA from plants, animals, and microorganisms can occur by lysis after their death (e.g., Widmer et al. 1997; Paget et al. 1998; Gebhard and Smalla 1999; Ceccherini et al. 2003), after infection of bacteria by bacteriophages (e.g., Widmer et al. 1996, 1997; Redfield 1988; Gebhard and Smalla 1999; Monier et al. 2007; Nielsen et al. 2007), and by active release of plasmid and chromosomal DNA by living bacteria (e.g., Lorenz and Wackernagel 1994; Nielsen et al. 2007). Such extracellular DNA can attain concentrations that could result in horizontal gene transfer (HGT) by transformation. Thus, over long periods of time, HGT is probably important in enhancing both microbial diversity in an environment such as soil (e.g., Neilson et al. 1994) and bacterial evolution (e.g., Yin and Stotzky 1997; Miller 1998; Bertolla and Simonet 1999; Sørensen et al. 2005; Fall et al. 2007; Heuer and Smalla 2007), as indicated in large-scale sequencing of bacterial genomes (Thomas and Nielsen 2005). Moreover, numerous bacterial species have been reported to be capable of natural transformation and of active or passive release of DNA (e.g., Lorenz and Wackernagel 1994; Paget and Simonet 1994; Heuer and Smalla 2007; Pontiroli et al. 2007).

The concept that extracellular DNA is rapidly degraded in soil (e.g., Greaves and Wilson 1970; Blum et al. 1997) has been challenged by studies that have shown that DNA is present in soil where free DNA or plant material had been deposited and to persist in nonsterile soil for several months (e.g., Romanowski et al. 1992, 1993; Widmer et al. 1996, 1997). Recombinant DNA was found in rhizosphere soil of transgenic potato plants, where it was apparently released by roots during plant growth and by pollen during flowering (De Vries et al. 2003). Plant roots provide microorganisms in the rhizosphere with large colonizable surface areas, and bacterial populations in the rhizosphere are presumably more stable than in bulk soil, as the result of the availability of nutrients and specific microniches (Sørensen et al. 2005). Studies in situ have shown that HGT occurs primarily in niches characterized by high bacterial densities, indicating that the available nutrients enable bacteria to form localized dense aggregates (i.e., microcolonies), wherein the probability of transfer of DNA is high (Sørensen et al. 2005; Pontiroli et al. 2007). In addition, DNA may be preserved in dead plant tissue, perhaps protected by cell walls, with retention of transforming capability for at least a few days (Nielsen et al. 2000). Moreover, the *cry1Ab* gene derived from fields of *Bt* corn was detected in nearby streams and rivers and sometimes several kilometers downstream, indicating contamination by agricultural transgenic DNA, and the gene persisted for more than 21 and 40 days in surface water and sediment, respectively. In contrast, the Cry1Ab protein was seldom detected in aquatic environments and then only in trace amounts (Douville et al. 2005, 2007).

The main factor affecting the persistence of DNA in soil appears to be the binding of DNA on surface-active particles (e.g., clay minerals, humic acids), which confers protection to the DNA against degradation by nucleases (e.g., Khanna and Stotzky 1992; Gallori et al. 1994; Lorenz and Wackernagel 1994; Vettori et al. 1996; Crecchio and Stotzky 1998; Khanna et al. 1998; Stotzky 2000; Crecchio et al. 2005). Proteins, peptides, amino acids, and viruses have also been shown to be protected against microbial degradation and inactivation when bound on such surface-active particles (e.g., Schiffenbauer and Stotzky 1982; Lipson and Stotzky 1985, 1986; Stotzky 1986, 2004; Dashman and Stotzky 1986; Khanna and Stotzky 1992; Vettori et al. 1999; Calamai et al. 2000; Lozzi et al. 2001). Although binding of DNA on surface-active particles protects the DNA from enzymatic degradation, it does not prevent transformation. Consequently, such protected cryptic genes can subsequently be expressed when a susceptible host comes into contact with soil particle – DNA complexes and HGT occurs.

HGT by transformation has been demonstrated in soil microcosms (e.g., Stotzky 1989; Gallori et al. 1994, 1998; Lorenz and Wackernagel 1994; Marsh and Wellington 1994; Nielsen et al. 1997, 2000; Pietramellara et al. 1997; Yin and Stotzky 1997; Crecchio and Stotzky 1998; Miller 1998; Bertolla and Simonet 1999; Vettori et al. 1999; Ceccherini et al. 2003; Crecchio et al. 2005; Brinkmann and Tebbe 2007; Heuer and Smalla 2007; Monier et al. 2007; Pontiroli et al. 2007; Richter and Smalla 2007). However, there have been few studies on HGT among bacteria and among and between other organisms in soil and other natural habitats in situ, and information on the range and frequency of HGT is needed for a more complete understanding of the potential effects of the releases of genetically modified organisms to the environment (e.g., Mohr and Tebbe 2007; Heuer and Smalla 2007).

Bacillus thuringiensis (*Bt*) corn (*Zea mays* L.) is transgenic corn into which *cry* genes from the bacterium *Bt* have been inserted to produce insecticidal Cry proteins to control insect pests of corn [e.g., European corn borer (*Ostrinia nubilalis* Hübner) (Lepidoptera), corn earworm (*Helicoverpa zea* Boddie) (Lepidoptera), corn rootworm complex (*Diabrotica* spp.) (Coleoptera)]. Cry proteins are released to soil from *Bt* plants in root exudates (Saxena et al. 1999, 2004), by the dispersal of pollen (Losey et al. 1999; Obrycki et al. 2001), and from decaying plant material (e.g., Stotzky 2002, 2004; Zwahlen et al. 2003).

Similar to extracellular DNA, the Cry proteins produced by *Bt* subsp. *kurstaki* (active against Lepidoptera), subspp. *morrisoni* (strain *tenebrionis*) and *kumamotoensis* (active against Coleoptera), and subsp. *israelensis* (active against Diptera) adsorbed and bound rapidly (in <30 min, the shortest time studied) on clay minerals [montmorillonite (M) and kaolinite (K)], on the clay-size fraction of soil, on humic acids, and on complexes of M-humic acids-Al hydroxypolymers (e.g., Venkateswerlu and Stotzky 1992; Tapp et al. 1994; Tapp and Stotzky 1995a, b, 1998; Crecchio and Stotzky 1998, 2001; Lee et al. 2003; Stotzky 2004; Fiorito et al. 2008). The binding of the Cry proteins on these surface-active particles reduced their availability to microbes, which was probably responsible for their persistence in soil (e.g., Koskella and Stotzky 1997; Crecchio and Stotzky 1998, 2001; Tapp and Stotzky 1998; Stotzky 2000, 2004; Saxena and Stotzky 2001a, b, 2002; Saxena et al. 2002a, b; Zwahlen et al. 2003; Muchaonyerwa et al. 2004; Wang et al. 2006; Icoz and Stotzky 2008a, b; Icoz et al. 2008). In addition to binding on surface-active particles, Cry proteins, after release into the soil solution, can be degraded by the soil microbiota, leached from the soil profile, abiotically destroyed, or taken up by plants, as has been observed with some pharmaceuticals from transgenic plants (e.g., Halling-Sørensen et al. 1998; Jørgensen and Halling-Sørensen 2000; Stotzky and Saxena 2009), some antibiotics from manure (Kumar et al. 2005; Boxall et al. 2006; Dolliver et al. 2007), and some inorganic and organic components of toxic wastes (Davies 1993; Kasai and Bayer 1995; Helgensen and Larsen 1998; Zayed et al. 1998; Engwall and Hjel 2000).

The major objectives of this study were to determine whether the Cry1Ab protein released in root exudates and from decaying plant residues of *Bt* corn was taken up by plants from soil in the field on which *Bt* corn had previously grown and by carrot, as a representative plant, in sterile hydroponic culture to which purified Cry1Ab protein had been added.

Materials and Methods

Experimental Design

In 2005, vegetables were grown in a field at the Rosemount Experiment Station of the University of Minnesota on which *Bt* corn expressing the Cry1Ab protein had been planted the previous year. The experimental design was a randomized complete block with 11 plant species (15 varieties) in four blocks, each on 1 m × 1 m plots for a total of 60 plots. The plants were basil (*Ocimum basillicum* L.), variety Genovese; beet (*Beta vulgaris* L.), varieties Early Wonder and Detroit Dark Red; carrot (*Daucus carota* L.), varieties Chantenay and Healthmaster; kale (*Brassica oleracea* L.), variety Dwarf Blue; lettuce (*Lactuca sativa* L.), variety Simpson; okra (*Hibiscus esculentus* L.), variety Clemson Spineless; parsnip (*Pastinaca sativa* L.), variety Hollow Crown; radish (*Raphanus sativus* L.), varieties Cherry Belle and White Icicle; snap bean (*Phaseolus vulgaris* L.), varieties Contender and Tendergreen; soybean [*Glycine max* (L.) Merr.], variety Edamame; and spinach (*Spinacia oleracea* L.), variety Bloomsdale.

In 2006, vegetables were grown in seven fields at the Rosemount Experimental Station. Each field was a randomized complete block design with a total of 32 plots, which were planted with four blocks of six plant species (eight varieties), each on 1 m × 1 m plots. The plants were basil (Genovese), carrot (Chantenay and Healthmaster), kale (Dwarf Blue), radish (White Icicle), snap bean (Contender and Tendergreen), and soybean (Edamame). Five of the seven fields had been planted to *Bt* corn expressing the Cry1Ab protein in 2005, and two had not been planted to *Bt* corn since 2002. The planting history of the fields was not revealed until after completion of the study. Mineralogically, the soils contain quartz, feldspars, amphiboles, some well crystallized mica-illite, low amounts of chlorite and kaolinite, and no smectites or other swelling clays. Some of the physicochemical properties of the soils, which belong predominantly to the Dakota and Waukegan series, are given by Icoz et al. (2008).

Sampling of Plants and Soils

Above-ground plant samples were collected randomly from each plot for a total of 60 plants in 2005 and a total of 224 plants in 2006. Plants (leaves plus stems) were thoroughly washed under a strong stream of water to remove all soil, patted dry, put into plastic bags, which were placed on dry ice and shipped to New York University on dry ice, where the samples were immediately stored at −20°C upon receipt and analyzed for the presence of the Cry1Ab protein by Western blot and ELISA. Soil samples were also collected randomly from some of the same plots, placed and shipped on dry ice, and analyzed for the presence of the Cry1Ab protein.

Western Blot Assays

Plants and randomly selected soils from each plot were analyzed for the presence of the Cry1Ab protein immunologically with commercial Western blot assay kits from two manufacturers: ImmunoStrips from Agdia, Elkhart, IN (Catalog # STX 06200), and Quickstix from Envirologix, Portland, ME (Catalog # AS 003 CRLS). Frozen plant material (1 g) was homogenized with a mortar and pestle on ice in 1 mL of extraction buffer provided in the kits, and

0.5 g of soil was vortexed in 0.5 mL of the extraction buffer, centrifuged at 10,500×*g* for 5 min, and the supernatants analyzed by following the protocol of the manufacturers. In 2006, 0.65 cm^2 of fresh plant material, taken from the first true leaf of seedlings from each plot and from a mature leaf of the plants shipped to New York University, was also analyzed at the University of Minnesota using Western blot kits from Agdia.

ELISA

In 2005, 44 samples (four replicates of 11 plant species) and, in 2006, 120 (four replicates of six plant species from five fields in which *Bt* corn had been planted previously) and 48 samples (four replicates of six plant species from two fields in which *Bt* corn had not been planted since 2002) were analyzed for the presence of the Cry1Ab protein with enzyme-linked immunosorbent assay (ELISA) kits from three manufacturers: Agdia (Catalog # PSP 06200), Envirologix (Catalog # AP 003 CRBS), and Abraxis LLC, Warminster, PA (Catalog # PN510001). The amount of the Cry1Ab protein in plant extracts was quantified by following the protocol of each manufacturer: briefly, 1 g of fresh plant material (leaves plus stems) was homogenized in 1 mL of extraction buffer provided in the kits in a mortar and pestle on ice, centrifuged at 10,500×*g* for 5 min, and the supernatants were added to each well of ELISA plates coated with the antibody to the Cry1Ab protein and containing an antibody-peroxidase conjugate; after incubation for 2 h at 27°C, the wells were washed with washing buffer provided in the kits, a substrate solution provided in the kits was added, the plates incubated at room temperature, a stop solution provided in the kits was added, and the optical density was measured at 450 nm with the kits from Envirologix and Abraxis and at 650 nm with the kit from Agdia in a Microplate reader (Bio-Rad, Model 550) using Microplate Manager 4 Software. A standard curve was constructed with purified Cry1Ab protein to estimate the amount of Cry1Ab protein in the plant samples.

Larvicidal Assay

The larvicidal activity of the plant material was determined with the larvae of the tobacco hornworm (*Manduca sexta* L.; Tapp and Stotzky 1998). In 2005, 60 plants (four replicates of 15 varieties) and, in 2006, 32 plants (four replicates of eight varieties) from a field that had been planted with *Bt* corn in 2005 and from a field that had not been planted with *Bt* corn since 2002 were analyzed for larvicidal activity. Eggs of *M. sexta* and food medium were obtained from Carolina Biological Supply Company (Burlington, NC). The eggs, dispensed on solidified medium in Petri plates, were incubated at 29 ± 1°C under a 40-W lamp for 3–5 days. The medium was dispensed, after microwaving, in 5-mL amounts into vials (3 cm diameter, 6 cm tall) and allowed to solidify. Aliquots (100 μL) of homogenized plant extracts (leaves plus stems) or of just the extraction buffer were uniformly distributed over the surface of the medium (8.55 cm^2) with disposable pipette tips (200-μL capacity) that had been cut ca. 1.5 cm from the tip, to ensure that all suspended plant particles were transferred. After air-drying, 4 second-instar larvae were added to each of four vials, resulting in 16 larvae for each plant sample. Mortality was determined after 3 and 7 days, and percent mortality was based on mortality after 7 days.

Uptake of Purified Cry1Ab Protein by Carrot in Sterile Hydroponic Culture

Seeds of carrot (Goldmine Hybrid) were surface-sterilized with a solution of 5.25% NaOCl:sterile distilled water (dH_2O) (1.5:1 v v^{-1}) for 15 min, washed repeatedly with sterile dH_2O for 15 min (Saxena et al. 1999), placed on nutrient agar (Difco) in Petri plates at 25 ± 2°C to check for sterility, and germinated for 7–9 days. Seedlings were transferred aseptically to 30 replicate sterile test tubes (3 cm diameter, 20 cm tall) containing a cone of filter paper (Whatman No. 2) at the bottom and 3 mL of sterile Hoagland's solution (Hoagland's No. 2 Basal Salt Mixture; Sigma); 100 μg of purified Cry1Ab protein dissolved in 1 mL of phosphate buffered saline (PBS) was added to 15 tubes, and 1 mL of PBS was added to the other 15 tubes (Tapp and Stotzky 1995a). The protein concentration was estimated by the Lowry assay (Lowry et al. 1951). Plants were grown at 25 ± 2°C under a 16-h light and 8-h dark cycle. The hydroponic solution was replenished aseptically as needed. When the carrot plants reached the top of the tubes (after 56 days), the Hoagland's solution and plant tissues (leaves plus stems and roots) from randomly selected test tubes were analyzed for the presence of the Cry1Ab protein by Western blot and ELISA.

Statistics

Each of four replicate field-grown plants was evaluated at least three times by Western blot and at least twice by ELISA, resulting in at least 12 Western blots and eight ELISAs plant $variety^{-1}$. At least five randomly selected carrot plants in hydroponic cultures were evaluated by Western blot (ImmunoStrips) and by ELISA. The results of Western blots are expressed as the presence (+) or absence (−) of the Cry1Ab protein. The results of ELISA (in ng g^{-1} of fresh plant tissue) are expressed as the means ± the standard errors of the means.

Results and Discussion

Uptake of Cry1Ab Protein from Soil by Plants Under Field Conditions

The Cry1Ab protein was detected by Western blot (Agdia) in some tissue samples of basil, carrot, kale, lettuce, okra, parsnip, radish, snap bean, and soybean but not in any tissue samples of beet and spinach in 2005 (Table 1). Trace amounts of the Cry1Ab protein, estimated by ELISA (Envirologix) to be 0.05 ± 0.003, 0.02 ± 0.014, and 0.34 ± 0.176 ng g^{-1} of fresh plant tissue, were also found in some samples of basil, okra, and snap bean, respectively, but not in those of carrot, kale, lettuce, parsnip, radish, and soybean. The mortality of larvae of *M. sexta* exposed to extracts of the plants ranged from $12.5 \pm 7.22\%$ to $37.5 \pm 7.22\%$; the background mortality of larvae exposed to only the extraction buffer was $6.3 \pm 6.25\%$. No Cry1Ab protein was detected in the soils on which the plants were grown (Table 1).

In 2006, some tissue samples of basil, carrot, kale, radish, snap bean, and soybean grown in soil on which *Bt* corn had been planted in the previous year tested positive for the presence of the Cry1Ab protein with the Western blot assay from Agdia, but they tested negative, except for some samples of basil and kale, with the Western blot assay from Envirologix (Table 2). However, the Cry1Ab protein in the tissues of basil and kale gave only a very faint band on the Western blot assay from Envirologix. The Cry1Ab protein was detected in trace amount in only the tissues of snap bean (0.04 ± 0.012 ng g^{-1} of fresh plant tissue) with an ELISA kit from Agdia, whereas the protein was detected with an ELISA kit from Abraxis and estimated to be 0.02 ± 0.014 ng g^{-1} of fresh plant tissue in basil, 0.19 ± 0.060 ng g^{-1} in carrot, 0.05 ± 0.018 ng g^{-1} in kale, 0.04 ± 0.022 ng g^{-1} in radish, 0.53 ± 0.170 ng g^{-1} in snap bean, and 0.15 ± 0.071 ng g^{-1} in soybean (Table 2). None of the 0.65-cm^2 fresh tissue samples from seedlings or mature plants, which were significantly smaller than the 1-g samples from the frozen tissues, tested positive with ImmunoStrips from Agdia.

Some samples of plants grown in soils on which *Bt* corn had not been planted since 2002 also tested positive, except for soybean, for the presence of the Cry1Ab protein with the Western blot assay from Agdia (Table 3). The protein was also detected, albeit in trace amounts, in some samples with an ELISA kit from Abraxis and estimated to be 0.03 ± 0.021 ng g^{-1} of fresh plant tissue in basil, 0.02 ± 0.008 ng g^{-1} in carrot, 0.04 ± 0.017 ng g^{-1} in kale, 0.02 ± 0.012 ng g^{-1} in radish, 0.05 ± 0.004 ng g^{-1} in snap bean, and 0.09 ± 0.015 ng g^{-1} in soybean (Table 3).

In 2006, the mortality of *M. sexta* exposed to extracts of the plants was lower than the mortality in 2005 (Table 1) and ranged from $3.1 \pm 3.13\%$ to $6.3 \pm 6.25\%$ for plants grown in soils on which *Bt* corn had been planted the previous year and from 0% to $12.5 \pm 7.22\%$ for plants grown in soils on which *Bt* corn had not been planted since 2002 (Tables 2, 3, respectively). The background mortality of *M. sexta* exposed to only the extraction buffer was 0% in 2006.

Table 1 Presence in 2005 of Cry1Ab protein in plants grown in soils on which *Bt* corn had been planted in the previous year and in the soils on which the plants were grown

Plant	Immunological assay[a]	ELISA[b] (ng g^{-1})	Mortality[c] (%)	Presence of protein in soil[a]
Basil	+(2/4)[d]	0.05 ± 0.003	28.2 ± 11.05	−(0/4)[d]
Beet	−(0/8)	ND	ND	ND
Carrot	+(6/8)	0	25.0 ± 4.77	−(0/4)
Kale	+(2/4)	0	28.2 ± 8.79	−(0/4)
Lettuce	+(1/4)	0	12.5 ± 7.22	−(0/4)
Okra	+(1/4)	0.02 ± 0.014	37.5 ± 7.22	−(0/4)
Parsnip	+(1/4)	0	18.8 ± 6.25	−(0/4)
Radish	+(2/8)	0	31.3 ± 7.86	−(0/4)
Snap bean	+(3/8)	0.34 ± 0.176	22.9 ± 8.40	−(0/4)
Soybean	+(1/4)	0	18.8 ± 11.97	−(0/4)
Spinach	−(0/4)	ND	ND	ND

ND not determined

[a] Western blot (Agdia). "+" = presence; "−" = absence of Cry1Ab protein

[b] Enzyme-linked immunosorbent assay (ELISA) (Envirologix)

[c] Mortality of *Manduca sexta* exposed to extracts of the plants. Each treatment had four replicates; at least 16 larvae assayed in each treatment, and data are expressed as the means of 16 larvae ± the standard errors of the means

[d] The presence of Cry1Ab protein (positive results/total plant or soil samples analyzed)

Table 2 Presence in 2006 of Cry1Ab protein in plants grown in soils on which *Bt* corn had been planted in the previous year and in the soils on which the plants were grown

Plant	Immunological assay[a]	Immunological assay[b]	ELISA[c] (ng g^{-1})	ELISA[d] (ng g^{-1})	Mortality[e] (%)	Presence of protein in soil[a]
Basil	+(13/20)[f]	+(2/6)[f,g]	0	0.02 ± 0.014	0	+(2/3)[f]
Carrot	+(25/37)	−(0/4)	0	0.19 ± 0.060	6.3 ± 4.10	+(3/5)
Kale	+(10/20)	+(1/7)[g]	0	0.05 ± 0.018	0	+(3/3)
Radish	+(3/19)	−(0/2)	0	0.04 ± 0.022	6.3 ± 6.25	+(2/3)
Snap bean	+(17/39)	−(0/9)	0.04 ± 0.012	0.53 ± 0.170	3.1 ± 3.13	+(2/4)
Soybean	+(4/20)	−(0/2)	0	0.15 ± 0.071	0	+(1/4)

[a] Western blot (Agdia). "+" = presence; "−" = absence of Cry1Ab protein

[b] Western blot (Envirologix). "+" = presence; "−" = absence of Cry1Ab protein

[c] Enzyme-linked immunosorbent assay (ELISA) (Agdia)

[d] ELISA (Abraxis)

[e] Mortality of *Manduca sexta* exposed to extracts of the plants. Each treatment had four replicates; at least 16 larvae assayed in each treatment, and data are expressed as the means of 16 larvae ± the standard errors of the means

[f] The presence of Cry1Ab protein (positive results/total plant or soil samples analyzed)

[g] The band was faint

Table 3 Presence in 2006 of Cry1Ab protein in plants grown in soils on which *Bt* corn had not been planted since 2002 and in the soils on which the plants were grown

Plant	Immunological assay[a]	ELISA[b] (ng g^{-1})	Mortality[c] (%)	Presence of protein in soil[a]
Basil	+(8/8)[d]	0.03 ± 0.021	6.3 ± 6.25	+(2/2)[d]
Carrot	+(12/16)	0.02 ± 0.008	10.7 ± 5.06	+(2/3)
Kale	+(4/8)	0.04 ± 0.017	12.5 ± 7.22	+(2/3)
Radish	+(2/8)	0.02 ± 0.012	0	−(0/2)
Snap bean	+(9/16)	0.05 ± 0.004	6.3 ± 4.10	+(4/5)
Soybean	−(0/8)	0.09 ± 0.015	0	−(0/3)

[a] Western blot (Agdia). "+" = presence; "−" = absence of Cry1Ab protein

[b] Enzyme-linked immunosorbent assay (ELISA) (Abraxis)

[c] Mortality of *Manduca sexta* exposed to extracts of the plants. Each treatment had four replicates; at least 16 larvae assayed in each treatment, and data are expressed as the means of 16 larvae ± the standard errors of the means

[d] The presence of Cry1Ab protein (positive results/total plant or soil samples analyzed)

In contrast to the results of 2005, some of the soils on which the plants were grown also gave positive results in 2006 for the presence of the Cry1Ab protein, albeit in trace amounts. For example, the Cry1Ab protein was detected in soils that had been planted to *Bt* corn in the previous year (Table 2), as well as in the soils, except for soils from radish and soybean, that had not been planted to *Bt* corn since 2002 (Table 3).

Vegetables purchased from supermarkets and farm-stands were also analyzed for the presence of the Cry1Ab protein, which was not detected in the tissues of carrot, kale, radish, and lettuce but was detected in the tissue of basil by the Western blot assay from Agdia. Different concentrations of purified Cry1Ab protein in solution tested positive with the Western blot assays from Agdia and Envirologix, indicating that these Western blot kits were functioning properly with purified protein.

Uptake of Purified Cry1Ab Protein by Carrot in Sterile Hydroponic Culture

The Cry1Ab protein was detected by Western blot (Agdia) in all tissues (i.e., leaves plus stems and roots) of all carrot plants analyzed after 56 days of growth in hydroponic solution to which 100 μg of purified Cry1Ab protein had been added, and its presence was confirmed by ELISA (Abraxis). The concentration of the protein in leaves plus stems and roots was estimated to be 0.08 ± 0.021 and 0.60 ± 0.148 ng g^{-1} of fresh plant tissue, respectively (Table 4). No Cry1Ab protein was detected by Western

Table 4 Uptake of Cry1Ab protein by carrot grown in sterile hydroponic culture to which 100 μg of purified Cry1Ab protein had been added

	Cry1Ab protein added		No Cry1Ab protein added	
	Immunological assay[a]	ELISA[b] (ng g^{-1})	Immunological assay[a]	ELISA[b] (ng g^{-1})
Leaves and stems	+(5/5)	0.08 ± 0.021	−(0/5)	0
Roots	+(5/5)	0.60 ± 0.148	−(0/5)	0
H.S.[c]	+(5/5)	0.20 ± 0.118	−(0/5)	0

[a] Western blot (Agdia).“+” = presence; “−” = absence of Cry1Ab protein. The presence of Cry1Ab protein (positive results/total plants or H.S. samples analyzed)

[b] Enzyme-linked immunosorbent assay (ELISA) (Abraxis)

[c] Hoagland’s solution (ng mL^{-1})

blot and ELISA in the tissues of carrots grown in hydroponic culture to which no protein had been added.

Plants can become contaminated with toxic organic chemicals, and their uptake by vegetation is a major cause of their bioaccumulation in the food chain and an important route of their exposure to human beings and other animals. For example, pesticides, herbicides, polychlorinated biphenyls, dioxins, and other organic compounds that are widely used and ubiquitous in the environment accumulate in grain crops (Duarte-Davidson and Jones 1996; Collins et al. 2006), and endosulfan compounds were present in the roots of lettuce (Esteve-Turrilas et al. 2005). Antibiotics, such as sulfamethazine, chlortetracycline, tylosin, florfenicol, and trimethoprim, commonly added to animal feed to promote growth of food animals, have also been shown to be taken up from manure-amended soil by corn, lettuce, potato, cabbage, green onion, and carrot (Kumar et al. 2005; Boxall et al. 2006; Dolliver et al. 2007). Chlortetracycline was taken up by cabbage, corn, and green onion from manure-amended soil, but tylosin was not, presumably because the large size of the tylosin molecule prevented mass flow or active uptake (Kumar et al. 2005). Enrofloxacin, florfenicol, and trimethoprim were detected in carrot, but only enrofloxacin was detected in lettuce (Boxall et al. 2006). A higher accumulation of antibiotics was found in carrot peels than in whole carrots, indicating that accumulation of antibiotics may be of particular concern with edible root and tuber crops grown in soil containing antibiotics (Boxall et al. 2006).

Although there have been numerous studies on the uptake by plants from soil of hydrophobic compounds, such as polychlorinated biphenyls, dioxins, furans, and polycyclic aromatic hydrocarbons (e.g., Hülster et al. 1994; Duarte-Davidson and Jones 1996; Esteve-Turrilas et al. 2005; Collins et al. 2006), and of antibiotics (e.g., Kumar et al. 2005; Boxall et al. 2006; Dolliver et al. 2007), there appears to have been only one study on the uptake of Cry proteins by plants (Saxena and Stotzky 2001c, 2002). Some of the results of the current study, in which the Cry1Ab protein was not detected in plant tissues by the Western blot kit from Envirologix, were in agreement with those of Saxena and Stotzky (2001c, 2002), who reported no uptake of the Cry1Ab protein, using the Western blot kit from Envirologix, by: (1) non-*Bt* corn, carrot, radish, and turnip grown in soil, in a plant-growth room, on which *Bt* corn had been grown or which had been amended with purified Cry1Ab protein or ground biomass of *Bt* corn; or (2) by non-*Bt* corn grown in sterile hydroponic culture in which *Bt* corn had previously been grown. However, some results of the current study were not in agreement with those of Saxena and Stotzky (2001c, 2002), as the Cry1Ab protein was detected in the tissues of various plants grown on soil previously planted to *Bt* corn by the Western blot kit from Agdia and by the ELISA kits from Agdia and Abraxis. The reasons for the differences in the results with kits from different manufacturers are not known. The differences may have been the result of the relative sensitivity of the kits from different manufacturers; of the possibility of false positives or negatives in the assays, despite the large number of replicate analyses of each field-grown plant variety (e.g., 12 Western blots and eight ELISAs plant $variety^{-1}$); or of differences in the chemical composition of the extracts from different plant species (i.e., perhaps some components in the extracts, other than the Cry1Ab protein, may have interacted with components of the kits to give false positive results). However, the latter possibility is remote, as numerous negative results were obtained with extracts from the same plants grown in soil that had not recently been planted with *Bt* corn.

In 2006, the Cry1Ab protein was detected in the soils on which the plants were grown, whereas it was not detected in the soils in 2005. In addition, the protein was also detected in most of the soils on which *Bt* corn had not been planted since 2002. Variable durations of persistence of Cry proteins in soil have been reported. In several studies in soil microcosms, the Cry proteins from *Bt* corn, *Bt* cotton, and *Bt* potato (Cry1Ab and Cry3Bb1, Cry1Ac, and Cry3Aa protein, respectively) did not persist and were

generally degraded within several weeks (e.g., Ream et al. 1994; Palm et al. 1996; Sims and Holden 1996; Head et al. 2002; Hopkins and Gregorich 2003; Dubelman et al. 2005; Icoz and Stotzky 2008a). By contrast, other studies have reported that the Cry1Ab protein from *Bt* corn persisted and was still detectable in plant residues after 240 days in the field (Zwahlen et al. 2003; Baumgarte and Tebbe 2005) and after 8 months (Tapp and Stotzky 1998) to 3 years in soil (Saxena and Stotzky 2002; Baumgarte and Tebbe 2005). The Cry2A protein from *Bt* cotton was still detectable after 120 days in soil in the field (Sims and Ream 1997). These differences in persistence and in rates of degradation of Cry proteins were probably the result of differences in plant species, Cry proteins, soil types, pH, microbial activity, temperatures, and methods used.

The pathways by which organic molecules enter vegetation depend on the chemical and physical properties of each molecule, such as hydrophobicity, water solubility, and vapor pressure; on environmental factors, such as temperature, pH, and organic matter content of the soil; and on the species of plant, its metabolism, and the composition of its roots and root exudates (e.g., Travis and Arms 1988; Paterson et al. 1990; Hellström 2003; Collins et al. 2006). The uptake of organic pollutants by plants is important when evaluating the risks associated with land contamination, the role of vegetation in the global cycling of persistent organic pollutants, and the potential of industrial discharges and agricultural practices to contaminate the food chain (e.g., Kumar et al. 2005; Collins et al. 2006). The current study indicated that some plant species grown in soils on which *Bt* corn had been grown take up the Cry1Ab protein, albeit in small amounts. More detailed studies with different methods and techniques are obviously needed to confirm the uptake by plants of Cry proteins from soil. If confirmed, the risks to the environment of such uptake will need to be evaluated. Furthermore, although HGT among microorganisms has been demonstrated in soil microcosms, and the *cry1Ab* gene has been shown to be transported in soil and waters, there is scant evidence for the uptake of *cry* genes by plants and other organisms from the environment. Because the uptake of *cry* genes could result in the expression of Cry proteins in plants, more studies on the uptake of *cry* genes by different plant species in soil in situ are also necessary. Such HGT of *cry* genes might also explain the discrepancies between the detection of the Cry1Ab protein in plants grown in soils either previously planted or not planted with *Bt* corn.

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