Release of Larvicidal Cry Proteins in Root Exudates of Transgenic Bt Plants

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Transgenic plants engineered to express larvicidal Cry proteins from *Bacillus thuringiensis* (Bt) can reduce the use of broad-spectrum chemical insecticides. However, there is some concern that Bt toxins (Cry proteins) released to soil may pose risks to the environment. The toxins could accumulate to concentrations that may constitute a hazard to nontarget organisms, such as the soil microbiota, beneficial insects, and other animal classes, and may result in the selection and enrichment of toxin-resistant target insects. Conversely, the accumulated toxins could increase the control of target pests. Accumulation is enhanced when the toxins are bound on surface-active particles in the environment (e.g., clays, humic substances) and, thereby, are rendered less accessible for microbial degradation but still retain their toxic activity. The toxins produced by *B. thuringiensis* subsp. *kurstaki* (Bt*k*; antilepidopteran), subsp. *morrisoni* strain *tenebrionis* (Bt*t*; anticoleopteran), and subsp. *israelensis* (Bt*i*; antidipteran) bound rapidly on these surface-active particles, persisted in soil and water, and remained larvicidal to the tobacco hornworm (*Menduca sexta*), the Colorado potato beetle (*Leptinotarsa decemlineata*), and a mosquito (*Culex pipiens*), respectively, which were used as assay species[1].

Although the major introduction of the toxins to soil occurs after harvest of a Bt crop, the Cry1Ab protein was released in root exudates from transgenic Bt corn plants throughout their growth in sterile hydroponic culture and in sterile and nonsterile soil[2]. The presence of the toxin was demonstrated by SDS-PAGE and confirmed by immunological and larvicidal assays. The toxin from root exudates was also detected in natural soil 180 days (the longest time evaluated) after growth of Bt corn in a plant-growth room, as well as in the field after harvest and after frost from Bt corn plants that had been dead for several months. The Cry1Ab protein was released in root exudates from all 13 Bt corn hybrids, representing three transformation events (Bt11, MON810, and 176) and evaluated in both the plant-growth room and the field[3].

To determine whether the release of Cry proteins in root exudates is a common phenomenon in transgenic Bt plants, the release of different Cry proteins (Cry1Ab, Cry3A, and Cry1Ac) in the root exudates of Bt corn (*Zea mays* L.), and rice (*Oryyza sativa* L.), potato (*Solanum tubersoum* L.), and canola *(Brassica napus* L.), cotton (*Gossypium hirsutum* L.), and tobacco (*Nicotiana tabacum* L.), respectively, was evaluated. Soil and hydroponic solution in which Bt corn, rice, or potato had been grown were both immunologically positive for the presence of the Cry proteins and toxic to the larva of *M. sexta* (corn and rice) and *L. decemlineata* (potato). No Cry proteins were detected immunologically or by larvicidal assay in any soil or hydroponic solution in which Bt canola, cotton, or tobacco, as well as all near-isogenic non-Bt plant counterparts or no plants, had been grown[4]. However, the Cry proteins were detected in the tissues of all Bt plants. There were apparent differences in exudation of the proteins (as evaluated immunologically and by mortality and weight of surviving larvae) between plant species: exudates from Bt corn were more larvicidal than those from Bt rice and potato. No green fluorescent protein (GFP) was detected in hydroponic solutions from canola and tobacco genetically modified to express GFP or both GFP and the Cry1Ac protein.

Cry proteins released in root exudates of Bt corn, rice, and potato accumulated in soil and retained larvicidal activity, probably as the result of the binding of the proteins on surface-active particles in soil, which rendered them resistant to rapid biodegradation[1,2,3]. Although some Cry proteins were probably released from sloughed and damaged root cells of corn, rice, and potato in soil, the major portion was apparently derived from root exudates, as there was no discernable root debris when plants were grown in hydroponic culture, and no Cry proteins from Bt canola, cotton, or tobacco were detected when grown in soil where some damage to roots probably occurred.

In addition to the introduction to soil of Cry proteins in plant biomass and some in pollen, the proteins will also be released in root exudates during the entire growth of some Bt plants. The continual presence of the Cry proteins in soil could improve the control of insect pests, enhance the selection of toxin-resistant target insects, and/or constitute a hazard to nontarget organisms. The Cry1Ab protein released in root exudates and from biomass of Bt corn had no apparent effects on earthworms, nematodes, protozoa, bacteria, and fungi in soil[5]; it was not taken up by radish, carrot, turnip, and non-Bt corn[6]; and it did not move far vertically in soil[7].

Why Cry proteins were released in root exudates of Bt corn, rice, and potato but not in exudates of Bt canola, cotton, and

tobacco is not known. The methods of transformation of the *cry* genes, somaclonal variation, differences in level of protein expression (although all species had the cauliflower mosaic virus 35S promoter, except rice, which had the ubiquitin promoter from corn), or location of the endoplasmic reticulum relative to the plasma membrane (in corn, this appears to be a close relation) may have been involved[1]. Although Cry1Ab and Cry1Ac proteins differ in some aspects, the differences are apparently small, as their insect targets are similar and they cross-react with antibodies to each other[1]. Nevertheless, the differences may be responsible for release of the Cry1Ab protein and the lack of release of the Cry1Ac protein in root exudates. Further studies, especially by plant physiologists and anatomists, are obviously necessary.
The relevance of these observation also requires clarification, especially as at least 26 plant species, including corn, cotton, potato, canola, rice, broccoli, peanut, eggplant, and other crop species, have been modified to express Cry proteins, and 8.1 million hectares of Bt corn or 26% of total corn acreage, 2.4 million hectares of Bt cotton or 45% of total cotton acreage, and 0.02 million hectares of Bt potato or 3.5% of total potato acreage were planted in the United States alone in 2000[4].

The release of Bt toxins in the root exudates of some plant species, and their persistence in soil, indicate that caution must be exercised before plants and animals genetically modified to express pharmaceuticals (e.g., vaccines, hormones, antibiotics, blood substitutes, enzymes) and other bioactive compounds ("pharms") are introduced to the environment[8,9]. In contrast to pesticidal transgenic plants, the targets of these compounds, which are seldom found in natural habitat[5] (i.e., they are essentially environmental xenobiotics), are human beings and other "higher level" eukaryotes rather than insects, nematodes, and protozoa. Inasmuch as the persistence in and the effects on the environment of these biomolecules have not been studied adequately, their potential hazards are not known and cannot be predicted. As with Bt plants, where only a portion of the plants is harvested and the remainder of the biomass is incorporated into soil wherein the toxins released from disintegrating biomass are rapidly bound on surface-active particles, a substantial portion of the biomass of plant pharms containing the products of introduced novel genes will also be incorporated into soil. With pharms of transgenic animals, the feces, urine, and subsequently even carcasses containing bioactive compounds will eventually reach soil and other natural habitats. If these bioactive compounds (including prions from diseased animal carcasses) bind on surface-active particles—and as many of these compounds are proteinaceous, they most likely will—they may also persist in these habitats. If they retain their bioactivity, they could affect the biology of these habitats. Consequently, before the large-scale use in the field of such plants and animals pharms, the persistence of their products and the potential effects of the products on the inhabitants of soil and other habitats must be thoroughly evaluated.

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