PL-2

Release, Persistence, and Biological Activity in Soil of Insecticidal Proteins from *Bacillus thuringiensis*

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TABLE 1. SUMMARY OF INTERACTIONS OF PURIFIED BT TOXINS WITH SURFACE-ACTIVE SOIL PARTICLES AND WITH SOIL: EFFECTS ON PERSISTENCE AND LARVICIDAL ACTIVITY.

- Larvicidal proteins from *Bacillus thuringiensis* subspp. *kurstaki* (*Btk*; antilepidopteran), *morrisoni*, strain *tenebrionis* (*Btt*; anticoleopteran), and *israelensis* (*Bti*; antidipteran) bound rapidly and tightly on clays, humic acids, and complexes of clayhumic acid-Al hydroxypolymers; binding was pH dependent and greatest near the isoelectric point (pI) of the proteins.
- Bound toxins retained their structure, antigenicity, and insecticidal activity.
- Intercalation of clays by the toxins was minimal; toxins probably too large (~66 kDa).
- Biodegradation of the toxins was reduced when bound; microbial utilization of the toxins as a source of carbon was reduced significantly more than use as a source of nitrogen.
- Lavicidal activity of the toxin from *Btk* was detected 234 days after addition to nonsterile soils (longest time studied).
- Persistence of larvicidal-activity of the toxin from *Btk* was greater in acidic soils, probably because microbial activity was lower than in less acid soils; persistence was reduced when the pH of acidic soils was raised to *ca*. 7.0 with $CaCO_3$.
- Persistence in soil was similar under aerobic and anaerobic conditions and when soil was alternately wetted and dried or frozen and thawed, which indicated tight binding.
- Persistence in soil was demonstrated immunologically (e.g., dot-blot ELISA, flow cytometry, Western blots) and by larvicidal assays.
- Toxins from *Btk*, *Btt*, and *Bti* had no microbiostatic or microbicidal effect against a spectrum of bacteria (gram-positive and gram-negative), fungi (filamentous and yeast), and algae, neither in pure nor in mixed cultures.

SUMMARY OF FATE AND EFFECTS IN SOIL OF Bt TOXINS IN ROOT EXUDATES AND BIOMASS OF TRANGENIC PLANTS

- Cry1Ab protein was released in root exudates of *Bt* corn (13 hybrids representing three transformation events) and persisted in rhizosphere soil *in vitro* and *in situ*; protein accumulated more in soil amended (3 to 12%) with montmorillonite than with kaolinite.
- Cry1Ab protein was also released in root exudates of rice, Cry3A protein was released in root exudates of *Bt* potato, and Cry3Bb1 protein was released in root exudates of *Bt* corn. Cry1Ac protein was not released in root exudates of *Bt* canola, cotton, and tobacco.
- Cry1Ab protein - purified, in root exudates, and from biomass of *Bt* corn -was not taken up from nonsterile soil or sterile hydroponic culture by non-*Bt* corn, carrot, radish, and turnip, even though the toxin released in root exudates persisted for at least 180 days and that from biomass for at least 3 years in soil (the longest times studied).
- Cry1Ab protein - purified, in root exudates, and from biomass of *Bt* corn - moved slightly through soil leached with water; movement was less in soils amended with montmorillonite than with kaolinite and decreased as the concentration of added clays increased.
- Biodegradation of biomass of transgenic *Bt* corn (measured by CO_2 evolution) was significantly lower in soil and *in vitro* (biomass inoculated with a soil suspension) than that of near-isogenic non-*Bt* corn.
- No consistent statistically significant differences in numbers of culturable bacteria and fungi and in activity of representative enzymes between soil amended with *Bt* or non-*Bt* corn or not amended.
- Lower metabolic activity of soil amended with *Bt* corn may have been result of significantly higher lignin content in *Bt* than in near-isogenic non-*Bt* corn.
- Biodegradation of biomass of *Bt* canola, cotton, potato, rice, and tobacco was also significantly lower than that of biomass of near-isogenic non-*Bt* plants, but lignin content of these plant species, which was considerably lower than that of corn, was not significantly different between *Bt* and non-*Bt* biomass.
- Cry1Ab protein released in root exudates or from biomass of *Bt* corn appeared to have no effect on numbers of earthworms, nematodes, bacteria, and fungi in soil.
- Toxins from *Bt* persist, accumulate, and remain insecticidal in soil as the result of binding on clays and humic substances and, therefore, could enhance control of insect pests, enhance selection of toxin-resistant target species, and/or pose a hazard to nontarget organisms.
- Molecular “pharming” utilizes transgenic plants and animals to produce drugs for use primarily in human beings (e.g., vaccines, hormones, antibodies, blood substitutes, antigens, enzymes, toxins).
- These pharmaceutical products are seldom found in natural habitats; hence, they are environmental “xenobiotics”.

· Their persistence in and effects on the environment have not been studied adequately; hence, potential hazards are not know.
· In contrast to pesticidal transgenic plants, the targets of these biomolecules are human beings and other "higher level" eukaryotes rather than insects, nematodes, and protozoa.
· The products of the foreign genes are usually expressed in leaves, stems, roots, and pollen of transgenic plants and in milk, blood, urine, sperm, eggs, and other tissues of transgenic animals.
· Many of the products have therapeutic purposes, such as treatment of cystic fibrosis, hemophilia, osteoporosis, arthritis, malaria, and HIV.
· Some vaccines produced in plants are edible (oral immunotherapy), which may be important in delivery and in prevention of spoilage, especially in developing countries where refrigeration is limited.
· Production of these pharmaceuticals in transgenic plants and animals in more economical than production by classical chemical and microbiological methods.
· Animals in "factory" farms are also exposed to toxic chemicals, artificial hormones, antibiotics, tranquilizers, appetite stimulants, insecticides, herbicides, etc.
· These compounds can be passed to human beings in meat and to the environment in urine, feces, and carcasses.
· Expressed proteins from transgenic plants will be released to the environment in root exudates, pollen, and biomass after harvest of crops.
· These biomolecules will probably persist and accumulate in the environment as the result of their binding on surface-active particles (e.g., clays, humic substances), which reduces their biodegradation, as has been observed with other proteins, DNA, viruses, etc.
· Persistence and potential effect of these biomolecules on indigenous organisms in recipient natural habitats (e.g., on microbial populations and processes in soil necessary for biogeochemical cycling and crop production) must be established before the increased release of such transgenic plant and animals.
· At present, about 400 products of transgenic plants are being considered for release to soil, and some of these transgenic plants have already been grown in 300 undisclosed sites throughout the USA.
· Studies of bioactive compounds released by transgenic plants and animals will also provide information about the persistence and potential effects of pharmaceuticals that reach soil and waters from numerous other sources.
· A broad spectrum of drugs has already been detected in sewage sludge and effluent, surface and ground waters (even in tap water), and soils of feedlots and under landfills: e.g., aspirin; caffeine; nicotine; estradiol; anti-inflammatory drugs, such as diclofenac; analesics, such as phenazone; anticonvulsive drugs, such as carbamazepine and phenosuximide; cholesterol-lowering drugs, such as clofibric acid; anticancer agents; psychiatric drugs, such as pentobarbital and meprobamate; antibiotics used in both

animal production and human health; numerous compounds used in cosmetics.

· Most of these compounds are probably released in minute amounts; however, they could accumulate and persist when bound on surface-active particles.

· Moreover, these bound compounds can be transported through soil and water by "colloid-facilitated transport".

INFORMATION NEEDED ABOUT THE FATE AND EFFECTS OF BIOMOLECULES PRODUCED BY TRANSGENIC PLANT AND ANIMAL "FACTORIES" IN NATURAL HABITATS, ESPECIALLY IN SOIL.

· Do these biomolecules bind no soil (especially on surface-active components)?

· Influence of physicochemical and biological characteristics of soil on binding.

· Dose binding affect persistence (i.e., resistance to biodegradation) and bioactivity?

· Development of rapid and quantitative methods to follow environmental fate of biomolecules.

· Effects of free and bound biomolecules on microbe-mediated processes and microbial community structure.

· Release, persistence, and ecological effects of biomolecules released in root exudates and transgenic biomass.

· Uptake of released biomolecules by plants and other organisms; potential effects on food webs.

· Movement of biomolecules through soil and waters (e.g., free or by "colloid-facilitated transport").

· Physicochemical properties of complexes between biomolecules and surface-active particles.

· Mechanisms involved in above phenomena.