

Review

Fate and effects of insect-resistant *Bt* crops in soil ecosystems

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Abstract

Recent applications of biotechnology, especially genetic engineering, have revolutionized crop improvement and increased the availability of valuable new traits. A current example is the use of the insecticidal Cry proteins from the bacterium, *Bacillus thuringiensis* (*Bt*), to improve crops, known as *Bt* crops, by reducing injury from various crop pests. The adoption of genetically modified (GM) crops has increased dramatically in the last 11 years. However, the introduction of GM plants into agricultural ecosystems has raised a number of questions, including the ecological impact of these plants on soil ecosystems. Crop residues are the primary source of carbon in soil, and root exudates govern which organisms reside in the rhizosphere. Therefore, any change to the quality of crop residues and rhizosphere inputs could modify the dynamics of the composition and activity of organisms in soil. Insect-resistant *Bt* crops have the potential to change the microbial dynamics, biodiversity, and essential ecosystem functions in soil, because they usually produce insecticidal Cry proteins through all parts of the plant. It is crucial that risk assessment studies on the commercial use of *Bt* crops consider the impacts on organisms in soil. In general, few or no toxic effects of Cry proteins on woodlice, collembolans, mites, earthworms, nematodes, protozoa, and the activity of various enzymes in soil have been reported. Although some effects, ranging from no effect to minor and significant effects, of *Bt* plants on microbial communities in soil have been reported, using both culturing and molecular techniques, they were mostly the result of differences in geography, temperature, plant variety, and soil type and, in general, were transient and not related to the presence of the Cry proteins. The respiration (i.e., CO₂ evolution) of soils cultivated with *Bt* maize or amended with biomass of *Bt* maize and other *Bt* crops was generally lower than from soils cultivated with or amended with biomass of the respective non-*Bt* isolines, which may have been a result of differences in chemical composition (e.g., the content of starch, soluble N, proteins, carbohydrates, lignin) between *Bt* plants and their near-isogenic counterparts. Laboratory and field studies have shown differences in the persistence of the Cry proteins in soil, which appear to be the result primarily of differences in microbial activity, which, in turn, is dependent on soil type (e.g., pH, clay mineral composition, other physicochemical characteristics), season (e.g., temperature, water tension), crop species (e.g., chemical composition, C:N ratio, plant part), crop management practices (e.g., till vs. no-till), and other environmental factors that vary with location and climate zones. This review discusses the available data on the effects of Cry proteins on below-ground organisms, the fate of these proteins in soil, the techniques and indicators that are available to study these aspects, and future directions.

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1. Introduction

Genetically modified (GM) plants possess a gene or genes that have been transferred from a different species. GM plants have been deliberately developed for a variety of reasons: e.g., longer shelf life, disease resistance, pest

resistance, herbicide tolerance, nutritional improvement, resistance to such abiological stresses as drought or nitrogen starvation. The first GM crop approved for use in the USA was the FlavrSavr tomato in 1994, which was developed to have a longer shelf life. Since GM crops were first commercialized in 1996, the planting of GM crops has consistently increased by 10% or more each year worldwide. It is generally expected that commercial cultivation of GM crops will further increase over the coming years

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(Sanvido et al., 2006). The global area of GM crops increased approximately 60-fold during the 11-year period from 1996 to 2006: from 1.7 million hectares to 102 million hectares (James, 2006).

GM crops are currently grown by more than 10.3 million farmers in 22 countries. In 2006, the USA, followed by Argentina, Brazil, Canada, India, and China, were the six principal users of GM crops globally, with 54.6 million hectares planted in the USA (53% of global GM crop area) of which approximately 28% contained stacked genes with two or three traits. Herbicide tolerance has consistently been the dominant GM trait followed by insect resistance and stacked genes for the two traits. In 2006, herbicide tolerance, used in soybean, maize, canola, cotton, and alfalfa, occupied 68% (69.9 million hectares) of the total global GM crop area, and 19% (19.0 million hectares) was planted with insect-resistant *Bt* crops and 13% (13.1 million hectares) with crops with stacked traits of insect resistance and herbicide tolerance (James, 2006). Soybean and cotton with herbicide-tolerant traits have been the most widely and rapidly adopted GM crops in the USA, followed by insect-resistant cotton and corn. Herbicide-tolerant soybean was the principal GM crop in 2006, occupying 58.6 million hectares (57% of the global GM crop area), followed by maize (25.2 million hectares at 25%), cotton (13.4 million hectares at 13%), and canola (4.8 million hectares at 5%). Herbicide-tolerant alfalfa, the first perennial GM crop to be introduced globally in 2006, was planted on 80,000 hectares in the USA, and RR[®] Flex herbicide-tolerant cotton was introduced on over 800,000 hectares in the USA and Australia. Insect-resistance based on *Bacillus thuringiensis* (*Bt*) is the second major trait used in commercial GM crops, and *Bt* maize occupied 11.3 million hectares, equivalent to 13% of the global GM crop area (James, 2005). The use of GM crops has been greatest in the USA, where there has been a 33-fold increase in the area of GM crops planted during the last 10 years (1.5 million hectares in 1996 to 49.8 million hectares in 2005). Over the last 11 years, 1996–2006, farmers have consistently increased their plantings of GM crops by double-digit growth rates every single year since GM crops were first commercialized in 1996 (James, 2006).

However, there are concerns that the commercial cultivation of GM crops could result in adverse effects on the environment. One of the potential adverse environmental effects of GM crops is a nontarget effect on soil organisms and a change in microbe-mediated processes and functions in soil, which could be affected by the presence of, for example, insecticidal Cry proteins derived from insect-resistant *Bt* crops in soils through cultivation of *Bt* crops (e.g., Masoero et al., 1999; Escher et al., 2000; Saxena and Stotzky, 2001a; Dinel et al., 2003; Manachini et al., 2004; Höss et al., 2004; Turrini et al., 2004; Gupta and Watson, 2004; Xue et al., 2005; Rui et al., 2005; Griffiths et al., 2005). Interactions between plants and soil ecosystems indicate that, similar to other

agricultural crops, GM crops will influence processes and functions in soil. Plants have a major influence on communities of micro- and other organisms in soil, which are fundamental to many functions of soil systems, such as nitrogen cycling, decomposition of wastes, and mobilization of nutrients. The type and amount of nutrients released will affect both the numbers of organisms and their diversity. The major carbon supply to soil systems is from plant litter incorporated after harvest and from root exudation. Saxena et al. (2004) showed that insect-resistant GM crops, such as *Bt* maize, potato, and rice, contributed to the presence and persistence of Cry proteins in soil via root exudation, whereas *Bt* cotton, canola, and tobacco did not. There appeared to be no significant differences in exudation from 12 different *Bt* maize hybrids, which included three transformation events (Bt11, MON810, and 176), expressing the Cry1Ab protein (Saxena et al., 2002). A 3-year field study with *Bt* maize (event MON810) confirmed that the release of Cry protein in root exudates continued throughout growth, and levels of the protein in soil did not correlate with a specific period of plant growth (Nguyen Thu, 2004; Baumgarte and Tebbe, 2005). The continuous release, via root exudates, leads to higher concentrations of Cry protein in rhizosphere than in bulk soil. Moreover, repeated and large-scale use of *Bt* crop plants and their residues after harvest could lead to accumulation and persistence of plant-produced Cry proteins in soil, as a result of their binding on soil components (e.g., Tabashnik, 1994; Crecchio and Stotzky, 1998; Tapp and Stotzky, 1998; Saxena and Stotzky, 2001a,b; Saxena et al., 2002; Zwahlen et al., 2003a; Muchaonyerwa et al., 2004; Stotzky, 2000, 2002, 2004).

This review, which supplements previous reviews of aspects discussed herein (e.g., Stotzky, 2000, 2002, 2004; Federici, 2002; Marvier, 2002; Shelton et al., 2002; Bruinsma et al., 2003; Kowalchuk et al., 2003; Benedict and Ring, 2004; De Maagd, 2004; Gupta and Watson, 2004; Motavalli et al., 2004; O'Callaghan et al., 2005; Liu et al., 2005; Lilley et al., 2006), summarizes the results of numerous studies conducted to determine the: (i) effects of insecticidal Cry proteins derived from GM *Bt* crops on soil ecosystems, including soil microorganisms, microbe-mediated processes and functions, and soil-dwelling invertebrates; and (ii) persistence and fate of Cry proteins in soil. The review does not discuss the environmental effects of herbicide-tolerant plants, primarily because herbicide-tolerant crops are considered not to have direct toxic effects on nontarget organisms, as the enzymes conferring herbicide tolerance are normally present in plants and are not known to have any toxic properties (APHIS-USDA, 1994; Carpenter, 2001). The use of herbicide-tolerant crops could, however, result in indirect environmental effects caused by changes in agricultural practices (e.g., Germida et al., 1998; Siciliano et al., 1998; Siciliano and Germida, 1999; Dunfield and Germida, 2003, 2004).

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