A brief survey regarding fate of Bt proteins synthesized by transgenic maize in soil

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Abstract Bt proteins are normally incorporated into soil together with plant residues, with sloughing of root cells, and potentially through the release of exudates from roots. Thus, Bt-toxins represent a possible risk for the soil ecosystem, and their potential impact on soil organisms may depend on their persistence. Under these circumstances, to understand the impact and risk of Bt-toxins on soil organisms, it is important to know how the different Bt-toxins vary in their degradation patterns. The differences in dissipation/persistence of Bt proteins in soil can be a function of soil type, environmental conditions, protein source (purified versus plant-produced), and the particular Cry protein examined. In this paper, we summarize insecticidal proteins synthesized by Bt plants commercially grown and the results of research regarding the persistence of Bt proteins in soil carried out in Romania and in other countries. These results provide evidence that Bt proteins do not accumulate in soil and reveal that the dissipation in soil of Bt proteins produced by genetically modified stacked maize was related to the type of endotoxin produced and not to the number of transgenes expressed.

Key words Environmental fate; Bt proteins; transgenic maize; Cry protein

Bt maize under the global perspective

In 2010, commercial cultivation of several Bt maize events has been approved in 16 different countries globally [17]. In the European Union, only MON810 maize event is commercially grown that is resistant to the European Corn Borer (ECB) and different GM maize events are imported for processing and use as food and feed. (http://europa.eu.int/comm/food/fs/sc/ssc/out327_en.pdf.).

Globally, currently there are a number of commercially available double- and triple stack- maize hybrids produced by traditional breeding of genetically modified parental inbred lines, that synthesize one or more toxins that confer resistance to pests belonging to Coleoptera and Lepidoptera [17].

The first generation of Bt maize plants approved for cultivation are 176, MON810 and Bt11 that synthesize the Cry1Ab protein active against the European Corn Borer. However, since then, other GM maize events synthesizing different Cry toxins (Table 1) were introduced to the market:

- 1507, that produces the Cry1F insecticidal protein, which impart protection against feeding damage caused by the European corn borer (ECB, Ostrinia nubilalis) and other lepidopteran insect pests;
- MON 863 şi 88017, that expresse Cry3Bb1 protein that provides protection against certain coleopteran insect pests including members of the corn rootworm (CRW) complex (Diabrotica spp.);
- MIR604, that expresses the modified Cry3A protein, with extended insecticidal activity on Diabrotica species;
- 59122, that produces the Cry34/35Ab1 binary insecticidal protein that provides a second mode of activity against corn rootworm larval feeding (Diabrotica spp.);
- 89034, that produces two distinct proteins, Cry1A.105 and Cry2Ab2, which provide a dual effective dose against feeding damage caused by the key lepidopteran pest complex in maize: European corn borer (ECB, Ostrinia nubilalis), southwestern corn borer (SWCB, D. grandiosella), and corn earworm (CEW, Helicoverpa zea); and other lepidopteran insect pests, such as the fall armyworm (FAW, Spodoptera frugiperda) sugarcane borer (SCB, D. saccharalis).
Table 1

Toxins encoded by the transgenes from *Bacillus thuringiensis* synthesized by commercially cultivated Bt maize

(http://europa.eu.int/comm/food/fs/sc/ssc/out327_en.pdf.)

<table>
<thead>
<tr>
<th>Bt toxins</th>
<th>Insects order with sensibility to the toxin</th>
<th>Target insects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cry1Ab</td>
<td>Lepidoptera (moths and butterflies)</td>
<td><em>Ostrinia nubilalis</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Ostrinia nubilalis</em> and <em>Sesamia spp.</em>, <em>Heliothis zea</em>, <em>Spodoptera frugiperda</em> and <em>Agrotis ipsilon.</em></td>
</tr>
<tr>
<td>Cry1F</td>
<td>Lepidoptera (moths and butterflies)</td>
<td><em>Ostrinia nubilalis</em></td>
</tr>
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<td></td>
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<td><em>Ostrinia nubilalis</em> and <em>Sesamia spp.</em>, <em>Heliothis zea</em>, <em>Spodoptera frugiperda</em> and <em>Agrotis ipsilon.</em></td>
</tr>
<tr>
<td>Cry1A.105</td>
<td>Lepidoptera (moths and butterflies)</td>
<td><em>Ostrinia nubilalis</em></td>
</tr>
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<td></td>
<td></td>
<td><em>Ostrinia nubilalis</em> and <em>Sesamia spp.</em>, <em>Heliothis zea</em>, <em>Spodoptera frugiperda</em> and <em>Agrotis ipsilon.</em></td>
</tr>
<tr>
<td>Cry2Ab2</td>
<td>Lepidoptera (moths and butterflies)</td>
<td><em>Ostrinia nubilalis</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Ostrinia nubilalis</em> and <em>Sesamia spp.</em>, <em>Heliothis zea</em>, <em>Spodoptera frugiperda</em> and <em>Agrotis ipsilon.</em></td>
</tr>
<tr>
<td>mCry3A</td>
<td>Coleoptera (insects)</td>
<td><em>Diabrotica virgifera virgifera</em></td>
</tr>
<tr>
<td>Cry3Bb1</td>
<td>Coleoptera (insects)</td>
<td><em>Diabrotica virgifera virgifera</em></td>
</tr>
<tr>
<td>Cry34Ab1/Cry35Ab</td>
<td>Coleoptera (insects)</td>
<td><em>Diabrotica virgifera virgifera</em></td>
</tr>
</tbody>
</table>

Cry proteins synthesized by transgenic maize have been widely characterized. According to regulatory provisions, the applicant requesting approval for market release of a genetically modified plant in the European Union must characterize the protein synthesized by the transgene, providing detailed information regarding mod of action, toxicity and allergenicity etc. [10].

**Persistence and accumulation of Bt proteins in soil where transgenic maize has been cultivated**

The dynamics of Bt proteins in plant residues and in soil has been studied using immunological methods, using biotests with sensible insect species and by degradation of Bt maize biomass (Table 2).

The persistency and potential accumulation of Bt proteins in soil samples depends on a multitude of factors that vary depending on the transgenic plant, type of protein synthesized by the plant, soil type, soil nutrient level, pH, type and quantity of mineral clay and organic material [25,26,27,8,18,28,29,22,3], the source of protein used in the study (purified protein versus plant tissue residue), type of plant tissue used [21,30,24,6,4], protein type [5], environmental conditions, especially temperature [30], the initial concentration of the toxin used [7] and finally the insects and methods used for biotests when determining the insecticidal effect to larvae, agricultural practices (with or without tillage) and other parameters that characterize the environment related to geo-climatic positioning [14].

For instance, results regarding the concentrations of Cry1Ab and Cry3Bb1 proteins in foliar residues, during an analysis interval beginning from 90 days after harvest and until the beginning of the next season, have shown that in senescent leaves, the initial concentration of Cry3Bb1 was higher than the initial concentration of Cry1Ab protein. Cry3Bb1 is more rapidly degraded and as consequence persists less in the soil [29].

Results of experiments carried out to analyze the degradation rate of Cry1Ab protein in three different soil types with different physico-chemical parameters have demonstrated that after an increase in the first 6-9 weeks from the incorporation of plant residues in soil, there is a gradual decrease of the protein concentration in the following three to four months. During the degradation period, average values of the quantities of Cry1Ab protein immunologically detected by ELISA varied in different samples, from 0.09 to 0.6ng/g of soil or have decreased under the limit of detection [3].

Soil monitoring results (Table 2) showed that all Bt proteins studied degrade rapidly after GM stacked maize plant tissues is incorporated into soil [3,4,5].

Dubelman et al. (2005) did not observe any persistance or accumulation of Cry1Ab protein in soil where Bt maize was cultivated for three consecutive years [11].

Baumgarte and Tebbe (2005) detected Cry1Ab protein after the winter season in a field where MON810 maize had been cultivated in the previous year, but in very small concentrations (0.21ng/g soil) [6].

Grüber et al. (2012) did not detect Cry1Ab protein in soil samples collected in spring, from four different field trials, following nine years of monoculture [13].
### Summary of results regarding studies on persistance of Cry proteins in soil

<table>
<thead>
<tr>
<th>Bt Protein</th>
<th>Event</th>
<th>Method</th>
<th>Persistence of proteins in soil</th>
<th>Bibliographic reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cry1Ab</td>
<td>Bt11, MON810 and 176</td>
<td>Immunological (Lateral Flow Quickstix EnviroLogi)</td>
<td>Protein is released through root exudates. Remains toxic for larvae for 180 days</td>
<td>Saxena and Stotzky, 2001</td>
</tr>
<tr>
<td>Cry1Ab</td>
<td>Bt11 maize</td>
<td>ELISA</td>
<td>Fate/degradation depends on the crop system (tillage/no tillage) and temperature; after 200 days, concentration found was 0.3% of the initial concentration</td>
<td>Zwahlen et al., 2003</td>
</tr>
<tr>
<td>Cry1Ab</td>
<td>MON810 maize</td>
<td>ELISA</td>
<td>Cry1Ab protein was detected even after winter in fields where MON810 maize was cultivated the year before, but in very small concentrations</td>
<td>Baumgarte and Tebbe, 2005</td>
</tr>
<tr>
<td>Cry1Ab</td>
<td>MON810 and Bt11 maize</td>
<td>Biotest</td>
<td>Did not persist for 3 consecutive years</td>
<td>Dubelman et al., 2005</td>
</tr>
<tr>
<td>Cry1Ab</td>
<td>MON810 maize</td>
<td>ELISA</td>
<td>The protein did not accumulate in soil during plant development period and neither after one year following harvest</td>
<td>Ezequiel et al., 2008</td>
</tr>
<tr>
<td>Cry1Ab</td>
<td>MON810 maize</td>
<td>ELISA</td>
<td>During an interval of 14-56 days, the concentration increased by 25% in comparison to the concentration detected in plant tissue; was undetectable after 112 days</td>
<td>Daudu et al., 2009</td>
</tr>
<tr>
<td>Cry1Ab</td>
<td>MON810 maize</td>
<td>ELISA</td>
<td>Fate/degradation rate depended on the type of soil</td>
<td>Badea et al., 2010a</td>
</tr>
<tr>
<td>Cry1Ab</td>
<td>MON810 maize</td>
<td>ELISA</td>
<td>Was not detected in spring, in four different type of soils, for 9 consecutive years</td>
<td>Gruber et al., 2012</td>
</tr>
<tr>
<td>Cry3Bb1</td>
<td>MON863 maize</td>
<td>ELISA</td>
<td>Undetectable levels in two fields locations</td>
<td>Ahmad et al., 2005</td>
</tr>
<tr>
<td>Cry3Bb1</td>
<td>MON863 maize</td>
<td>Immunological (ImunoStrip)</td>
<td>The protein does not persist and does not accumulate in soil, but is rapidly decomposed</td>
<td>Icoz and Stotzky, 2008</td>
</tr>
<tr>
<td>Cry1Ab and Cry3Bb</td>
<td>Bt11 and MON810; MON863 maize</td>
<td>Immunological (ImunoStrip) Insecticidal activity (Manduca sexta)</td>
<td>Cry1Ab protein was detected in soil following 4 years of maize monoculture; Cry3Bb was not detected</td>
<td>Icoz et al., 2008</td>
</tr>
<tr>
<td>mCry3A and Cry1Ab</td>
<td>Bt11 x MIR604 x GA21</td>
<td>ELISA</td>
<td>All Cry proteins studied degrade rapidly after GM stacked maize plant tissues are incorporated into soil</td>
<td>Badea et al., 2010b</td>
</tr>
<tr>
<td>Cry1Ab, Cry2Ab2, CryA.105, Cry3Bb</td>
<td>MON810 x NK603, MON89034, MON89034 x MON88017</td>
<td>ELISA</td>
<td>The soil concentration of all Bt proteins was below the limit of quantification at 40 days after harvest and was non-detectable six months after harvest.</td>
<td>Badea et al., 2011</td>
</tr>
</tbody>
</table>

Similar results were reported in the case of Cry3Bb1 protein [14,15,20]. Results from studying the persistance and accumulation in soil of Cry3Bb protein produced by MON863 and MON 88017 maize events, in three years of monoculture [20] and four years of monoculture [16] revealed that this toxin is very unstable in plant residue and the amount of protein entering the soil are extremely small and do not accumulate during consecutive cultivation of Bt maize.

Even though Cry proteins can bind rapidly to mineral clay and humus substrates, evidence does not exist regarding their accumulation in soil even following several years of monoculture with Bt maize [6,19,16,20,13].

The recovery of C\(^{14}\)O\(_2\) from radioactively labeled (C\(^{14}\)) Cry1Ab protein was 95% following 20 days of incubation under controlled conditions, providing evidence that the risk for accumulation in soil is negligible, especially due to microbiological processes [1]. Moreover, Schrader et al., (2008) and Emmerling et al. (2011) have demonstrated that worms (Lumbricus terrestris, Aporrectodea caliginosa)
amplify the degradation process of Cry1Ab protein from maize residue [23].

**Conclusions**

The research results have demonstrated the rapid degradation of Cry proteins in soil where Bt maize plants have been cultivated. Differences observed regarding the degradation and their persistence in soil are determined by the type of toxin studied, soil type, pH, microbial activity, temperature, and analytical method used. Although Cry proteins can bind rapidly to mineral clay and humus substrates, there is no evidence of their accumulation in soil samples from fields where Bt plants have been cultivated in monoculture for long time.

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