Soil Biology & Biochemistry 38 (2006) 195–199

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Short communication

Decomposition processes under *Bt* (*Bacillus thuringiensis*) maize: Results of a multi-site experiment

Jérôme Cortet[a,*], Mathias N. Andersen[b], Sandra Caul[c], Bryan Griffiths[c], Richard Joffre[d], Bernard Lacroix[e], Christophe Sausse[f], Jacqueline Thompson[c], Paul Henning Krogh[g]

[a] *Institut Méditerranéen d'Ecologie et Paléoécologie, Europôle de l'Arbois, BP 80, Bâtiment Villemin, 13545 Aix-en-Provence Cedex 04, France*
[b] *Department of Agroecology, Danish Institute of Agricultural Sciences, Research Centre Foulum, P.O. Box 50, DK-8830 Tjele, Denmark*
[c] *Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK*
[d] *CEFE-CNRS, 1919 route de Mende, FR-34000 Montpellier, France*
[e] *ARVALIS-Institut du végétal, Station Inter-instituts, 6, chemin de la côte vieille 31450 Baziège, France*
[f] *Centre Technique Interprofessionnel des Oléagineux Métropolitains, Centre de Grignon BP 4, 78850 Thiverval Grignon, France*
[g] *Soil Fauna and Ecotoxicology Research Unit, Department of Terrestrial Ecology, National Environmental Research Institute, P.O. Box 314, Vejlsoevej 25, DK-8600 Silkeborg, Denmark*

Received 6 January 2005; received in revised form 4 April 2005; accepted 26 April 2005

Abstract

The effects of maize expressing the *Bacillus thuringiensis* Cry1Ab protein (*Bt* maize) on decomposition processes under three different European climatic conditions were assessed in the field. Farming practices using *Bt* maize were compared with conventional farming practices using near-isogenic non-*Bt* maize lines under realistic agricultural practices. The litter-bag method was used to study litter decomposition and nitrogen mineralization dynamics of wheat straw. After 4 months incubation in the field, decomposition and mineralization were mainly influenced by climatic conditions with no negative effect of the *Bt* toxin on decomposition processes.

Keywords: Wheat straw decomposition; Nitrogen mineralization; Maize; Genetically modified plants; *Bacillus thuringiensis* toxin

Maize (*Zea mays* L.) has been genetically modified to express the Cry1Ab protein from the bacterium *Bacillus thuringiensis* to produce a protein that is toxic to some lepidopteran insect pests, particularly the European corn borer *Ostrinia nubilalis*. As the toxin, released to soil from *Bt* maize in root exudates, has been shown to degrade slowly and to accumulate in soil (Tapp and Stotzky, 1995, 1998; Saxena et al., 2002; Zwahlen et al., 2003a) it is desirable to assess the effects of *Bt* maize cultivation on non-target soil organisms, similar to the assessment of any other kind of pesticide. Under laboratory conditions, no effect of the Cry1Ab protein was found on Collembola (Sims and Martin, 1997), isopods (Escher et al., 2000), protozoa, nematodes, fungi, bacteria, algae, and earthworms (Saxena and Stotzky, 2001; Koskella and Stozky, 2002). However, a small significant effect of this toxin was noticed on soil microbial community structure (using community-level physiological profiles) in a high-clay soil (Blackwood and Buyer, 2004). Studies of microbial and microfaunal communities under two years of *Bt* maize revealed differences between the *Bt* and non-*Bt* crops, but non that were outside the expected variation of agricultural practices (Griffiths et al., 2005). A combination of laboratory, glasshouse and field studies (the so-called 'tiered' approach, Jepson et al., 1994) is required as the toxin can have a different behavior in the field than in the laboratory, and insecticidal activity can vary with climatic, soil texture and pH conditions (Tapp and Stotzky, 1995; Zwhalen et al., 2003b).

Other studies have examined the effects of *Bt* crops on ecosystem functions, such as decomposition. Such a functional effect could have a direct impact on soil fertility, which is important for agriculture (Eijsackers and Zehnder, 1990). Most of these studies have compared

---

* Corresponding author. Tel.: +33 4 4290 8459; fax: +33 4 4290 8448.
E-mail address: jerome.cortet@univ.u-3mrs.fr (J. Cortet).

0038-0717/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2005.04.025

the decomposability of *Bt* and non-*Bt* plant residues. Hopkins and Gregorich (2003) did not observe any detectable difference in the decomposition of plant material from *Bt* and non-*Bt* maize lines, as determined by CO_2 production. Flores et al. (2005) noticed a higher CO_2 soil production with near-isogenic non-*Bt* maize residues than with *Bt* maize residues that could not be explained by differences in C/N ratio, lignin maize content, or even soil microbiota. This approach is useful for determining whether there is any inherent difference in the decomposability of plant material containing the *Bt* toxin, but could not assess whether the presence of *Bt* crops would influence the decomposition of exogenous organic matter. We addressed this using a litter-bag technique. Litter-bags containing wheat straw have been suggested as a suitable field test method for assessing the effects of pesticides on decomposition processes (Kula and Römbke, 1998; Cortet et al., 2002; Knacker et al., 2003) and were recently presented as a standard test for soil functioning employed in arable fields (Römbke et al., 2002). We focus here on the effects of *Bt* maize on decomposition processes by using this litter-bag methodology under realistic agricultural conditions. The main objective was to assess the overall crop effects (i.e., *Bt* or non-*Bt* maize) using wheat straw (*Triticum aestivum* L.) as a standard exogenous material in the litter-bags.

Field locations, selected to represent diverse climatic and soil conditions, were part of the ECOGEN project (www.ecogen.dk) and also used for the analysis of soil communities (Griffiths et al., 2005). Foulum (Jutland, Denmark) was the most northerly site at 56°30′N, 9°35′E. Varois (Bourgogne, France) was an intermediate geographic site at 47°34′N, 5°13′E. Narbons (Midi-Pyrénées, France) was the most southerly site at 43°26′N, 1°27′E. Soil properties are summarised in Table 1. At Foulum and Varois two maize cultivars were planted: MEB307 (a *Bt* variety producing the Cry1Ab toxin) and Monumental (the conventional near-isogenic variety without the *Bt* trait) each randomised in plots of at least 12 m × 19 m in a four-block design. Fields were ploughed in the autumn of 2002, seeded on 22 and 28 May 2003, and harvested on 5 November and 18 October, in Foulum and Varois, respectively. All fertilization and pesticide applications were done according to current agricultural practices and were identical for the two treatments. The same protocols (with *Bt* and near-isogenic varieties) had been previously applied in 2002 on the same plots in Foulum and Varois. A similar design was used in Narbons but the maize cultivars, suited for the regional climate, were DK552Bt (a *Bt* variety expressing the Cry1Ab toxin) and DK532 (the conventional near-isogenic variety without the *Bt* trait). The field was ploughed and seeded on 4 June 2003 and harvested on 10 October 2003. In Narbons, the seeds of the near-isogenic maize variety were treated with Gaucho (0.112 kg/ha, active ingredient imidaclopride), whereas the *Bt* maize seeds received no insecticide. All field operations were identical for both treatments. Plots were irrigated regularly throughout the growing season at Narbons but not at Foulum and Varois. Wheat straw decomposition was assessed using litter-bag methodology (Cortet et al., 2002). The same sample of straw, hand-picked to select only internodes, was used for all three sites. It was cut into small pieces (5–10-cm pieces), oven-dried (45 °C for 48 h) and 5 g portions sealed in 12 cm × 12 cm Nylon bags (5 mm diameter mesh). Chemical analysis of five replicates of initial straw gave coefficient of variation (%) of 0.6, 2.8 and 2.9 for C, N and ash, respectively. Bags were installed in the field just after maize emergence and collected at regular intervals until harvest. Twenty-four bags were placed on top of the soil in the centre of each plot, within rows but between the plants. In Foulum, eight bags per plot were destructively sampled 51, 101, and 142 days after exposure. In Varois and Narbons four bags per plot were destructively sampled 43, 93, and 126 days and 43, 85, and 121 days after exposure, respectively. Wheat straw from the bags was oven-dried (24 h, 45 °C), sieved (0.5 mm) to eliminate most soil particles, weighed and ground (1 mm mesh filter) with a Cyclotec-1093 mill (Tecator, Höganäs, Sweden). All samples (total of 384) were analyzed using a near-infrared spectrophotometer (NIRS system 6500, Foss Nirs systems, Inc., Silver Spring, MD, USA). Ash-free litter mass remaining (LMR), N concentrations and C/N were predicted from calibrations equations derived from ash and wet chemistry measurements done on 52 representative samples (see Joffre et al., 1992; Coûteaux et al., 1998; Cortet et al., 2003 for methodological details). As litter-bags collected at a given date $(t+n)$ cannot be considered as independent from litter-bags collected at the previous date (t), samples were considered as repeated measures (Grafen and Hails, 2002). LMR, N concentrations, and C/N were each compared using a global two-way MANOVA (Analysis of variance with multiple variables), with sites (Foulum, Varois and Narbons) and crops (*Bt*/non-*Bt*) being fixed effect factors, and dates being the multiple variable (three sampling dates). We considered the intervals between the three sampling dates to be equivalent for each site. For each site, LMR, N concentration and C/N were also each compared using a two way MANOVA, with crops (*Bt*/non-*Bt*) being fixed effects factors, blocks being a random factor, and dates being the multiple variable (three sampling dates) (Statgraphics plus 5.0 software, Manugistics Corp., Rockvile, MD, USA).

The time-course of LMR, N concentrations, and C/N showed similar trends among the three sites (Fig. 1). There was a significant site effect (Table 2) with a greater

Table 1
Soil properties of the three sites, Foulum, Varois and Narbons

	Clay (%)	Silt (%)	Sand (%)	Organic matter (%)	Calcium (%)	pH (H_2O)
Foulum	8.7	24.8	66.5	6.4	0.0	6.2
Varois	43.1	39.0	17.9	4.8	18.8	8.1
Narbons	27.9	38.3	33.8	1.5	7.0	8.2