Soil Biology and Biochemistry 119 (2018) 194–202

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

# The responses of soil nematode communities to *Bt* maize cultivation at four field sites across Europe

Andrea Čerevková[a,*], Dana Miklisová[a], Márton Szoboszlay[b], Christoph C. Tebbe[b], Ľudovít Cagáň[c]

[a] Institute of Parasitology, Slovak Academy of Science, Košice, Slovakia
[b] Thünen Institute of Biodiversity, Bundesallee 65, 38116 Braunschweig, Germany
[c] Department of Plant Protection, Slovak Agricultural University, Nitra, Slovakia

ARTICLE INFO

*Keywords:*
MON810
GMO biosafety
Nematodes community composition
*Ostrinia nubilalis*

ABSTRACT

Transgenic maize expressing the *Bacillus thuringiensis* (*Bt*) insecticidal crystal (Cry1Ab) protein is poisonous to lepidopterans including the European Corn Borer (*Ostrinia nubilalis*). In many European countries, commercial cultivation of *Bt* maize is not allowed. One major reason is the potential variation of the environmental risk across different biogeographical regions. The aim of this study was to collect data about soil nematode communities as bioindicators of unintended effects across geographically diverse maize growing regions in Europe by sampling field sites in Denmark, Slovakia, and Sweden during 2013–2014, and in Spain during 2013. DKC3872YG (*Bt* maize line MON810) and its near-isogenic line DKC3871 were grown at the sites in Slovakia, Denmark, and Sweden and hybrids DKC6451YG (*Bt* maize line MON810) and its near-isogenic line DKC6450 were cultivated at the site in Spain. Dominating nematode genera in the maize fields regardless of the field site or maize variants were bacterial feeders *Rhabditis, Acrobeloides*; root-fungal feeders *Filenchus*; fungal feeders *Aphelenchoides, Aphelenchus*; and omnivores *Eudorylaimus*. A significant effect of the field site location on the total nematode abundance, nematode abundance in trophic groups, diversity of nematode genera, and ecological and functional nematode indices was detected. Significant annual variation was found in the Plant parasite and Structure indices. There were significant differences in the abundances of omnivores and root-fungal feeders and in the Maturity, Channel, and Enrichment indices between *Bt* and non-*Bt* maize plots in Denmark in 2013, and in the abundance of fungal feeders in Sweden (2013). On the other hand, no difference was found between the *Bt* and non-*Bt* plots at the sites in Spain and Slovakia or at any of the sites in 2014. The effect of the field site location and season on the soil nematode community was more pronounced than that of the *Bt* genetic modification. We conclude that *Bt* maize had only a limited or no effect on soil nematode communities.

## 1. Introduction

*Bt* maize is genetically modified maize (*Zea mays* L.) containing genes of the bacterium *Bacillus thuringiensis* that codes for insecticidal proteins (*Bt*-toxins; *Bt*). *Bt* maize expressing *Bt*-toxin Cry1Ab is one of the most widely cultivated *Bt* crops worldwide (Benedict and Ring, 2004). It is able to protect itself against feeding by the European corn borer (*Ostrinia nubilalis* Hübner). The fate and effect of insect resistant *Bt* maize in soil ecosystems has intensively been studied (Baumgarte and Tebbe, 2005; Icoz and Stotzky, 2008; Tabashnik et al., 2013), but there is still a lack of information about the importance of different environmental conditions i.e. soil type or agroecosystem, on its ecological impact. In fact, the environmental persistence of *Bt* protein is known to depend on factors such as the type of *Bt* proteins, its expression pattern in plants, soil types, temperature, or precipitation (Tank et al., 2010; Feng et al., 2011; Xue et al., 2014). Saxena et al. (1999) found that Cry1Ab *Bt* toxin was released from corn plants into the rhizosphere soil in root exudates. Baumgarte and Tebbe (2005) reported that *Bt* proteins mainly enter soil by decomposition of roots and can persists during winter until the following growing season, and Tapp and Stotzky (1998) observed that the bound state of the *Bt* toxin persist in soil for up to 234 days. In fact, active *Bt* toxins can persist and remain insecticidal in soil as a result of binding to clay surfaces (Saxena et al., 2002) or humic substances (Crecchioa and Stotzkyb, 1998). Other studies, however, emphasize that *Bt* proteins will not accumulate in soils (Tank et al., 2010; Wang et al., 2013).

Soil nematodes are one of the most abundant groups of soil metazoans with densities reaching up to 50 million per $m^2$ (Bongers and Bongers, 1998) and with important ecosystem functions (Yeates, 1981). In numerous studies, soil nematodes were successfully used as

* Corresponding author.
*E-mail address:* cerev@saske.sk (A. Čerevková).

https://doi.org/10.1016/j.soilbio.2018.01.023
Received 21 September 2017; Received in revised form 22 January 2018; Accepted 23 January 2018

indicators of environmental conditions and for general ecosystem health (Bongers, 1990; Ettema and Bongers, 1993; Neher, 2001; Ciobanu et al., 2015; Renčo and Baležentienė, 2015).

Nematode communities under maize crops may be influenced by many different factors, including crop species, plant age, and environmental variables (Karuri et al., 2013). Previously, nematode numbers have been shown to be similar in soils planted with *Bt* maize and its isogenic equivalent (Saxena and Stotzky, 2001; Al-Deeb et al., 2003). Höss et al. (2011, 2015) did not find significant differences in the abundance and diversity of field nematodes in soil planted with *Bt* and non-*Bt* maize. Griffiths et al. (2005, 2006) compared *Bt* maize expressing the Cry1Ab to the near-isogenic non-*Bt* cultivar, another conventional maize cultivar, and grasslands in three European sites (Denmark, Eastern France, South-West France). The authors stated that the effect of *Bt* maize on soil nematodes was relatively small compared to the effects of soil type, plant growth stage, and applications of an insecticide. In contrast, another study reported that nematode diversity values were greater in a *Bt* hybrid maize field versus the non-*Bt* isoline maize field treated with insecticide (Neher et al., 2014). Höss et al. (2011, 2013) noted that Cry proteins (Cry1A.105; Cry2Ab2) could potentially also harm free-living nematodes in similar mode of action than the insects. Furthermore, a soil microcosm study using a mixture of the Cry-proteins expressed by MON89034 × MON88017 found significantly deleterious effects on nematode communities at a nominal concentration of 1 μg Cry-proteins per gram of soil (Höss et al., 2014).

While abundant literature is available concerning the effects of *Bt* maize on nematode communities across individual sites, a deficit remains in regards to baseline data on nematode communities and their response to GM maize across multiple geographically distinct regions. Only one study from Europe describes the temporal and spatial impact of *Bt* maize on nematode diversity (Griffiths et al., 2005, 2007), which examined sites in Denmark and France from 2002 to 2005. In this study, the objective was to consider the impact of the biogeographical diversity which exists in Europe by selecting four sites located in distinct regions. This is of special importance for the approval of *Bt* maize for cultivation in Europe due to its diversity of biogeographical regions in which maize is grown, ranging from Spain to Scandinavia. The objective of this study was, therefore, to assess the diversity of nematodes in maize fields from different European regions and analyze the response of the nematode communities to the cultivation of *Bt* maize. The results should enhance our understanding of the importance of the European biogeographical diversity in assessing the effects of GM plants on non-target organisms and thus support the environmental risk assessment of future genetically modified crops in the European Union (Arpaia et al., 2014).

## 2. Materials and methods

### 2.1. Sites, maize variants, and experimental design

The study was carried out in four sites located in different countries during 2013, i.e., in Denmark, Spain, Slovakia, and Sweden. All sites except for Spain were also analysed in the following year 2014.

In Denmark, the field site was located at the Experimental farm of the University of Aarhus Flakkebjerg Research Centre, near the town of Slagelse, on the island of Zealand. Coordinates of the site were 55°19′N, 11°23′E. Altitude was 35 m a.s.l. The soil at the site was classified as an Udoll (USDA soil taxonomy) based on https://soilgrids.org (Hengl et al., 2017) and the European digital archive on soil maps (EuDASM) (Panagos et al., 2011). The preceding crops were barley in 2012 and maize in 2013. Sowing dates were May 15, 2013 and May 20, 2014. Herbicides were used one week before sowing and again three weeks later. Soil for analysis of selected soil chemical parameters and nematode communities was collected on August 26, 2013 and August 13, 2014.

In Spain, the field site was located southeast of Madrid, municipality of Seseña, in the province of Toledo, central Spain. Coordinates of the site were 40°05′N, 3°40′W. Altitude was 495 m a.s.l. The soil was classified as a Fluvent. The preceding crop was maize in 2012. The sowing date was May 9, 2013. Herbicides were used one week before sowing and again three weeks later. Soil for analyses was collected on July 24, 2013.

In Slovakia, the field site was located in Borovce in western Slovakia. Coordinates of the site were 48°34′N, 17°43′E. Altitude was 181 m a.s.l. The soil was classified as an Udoll. The preceding crops were winter wheat in both 2012 and 2013. Sowing dates were May 9, 2013 and April 28, 2014. Herbicides were used one week before sowing and again three weeks later. Soil for analyses was collected on July 30, 2013 and July 21, 2014.

In Sweden, the field site was located northwest of Lund. Coordinates of the site were 55°45′N 13°2′E. Altitude was 10 m a.s.l. The soil was classified as an Ochrept. The preceding crops were winter wheat in both 2012 and 2013. Sowing dates were May 15, 2013 and May 20, 2014. Herbicides were used one week before sowing and again three weeks later. Soil for analyses was collected on August 22, 2013 and August 18, 2014.

In Slovakia, Denmark, and Sweden, the maize *Bt* and isogenic (ISO) hybrids included in the experiment were DKC3872YG (*Bt* maize line MON810) and its near-isogenic line DKC3871. In Spain, hybrid DKC6451YG (*Bt* maize line MON810) and its near-isogenic line DKC6450 were cultivated. At each location, hybrids were sown in 10 replicated plots, each measuring 10 m × 10 m. Each plot was isolated from the adjacent plots by a 5 m wide strip of barley. The location of the respective *Bt* and ISO plots were completely randomized. The experiments were conducted during seasons 2013 and 2014 with the identical plot arrangements.

### 2.2. Soil sampling and chemical analyses

Soils samples were taken at all sites during the flowering stage of maize with three sub-samples from each field plot. At each plot, three representative plants were uprooted, the roots with the adhering soil were placed into plastic bags and shaken to separate a major part of the soil from the roots. The soil samples from the three plants were pooled, thus both *Bt* and ISO maize cultivation were represented with 10 independent (biological) replicates from each site in each year. All samples were transferred to the laboratory in sealed plastic bags and stored at 5 °C until processing.

To determine the chemical parameters, the soil samples were dried at 105 °C for 17–18 h. Soil pH was measured from 10g soil in 0.01 M $CaCl_2$ with 1:2 (w/v) soil-to-solution ratio using a Professional Meter PP-25 electrode (Sartorius, Germany). Total soil carbon (C %) and nitrogen (N %) content were determined from 3 g grounded soil samples by dry combustion with a LECO TruMac elemental analyzer (Elementar, Germany).

### 2.3. Analyses of nematodes

Each soil sample was homogenised by gentle hand mixing, and then 50 g of soil was processed by a modified Baermann technique. Nematodes were extracted from the aqueous soil suspensions using a set of two cotton-propylene filters. Sub-samples were then collected after a 24 h extraction at 22 °C. The aqueous suspensions were subsequently examined under a stereomicroscope (40× and 60× magnification), excessive water was removed, and the nematodes were fixed in Ditlevsen´s FAA solution (95% ethanol, 40% formaldehyde, glacial acetic acid, distilled water) (Southey, 1986). The nematodes were then microscopically (100, 200, 400, 600, and 1000 × magnification) identified at genus-level using an Eclipse 90i light microscope (Nikon, Japan).

Identified nematode genera were partitioned to six trophic groups based on their feeding habits: bacterial feeders (B), fungal feeders (F),