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ScienceDirect
Ecotoxicology and Environmental Safety 70 (2008) 334–340
Ecotoxicology and Environmental Safety
www.elsevier.com/locate/ecoenv

Effects of transgenic corn and Cry1Ab protein on the nematode, *Caenorhabditis elegans* ☆

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Received 18 January 2007; received in revised form 11 October 2007; accepted 21 October 2007
Available online 20 February 2008

Abstract

The effects of the insecticidal Cry1Ab protein from *Bacillus thuringiensis* (*Bt*) on the nematode, *Caenorhabditis elegans*, were studied with soil from experimental fields cultivated with transgenic *Bt* corn (MON810) and with trypsinized Cry1Ab protein expressed in *Escherichia coli*. The content of Cry1Ab protein was above the detection limit of an ELISA test in only half of the soil samples obtained from transgenic plots, ranging from 0.19 to 1.31 ng g^{-1} dry weight. In a laboratory bioassay, *C. elegans* was exposed to rhizosphere and bulk soil from fields with isogenic or transgenic corn or to solutions of Cry1Ab protein (0, 24, 41, 63, 118, and 200 mg l^{-1}) over a period of 96 h, with growth and reproduction serving as the test parameters. Nematode reproduction and growth were significantly reduced in rhizosphere and bulk soil of *Bt* corn compared with soil from isogenic corn and were significantly correlated with concentrations of the Cry1Ab protein in the soil samples. Moreover, the toxicity of pure Cry1Ab protein to the reproduction and growth of *C. elegans* was concentration-dependent. As significant inhibition occurred at relatively high concentrations of the Cry1Ab protein (41 mg l^{-1}), the effects of the soil samples from *Bt* corn could not be assigned directly to the toxicity of the Cry1Ab protein. The results demonstrate that bioassays with the nematode, *C. elegans*, provide a promising tool for monitoring the potential effects of *Bt* toxins in aqueous medium and soils.

Keywords: *Bt* corn; Cry1Ab; Nematodes; *Caenorhabditis elegans*; Growth; Reproduction

1. Introduction

The genetic modification of corn (*Zea mays* L.) has resulted in varieties (*Bt* corn) containing genes for insecticidal δ-endotoxins of the bacterium, *Bacillus thuringiensis* (*Bt* toxins). *Bt* corn expressing the Cry1Ab protein is able to protect itself against feeding by the European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera: Pyrelidae). *Bt* toxins produced by transgenic corn can enter soil in root exudates (Saxena et al., 1999), plant residues (Zwahlen et al., 2003a), or the feces of animals that have fed on *Bt* plant material (Weber and Nentwig, 2006). Once in soil, the toxins can be bound on clay and humus particles (Crecchio and Stotzky, 1998; Tapp and Stotzky, 1998), which protects them from biodegradation and preserves their insecticidal activity (Koskella and Stotzky, 1997). If the toxins are able to accumulate in soils in their bioactive form, they may pose a potential, inadvertent hazard for soil-dwelling organisms.

Studies on the effects of *Bt* toxin and of *Bt* crops on non-target organisms have produced contrasting results. The green lacewing, *Crysoperla carnea* (Neuroptera: Chrysopidae), showed higher mortality and delayed development after feeding on prey reared on *Bt* corn (Hilbeck et al., 1998). However, Romeis et al. (2004) showed that

☆ All experiments were conducted according to national guidelines for the protection of animal welfare.

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doi:10.1016/j.ecoenv.2007.10.017

C. carnea is not sensitive to Cry1Ab protein and concluded that direct effects of the *Bt* toxin on this organism are not likely to occur under field conditions. Bakonyi et al. (2006) showed that *Bt* corn was less preferred as food by the springtail, *Folsomia candida* (Collembola: Isotomidae), than isogenic corn, but this was not the case for other collembolan species (Bakonyi et al., 2006). The growth and reproduction of *Protaphorura armata* (Collembola: Onychiuridae) were not affected in the presence of *Bt* corn or food contaminated with Cry1Ab protein (Heckmann et al., 2006). In earthworms only minimal effects of the Cry proteins were detected (Saxena and Stotzky, 2001; Zwahlen et al., 2003b; Vercesi et al., 2006). Results concerning the effects of *Bt* corn on nematodes are conflicting: although adverse effects of *Bt* corn on total abundance of nematodes or the abundance of certain feeding types were reported in field studies (Manachini and Lozzia, 2002; Griffiths et al., 2005), these findings could not be confirmed in other field and glasshouse experiments (Saxena and Stotzky, 2001; Griffiths et al., 2007). Several studies with single species have shown that some *Bt* toxins may have deleterious effects on nematodes (Wharton and Bone, 1989; Meadows et al., 1990; Leyns et al., 1995; Borgonie et al., 1996; Belair and Cote, 2004). Specific classes of nematicidal *Bt* toxins (Cry5, Cry6) have potent toxic effects on various nematode species, such as *Caenorhabditis elegans*, *Panagrellus redivivus*, and *Pristionchus pacificus* (Wei et al., 2003).

Free-living, non-parasitic nematodes have an important role for the functions of soils, being the most abundant and species-richest metazoans (Yeates, 1981; Andrassy, 1992). By evolving various feeding types, these invertebrates have been able to occupy key positions in terrestrial food webs (Yeates et al., 1993), thus influencing nutrient cycling in soils (Yeates and Coleman, 1982; Ingham et al., 1985; Beare, 1997). The presence of nematodes and the structure of nematode communities are, therefore, important to agricultural production and sustainability (Fiscus and Neher, 2002). Accordingly, nematodes are suitable ecological indicators for monitoring and assessing agricultural areas (Hess et al., 2000; Neher, 2001). In environmental studies, the soil-dwelling bacterivorous nematode, *C. elegans*, has been successfully used as a test organism for investigating complex matrices, such as soils (Donkin and Dusenbery, 1993; Peredney and Williams, 2000; ASTM, 2001) and freshwater sediments (Traunspurger et al., 1997; Höss et al., 1999).

The aim of the present study was to determine whether the cultivation of *Bt* corn under field conditions affects the reproduction and growth of *C. elegans*, as a model organism for studying the environmental effects of transgenic crops. The nematode was exposed to rhizosphere and bulk soil from experimental fields planted with transgenic corn (MON810) and the respective near-isogenic cultivar. Potential effects on *C. elegans* caused by the Cry1Ab protein were evaluated by analyzing its sensitivity to toxin concentrations present in soil samples and to purified Cry1Ab protein in aqueous medium.

2. Materials and methods

2.1. Experimental fields

Transgenic (MON810, Novelis) and near-isogenic (Nobilis) corn (*Z. mays* L.) varieties were cultivated over a period of 5 years (2000–2004) on four plots that were part of an experimental field located at Baumannshof (southern Germany; 11°32′16″E, 48°42′19″N). Each of the cultivars was grown on two separate plots (Nobilis 1 and 2, Novelis 1 and 2; Table 1).

2.2. Soil sampling and preparation

Rhizosphere soil was sampled in July 2002 (within the third year of cultivation) and October 2003 (end of the fourth year of cultivation) by collecting the soil directly from the roots of plants that had been removed from the plots. For each cultivar, soil samples of 10 plants were pooled. Bulk soil was collected in May 2003 and May 2004 (beginning of fourth and fifth year of cultivation, respectively) by using a soil corer (4.5 cm diameter) to remove the upper 20 cm. Soil samples were air-dried and sieved through a 200-μm sieve to avoid soil matrix effects on the nematodes. The samples were stored at −20 °C until used for the toxicity tests and for ELISA analysis. The number of indigenous nematodes in the soil samples was negligible, and species could easily be distinguished from *C. elegans* under the microscope.

2.3. Preparation of Cry1Ab protein expressed in Escherichia coli

Cry1Ab was expressed in *E. coli* XL1-Blue containing the plasmid pBD140, which carries the full-length of the *cry1Ab* gene. The major purification and solubilization method of Cry1Ab protein was modified according to De Maagd et al. (1996). Briefly, *E. coli* was cultured in a 2-l Erlenmeyer flask containing 500 ml of Terrific Broth medium (12 g bacto-tryptone, 24 g bacto-yeast extract, 4 ml glycerol, 2.31 g KH_2PO_4, and

Table 1
Concentrations of Cry1Ab protein in soil samples taken from rhizosphere soil (RS) and bulk soil (BS) of Nobilis (isogenic) and Novelis (MON810, transgenic)

Cultivar	Treatment	Cry1Ab	RS July 02	BS May 03	RS October 03	BS May 04
			Concentrations of Cry1Ab protein (ng g^{-1} dry wt)			
Nobilis 1	Isogenic	−	<0.07	<0.07	<0.07	<0.07
Nobilis 2	Isogenic	−	<0.07	<0.07	<0.07	<0.07
Novelis 1 (MON810)	*Bt*	+	0.51	0.19	<0.07	<0.07
Novelis 2 (MON810)	*Bt*	+	1.31	<0.07	<0.07	0.22

The detection limit of the ELISA was 0.07 ng Cry1Ab protein g^{-1} soil dry weight (dry wt).

12.54 g K_2HPO_4 l^{-1}) and ampicillin (100 μg ml^{-1}) at 28 °C with shaking (220 rpm). After 3 days of incubation, the cells were harvested by centrifugation at 4753*g* for 10 min at 4 °C using a SLA-1500 rotor (Sorvall RC5B Plus). The cells were frozen at −20 °C for at least 30 min and resuspended in 3 ml of lysis buffer (50 mM Tris/HCl, pH 8.0, 5 mM EDTA, 100 mM NaCl) g^{-1} of *E. coli*. The bacterial cells were then lysed by adding 800 μg of lysozyme g^{-1} of *E. coli*, followed by incubation at room temperature for 20 min. Deoxycholic acid (4 mg sodium salt g^{-1} *E. coli*) was added, and the samples were further incubated at 37 °C for 10 min, after which they were treated with DNase I (200 μg of DNase g^{-1} of *E. coli*) for 30 min at 37 °C. The suspension was cooled on ice and sonicated for 30 s at full power (Sonorex RK 255H) to complete cell lysis. Inclusion bodies of Cry1Ab protein were separated from solubilized proteins by centrifugation (9699*g*, 10 min, 4 °C) and washed three times in washing buffer (20 mM Tris/HCl, pH 7.5, 1 M NaCl, 1% Triton X-100), three times in PBS (10 mM Na_2HPO_4/KH_2PO_4, pH 7.4; 0.8% (w/v) NaCl), and three times in distilled H_2O. Inclusion bodies were solubilized in 50 mM cyclohexylaminopropane sulfonic acid (CAPS) buffer (pH 10.5) containing 0.25% β-mercaptoethanol (β-ME) and incubated at 37 °C for 2 h. The solubilized Cry1Ab protoxin (131 kDa) was trypsinized overnight at room temperature with L-1-tosylamido-2-phenylethyl chloromethyl ketone (TPCK)-treated bovine pancreas trypsin (1 mg ml^{-1}). The resulting 60-kDa Cry1Ab toxin core was ultrafiltered through a Millipore polyethersulfone membrane [nominal molecular weight limit (NMWL): 50,000]. The concentration of Cry1Ab protein in the stock solution was measured by the Bradford procedure, diluted with 50 mM CAPS buffer to a concentration of 1.6 mg ml^{-1}, and then loaded on a polyacrylamide gel alongside bovine serum albumin (BSA) to confirm the identity and purity of the toxin by SDS/PAGE. The purity of the *E. coli*-expressed Cry1Ab was determined to be 97% (Nguyen, 2004), and an artificial diet overlay bioassay with *O. nubilalis* showed good activity of the protein, with a toxicity of 29 ng cm^{-2} (Hang Thu Nguyen, unpublished).

The Cry1Ab stock solution was further diluted 1:8 with K-medium (nematode test medium: 3.1 mg NaCl and 2.4 mg KCl l^{-1}) to yield a nominal concentration of 400 mg l^{-1} (in 6.25 mM CAPS). Starting with this solution of Cry1Ab protein, a dilution series was prepared using 6.25 mM CAPS (50 mM CAPS diluted 1:8 with K-medium). This resulted in nominal concentrations of 400, 236, 126, 82, and 48 mg Cry1Ab protein l^{-1}.

2.4. Quantification of Cry1Ab protein in soil samples

The concentration of Cry1Ab protein in soil was determined using a commercially available ELISA test kit for detecting Cry1Ab/Cry1Ac proteins (Envirologix Cry1Ab/Cry1Ac Plate Kit; Envirologix, Portland, ME). The protocol originally described by the manufacturer for the analysis of plant tissue was modified for the extraction and analysis of Cry1Ab protein from soil samples. Soil (1 g wet weight) was mixed with 3 ml of the extraction/dilution buffer provided with the ELISA kit and homogenized on a VF2-vortex (Janke-Kunkel IKA-Labortechnik, Staufen, Germany) at maximum speed. These soil suspensions were incubated for 60 min at room temperature and centrifuged for 20 min at 16,000*g* and 15 °C. Supernatants were concentrated 5-fold by ultrafiltration with Microcon YM-10 centrifugal filter devices (Millipore, Eschborn, Germany) before ELISA. The sensitivity of the ELISA was increased by using quantitation standards diluted with extraction/dilution buffer to concentrations of 0.1, 0.5, 1.0, and 1.5 μg l^{-1} and by extending the recommended 1-h incubation period of the Cry1Ab–enzyme conjugate to 3 h. The limit of detection for immunoreactive Cry1Ab protein was 0.02 ng ml^{-1} of soil extract, corresponding to 0.07 ng Cry1Ab protein g^{-1} of soil for a single sample.

2.5. Nematode bioassay

C. elegans var. Bristol, strain N2, was maintained as stocks of dauer larvae (an alternative juvenile stage that occurs with a lack of food) on nematode growth medium (NGM) agar (17 g bacto agar, 2.5 g bacto peptone, and 3 g NaCl l^{-1}, after autoclaving, add 1 ml 1 M $CaCl_2$, 1 ml 1 M $MgSO_4$, 25 ml 1 M KH_2PO_4, and 1 ml of 5 mg ml^{-1} cholesterol solution in ethanol; Brenner, 1974) according to standard procedures (Sulston and Hodgkin, 1988; Lewis and Fleming, 1995). The nematode bioassay with *C. elegans* was carried out, with few modifications, according to standard methods (ISO, 2007). For the soil test, 0.3 g of soil (air-dry weight) was moistened with 0.2 ml of K-medium in test wells (12-well polystyrene multidishes; Nunc, Wiesbaden, Germany) and then mixed with 0.5 ml of *E. coli* (OP50, approximately 10^{10} cells ml^{-1}) suspended in K-medium as the food supply (ASTM, 2001; ISO, 2007). At the start of the test, 10 first-stage (J1) juvenile worms were transferred to each test well. The mean initial body length of the test organisms was 270 μm (±16 μm, SD). Six replicates were set up for each treatment. After 96 h of incubation at 20 °C, the test was stopped by heat-killing the worms at approximately 50 °C. The samples were then mixed with 0.5 ml of an aqueous solution of Rose Bengal (0.5 g l^{-1}) to stain the worms for easier counting and stored at 4 °C until further use.

For the aqueous test with pure Cry1Ab protein, 0.5 ml of the respective solution of protein (400, 236, 126, 82, and 48 mg l^{-1}) were mixed with 0.5 ml of *E. coli* suspension. As the result of the 1:1 dilution, the actual concentrations of the Cry1Ab protein were 200, 118, 63, 41, and 24 mg l^{-1}. Based on a molecular mass of 60 kDa, the tested concentrations of Cry1Ab protein corresponded to molar concentrations of 0.4, 0.7, 1.1, 2.0, and 3.3 μM. Two conditions were set up as negative controls: (i) K-medium and (ii) buffer (6.25 mM CAPS). Five first-stage (J1; initial body length: 315±26 μm, SD) juvenile worms were transferred to each test well and four replicates per treatment were set up. The other test conditions were identical to the soil test.

Nematodes were easily recovered and counted by transferring the aqueous medium (Cry1Ab solutions) from the test wells into a Petri dish using a Pasteur pipette. For the soil tests, nematodes were extracted from the soil according to standard procedures (ASTM, 2001; ISO, 2007), using a mixture of a suspension of colloidal silica (Ludox TM50; Sigma-Aldrich, Munich, Germany) and deionized water (density: 1.13 g cm^{-3}). Sediment and nematodes were removed from the test wells with a Pasteur pipette by washing with approximately 5 ml of Ludox. This suspension was transferred to a centrifuge tube (15 ml), thoroughly mixed, and centrifuged for 10 min at 800*g*. The supernatant, which contained the nematodes, was poured into a Petri dish, and the pellet, containing sediment particles, was resuspended with diluted Ludox and again centrifuged to extract any remaining nematodes. On average, 84% (±14% SD; $n = 144$) of the test organisms were recovered by this procedure. Nematode reproduction was quantified by counting the juvenile offspring under a dissecting microscope at 25-fold magnification. Nematode growth was determined by measuring the body length at 100-fold magnification using a light microscope. Growth was calculated by subtracting the mean initial body length of the test organisms from the mean body length after incubation.

For statistical analysis, Mann–Whitney *U*-test and one-way analysis of variance (ANOVA) were performed using SPSS® microcomputer software (Munich, Germany). To test for differences between the various treatments multiple comparison post hoc tests (Tukey; Dunnett) were carried out.

3. Results

3.1. Concentration of Cry1Ab protein in soil samples

Cry1Ab protein was detected only in soil cultivated with transgenic corn (Table 1). The highest concentrations of Cry1Ab protein, 0.51 and 1.31 ng g^{-1} dry weight, were found in rhizosphere soil collected in July 2002 from the two MON810 replicates. In the bulk soil samples of May 2003 and 2004, measurable concentrations of Cry1Ab protein (0.19 and 0.22 ng g^{-1} dry weight, respectively) were present in only one of the two MON810 replicates. In the

rhizosphere soil samples of October 2003, the concentrations of Cry1Ab protein were below the detection limit.

3.2. Bioassay with soil samples

Reproduction and growth of *C. elegans* were significantly lower in soils from fields with *Bt* corn than in soils from fields with the near-isogenic variety (Fig. 1a and b; $p<0.05$; one-way ANOVA, post hoc Tukey). Differences in reproduction were most pronounced in the rhizosphere soil of July 2002, when samples from fields with *Bt* corn yielded only 13% and 17% of the reproduction observed in non-*Bt* samples (Fig. 1a). In the rhizosphere soil samples taken from the transgenic plots in October 2003, *C. elegans* reproduction was only 64% and 51% of that from isogenic plots. In bulk soil from May 2003, only one of the two *Bt* replicates resulted in significantly different levels of reproduction (57% of the level obtained with isogenic soil), while in bulk soil from May 2004, the differences between transgenic and isogenic plots were not statistically significant. Growth was generally less affected in *Bt* soils than reproduction (Fig. 1b). The mean body length of *C. elegans* in soils from fields with *Bt* corn was 55 to 93% of that in soils from fields with isogenic corn. Nonetheless, with one exception (bulk soil May 2004), growth in soil from fields with the *Bt* cultivar was consistently and significantly reduced compared with that from fields with the near-isogenic variety. There was a significant correlation between the contents of the Cry1Ab protein of the soil samples and reproduction ($r^2 = 0.67$; $p<0.001$) or growth ($r^2 = 0.42$; $p = 0.019$) of *C. elegans*.

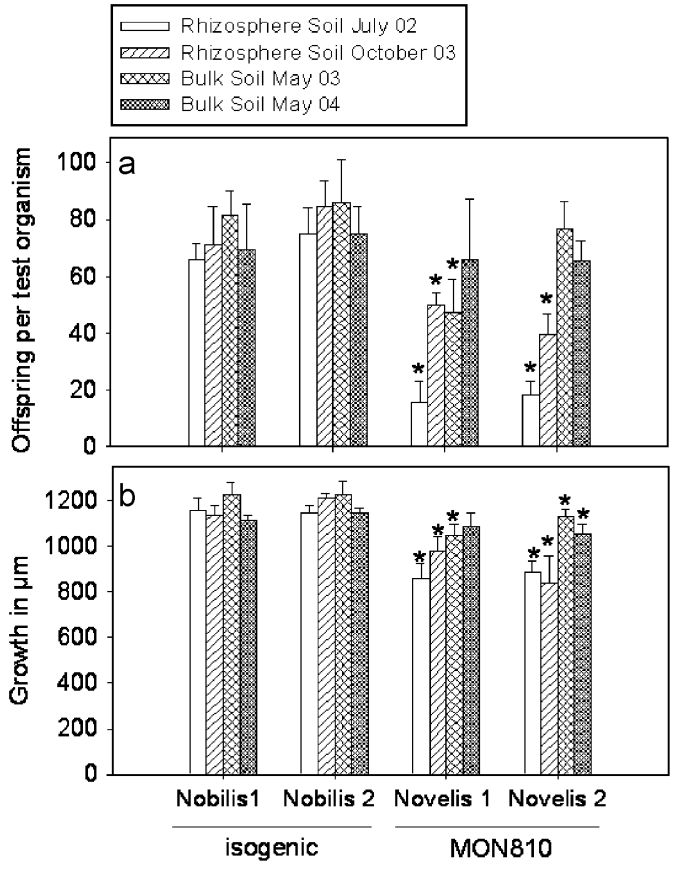

Fig. 1. (a) Reproduction (offspring per test organism) and (b) growth (µm) of *C. elegans* exposed to rhizosphere and bulk soil that had been cultivated with MON810 (Novelis) or the near-isogenic corn cultivar (Nobilis); Nobilis (Novelis) 1 and 2 represent two separate field plots; bars = mean, error bars = standard deviation ($n = 6$); *significantly different compared with the respective soil samples from near-isogenic corn cultivars ($P<0.05$; one-way ANOVA, post hoc Tukey).

3.3. Bioassay with solutions of Cry1Ab protein

No significant differences in growth and reproduction were found between the two negative controls, K-medium and buffer ($p>0.05$, Mann-Whitney U-test; Fig. 2). However, the toxicity of *E. coli*-expressed Cry1Ab protein on the growth and reproduction of *C. elegans* was concentration-dependent (Fig. 2). A significant reduction in nematode reproduction occurred with 63 mg l^{-1} (63% inhibition compared with the buffer control; $p<0.001$, one-way ANOVA, post hoc Dunnett). Compared with the pooled control (K-medium and buffer), the effect on reproduction was statistically significant at a concentration of protein of 41 mg l^{-1}, which produced 36% inhibition ($p<0.001$, one-way ANOVA, post hoc Dunnett). A

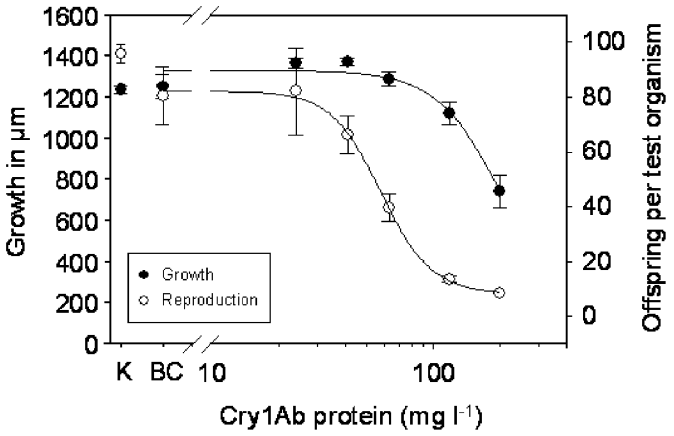

Fig. 2. Growth (µm) and reproduction (offspring per test organism) of *C. elegans* exposed to *E. coli*-expressed Cry1Ab protein in aqueous medium; K = K-medium control; BC = buffer control (6.25 mM CAPS). The means ($n = 4$) and standard deviations (error bars) are shown.

Table 2
EC50 (concentration at 50% inhibition of growth and reproduction), lowest observed effect concentration (LOEC), and no observed effect concentration (NOEC) of pure Cry1Ab protein in aqueous medium; molar concentrations were calculated based on a molecular mass of 60 kDa

Toxicity threshold	Growth	Reproduction
NOEC	63 mg l^{-1} (1.1 µM)	24 mg l^{-1} (0.4 µM)
LOEC	118 mg l^{-1} (2.0 µM)	41 mg l^{-1} (0.7 µM)
EC50	224 mg l^{-1} (3.7 µM)	54 mg l^{-1} (0.9 µM)

significant inhibitory effect on the growth of *C. elegans* occurred with 118 mg l^{-1}, with 11% inhibition ($p<0.01$, one-way ANOVA, post hoc Dunnett). However, significant stimulation of growth occurred at concentrations as low as 24 and 41 mg l^{-1}. EC50 values (concentration at which 50% inhibition occurs) for growth and reproduction were 225 and 54 mg l^{-1}, respectively (Table 2).

4. Discussion

The nematode, *C. elegans*, showed reduced growth and reproduction in rhizosphere and bulk soil from fields with *Bt* corn compared with soils from fields with the near-isogenic variety (Fig. 1). This effect was consistent at four different sampling dates over two vegetation periods, but it was most pronounced in rhizosphere soil sampled in July 2002, when the highest concentrations of Cry1Ab protein were also detected (Fig. 1, Table 1). The significant, but weak, correlation of nematode growth and reproduction with the concentrations of the protein in the soil samples raises the question whether Cry1Ab protein released into soil directly caused the subsequent observed effects on *C. elegans*.

Toxicity testing with pure Cry1Ab protein in aqueous medium showed initial deleterious effects on reproduction of *C. elegans* at a concentration of 41 mg l^{-1} (0.7 μM). The EC50 was 54 mg l^{-1} (0.9 μM), which was comparable to the toxicity of other environmental toxicants, such as cadmium (EC50 for reproduction: 1.7 μM; Traunspurger et al., 1997). However, the toxicity of the Cry1Ab protein was one-order of magnitude lower than that of nematicidal pesticides, such as abamectin (EC50 for reproduction: 0.1 μM; Höss et al., 2006), and two- to three-orders of magnitude lower than those of nematicidal Cry proteins (Wei et al., 2003), such as Cry14A (0.016 mg l^{-1}), Cry21A (0.047 mg l^{-1}), Cry5B (0.066 mg l^{-1}), and Cry6A (0.23 mg l^{-1}). Cry proteins affect nematodes by damaging the nematodal gut (Marroquin et al., 2000; Wei et al., 2003). Griffitts et al. (2003) showed that carbohydrate receptors, encoded by *bre* genes, on the intestinal cells of *C. elegans* interact with Cry proteins, allowing them to enter the cells and subsequently affect the nematode. The mode of action is, thus, analogous to that in insects (Schnepf et al., 1998). However, it is not clear whether the mechanism of toxicity of the Cry1Ab protein towards *C. elegans* is the same as that of nematicidal Cry proteins. A toxic effect of β-ME, a residual component of the solubilization buffer in the protein preparation, could be excluded, as the expected β-ME concentrations in the test (<0.0001%) were considerably lower than the tested no observed effect concentration (NOEC); 0.001%; data not shown).

The ratio of the measured concentrations of protein in the soil to the lowest observed effect concentration (LOEC) of Cry1Ab protein for *C. elegans* ($[Cry1Ab]_{soil}/[LOEC]_{aq}<10^{-4}$) was too low to account directly for the inhibitory effects of the *Bt* soil samples on growth and reproduction of the nematode (Fig. 1). Even if the determined concentrations of Cry1Ab protein in soil underestimated the real concentrations in soil (the extraction efficiency for the ELISA analysis was estimated to be approximately 40%; Baumgarte and Tebbe, 2005), concentrations of Cry1Ab protein would still not be high enough to produce direct toxic effects on *C. elegans*. Although *Bt* proteins may accumulate in soils by binding on clay and humus particles (Tapp and Stotzky, 1998), it is not likely that concentrations of Cry1Ab protein in the field could reach levels that are high enough to affect *C. elegans* directly. Baumgarte and Tebbe (2005) measured mean concentrations of Cry1Ab protein of 1.6 and 1.7 ng g^{-1} in two types of rhizosphere soil, with peak values of 3–10 ng g^{-1}, and much lower concentrations in bulk soil (mean concentrations of 0.05–0.12 ng g^{-1}). These were comparable with the soil concentrations of Cry1Ab protein measured in this study. Accumulation of the toxin was not observed during the third and fourth year of cultivation, neither for rhizosphere nor for bulk soil; instead, the highest concentrations of Cry1Ab concentration were measured at the first sampling date (Table 1).

Thus, the reasons for the effects of soil from *Bt* fields on *C. elegans* remain unclear. Indirect effects involving food quantity or quality due to *Bt*-induced changes in soil bacterial communities are not likely because: (i) major effects of Cry1Ab protein on the indigenous bacterial community were not observed in other studies (Saxena and Stotzky, 2001; Baumgarte and Tebbe, 2005), and (ii) under the test conditions, *C. elegans* mainly fed on added food bacteria (*E. coli*) that were present in amounts large enough to rule out indirect food effects.

Effects of *Bt* corn on the reproduction of indigenous nematodes may disturb their population dynamics and ultimately their community structure. Although it is difficult to generalize results of single-species tests, impairments of other nematode taxa by *Bt* corn cannot be excluded. *C. elegans* belongs to the family *Rhabditidae*, which are classified as typical colonizers that are relatively tolerant to disturbances, such as pollution (Bongers, 1990). Even within the family *Rhabditidae*, species can differ in their sensitivity to environmental chemicals (Boyd and Williams, 2003). Also, for Cry proteins there is a broad range of susceptibility between various nematode species (Wei et al., 2003).

Results of studies observing nematode communities in *Bt*-treated soils *in situ* are not unanimous. Saxena and Stotzky (2001) did not find any effects of Cry1Ab protein released in root exudates and from biomass of *Bt* corn on nematode abundances in soils. A more recent study showed that nematode abundances in soils from three different European climatic zones were significantly lower in fields with *Bt* corn than in those with the respective isogenic corn and with other conventional crops (Griffiths et al., 2005). These results were put into perspective by a glasshouse study involving eight different paired varieties of corn (*Bt* and near-isogenic). Nematode abundance varied mainly between the corn varieties, rather than between *Bt*

and non-*Bt* corn (Griffiths et al., 2007). Manachini and Lozzia (2002) found clear differences in the composition of feeding types, with bacterivorous species dominating in soil from isogenic corn fields and fungivorous species dominating in soil from *Bt* corn fields (Event 176, Novartis). However, this difference only occurred at one location, whereas at the other investigated locations no difference in nematode community structure occurred between soils from *Bt* and non-*Bt* fields.

In summary, a readily standardized soil bioassay with the nematode, *C. elegans*, as test organism was able to identify deleterious effects of rhizosphere and bulk soil samples from fields with *Bt* corn (Novelis, MON810). Although the observed effect of the soil samples on the nematodes could not be explained by a direct toxicity of the Cry1Ab protein, the effects, nonetheless, correlated with concentrations of the protein in soil. Whether indirect effects of the protein can explain the reduced reproduction and growth of the nematodes needs further investigation.

Toxicity tests using *C. elegans* offer several advantages as a tool for monitoring *Bt* effects on non-target soil organisms and of the potential field effects of commercial *Bt* crops: first, the test can be carried out in soil, as well as in aqueous medium, allowing a comparison between the effects of soil samples from the field and solutions of the Cry protein. Second, there are standardized test protocols (ASTM, 2001; ISO, 2007) supporting the reliability of the test systems; Third, the nematode toxicity test is quick and cost-effective, hence allowing the establishment of routine applications for an efficient toxicity screening during early stages of testing the effects of new transgenic crops.

Acknowledgments

This study was supported by the Bavarian Ministry of Environment, Health and Consumer Protection (StMUGV) and the German Federal Ministry of Education and Research (BMBF, Projects 0312631C and 0313279B). The gifts of *Caenorhabditis elegans* and *Escherichia coli* from the Caenorhabditis Genetic Center (Theresa Stiernagle) are gratefully acknowledged. We thank an anonymous reviewer for valuable comments on earlier versions of the manuscript.

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