



# Can *Bacillus thuringiensis* (*Bt*) corn residues and *Bt*-corn plants affect life-history traits in the earthworm *Aporrectodea caliginosa*?

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## Abstract

*Bt*-corn is genetically engineered to express proteins from the common soil bacterium *Bacillus thuringiensis* which presents specific toxicity to targeted insect pests. One type of *Bt*-corn expresses the Cry1Ab protein that provides protection against certain lepidopteran pests, mainly the European corn borer (*Ostrinia nubilalis*) and the Mediterranean stalk borer (*Sesamia nonagroides*). Vast areas of agricultural soils worldwide are grown with transgenic *Bt*-corn. With such widespread use of *Bt*-corn, it is important to evaluate the potential risks of *Bt*-protein to non-target organisms in agro-ecosystems such as earthworms. In this study, we investigated the effects of *Bt*-corn on important life-history traits (survival, reproduction and growth) of the earthworm *Aporrectodea caliginosa* under various experimental conditions. Finely ground *Bt*-corn leaves added to soil had no deleterious effects on survival, growth, development or reproduction in *A. caliginosa*, even in high concentrations that could be considered as a worst-case scenario. Also, growth of juvenile *A. caliginosa* was unaffected when worms were kept in pots with a growing *Bt*-corn plant. Only when considering cocoon hatchability did we see a slight, but statistically significant, negative effect of *Bt*-corn residues. The implications of these results for risk assessment of *Bt*-corn are discussed.

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## 1. Introduction

*Bt*-corn is genetically engineered to express proteins from the common soil bacterium *Bacillus thuringiensis*, which presents specific toxicity to

targeted insect pests. One type of *Bt*-corn expresses the Cry1Ab protein that provides protection against certain lepidopteran pests, mainly the European corn borer (*Ostrinia nubilalis*) and the Mediterranean stalk borer (*Sesamia nonagroides*). *Bt*-corn provides for an alternative to insecticides as a way of pest management in cornfields. In 2002, about 12 million ha were grown with transgenic *Bt*-corn, primarily in America

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and China (James, 2003). With such widespread use of *Bt*-corn, it is important to evaluate the potential risks of *Bt*-protein to non-target species in agro-ecosystems.

Earthworms are considered beneficial organisms in agricultural soils because of their functional roles in the breakdown of dead plant tissue and recycling of plant nutrients and in their influence on soil drainage and aeration (Lee, 1985; Edwards and Bohlen, 1996). Where corn is grown with reduced tillage practices, earthworms are particularly important in maintaining or improving soil physical conditions (Lachnicht et al., 1997; Kladvik et al., 1997). *Bt*-protein from *Bt*-corn can reach the soil by deposition and degradation of corn residues and by root exudation (Saxena et al., 1999; Zwahlen et al., 2003a). Most studies show that *Bt*-protein is degraded or inactivated in soil within weeks (Head et al., 2002; Hopkins and Gregorich, 2003). However, under certain field conditions, *Bt*-protein may persist for up to 200 days (Zwahlen et al., 2003a), implying that earthworms consuming corn residues and soil may be exposed directly to the protein for a significant part of their life-cycle.

Only a few studies have dealt with effects of *Bt*-protein on earthworms. Ahl Goy et al. (1995) observed no effects of *Bt*-corn on mortality or weight gain in the epigeic *Eisenia fetida* in a 14-day acute toxicity test. Saxena and Stotzky (2001) observed no negative effects on survival or body weight in *Lumbricus terrestris* in a 45-day study when worms were kept in pots with growing *Bt*-corn plants or in soil amended with *Bt*-corn biomass as compared to non-*Bt*-corn. Recently, Zwahlen et al. (2003b) reported that mortality and growth of juvenile and adult *L. terrestris* was largely unaffected when fed *Bt*-corn during 160 days. Only after a final sampling at 200 days was a significantly lower adult weight observed in worms fed *Bt*-corn litter as compared to controls.

Based on the results published so far, it seems that *Bt*-corn poses little threat to earthworm populations. However, as pointed out by Zwahlen et al. (2003b), more studies are needed to evaluate the effects of *Bt*-corn on more subtle life-history characteristics of earthworms than provided by acute toxicity studies, such as cocoon production, growth of juveniles and the time it takes for juveniles to reach the reproductive stage. This is consistent with recommendations by experts in earthworm ecotoxicology (Van Gestel and Weeks, 2004; Spurgeon et al., 2003). So far, effects of

*Bt*-corn have only been investigated in two species of earthworms of which one (*E. fetida*) is not relevant for agricultural soils, and the other (*L. terrestris*) is rarely found in high numbers in such soils (Edwards and Bohlen, 1996). Thus, there is a pressing need to examine earthworm species that are abundant and ecologically important in agricultural soils.

The aim of the present study was to assess the effects of *Bt*-corn on important life-history traits (survival, reproduction and growth) of the earthworm *Aporrectodea caliginosa*. This species is probably the most widespread in temperate agricultural soils, often comprising a major proportion of the total earthworm biomass (Lee, 1985; Edwards and Bohlen, 1996). In a series of experiments, we investigated the growth of juveniles until maturity, cocoon production and cocoon hatchability. Effects of *Bt*-corn litter mixed into the soil as well as the effects of growing corn plants were determined.

## 2. Materials and methods

### 2.1. Test soil

A soil from a Danish agricultural field was used as test soil. This soil, collected at the Danish Institute for Agricultural Science, Foulum, consisted of 28% coarse sand, 34% fine sand, 23% silt, 8% clay, 6% organic matter, and had a pH of 5.7. The sampled soil was dried at 80 °C and stored at room temperature until used in the experiments.

### 2.2. Earthworms

Adult specimens of the earthworm *A. caliginosa* var *tuberculata* (Savigny) were collected at an agricultural site. The earthworms were acclimated at 15 °C in the test soil for 7 days and then used in reproduction experiments, or kept in laboratory cultures for cocoon production (Holmstrup et al., 1991). In the cultures, the soil was changed every 3 weeks. Cocoons were collected by sieving and washing the culture soil through a 1 mm mesh. The cocoons were stored at 5 °C for a few weeks and then were hatched at 20 °C. Newly hatched juveniles, all within 1 week of age, were also acclimated at 15 °C in the test soil for 7 days before they were used in growth experiments.

### 2.3. Plant material

Genetically modified *Bt*-corn (MEB307 cultivar), a near-isogenic cultivar and a conventional variety were grown in experimental plots at the Danish Institute of Agricultural Sciences, Foulum, in 2002 and stored at  $-18\text{ }^{\circ}\text{C}$  after harvest. The near-isogenic cultivar was used as a control in the experiments and the conventional variety was used to indicate if possible effects could be related to the variety. Green leaves were air-dried at room temperature and then finely ground. Using an ELISA kit from Agdia Inc., Indiana, the content of *Bt*-toxin, Cry1Ab, was determined to be  $9.6\text{ }\mu\text{g g}^{-1}$  (95% C.L.: 8.4–10.8) in MEB307. The corn plants were characterised in terms of fodder constituents (Table 1). The two isogenic lines did not differ significantly from each other for any of the selected constituents, while the conventional variety differed significantly from the isogenic varieties in dry matter, nitrogen, fibre content and cattle enzyme digestible organic matter content.

### 2.4. Reproduction experiment with finely ground corn leaves

Finely ground corn leaves ( $5\text{ g D.M. kg}^{-1}$  dry soil) were added to the test soil. *Bt*-corn (MEB307) was tested in increasing concentrations (1, 2, 3, 4 or  $5\text{ g kg}^{-1}$  dry soil) by dilution with non-*Bt* plant material (the near-isogen to MEB307), named ‘Bt 1’–‘Bt 5’, respectively. The two control treatments were used with only the near-isogen to MEB307 or the conventional variety plant material. The earthworms were fed with a mixture of dried cow dung and test soil (1:1 dry vol.%) moistened to 50% water content. The

finely ground plant material, water ( $270\text{ ml kg}^{-1}$  dry soil) and food ( $10\text{ g kg}^{-1}$  dry soil) were thoroughly mixed using an electric mixer. Amounts of  $642.5\text{ g}$  mixed soil were then distributed to 1200 ml plastic pots with perforated lids allowing for ventilation. A reference treatment (or positive control) with benomyl ( $0.5\text{ mg a.i. kg}^{-1}$  dry soil) was also included to verify the sensitivity of the test. Previous studies have revealed that this concentration in arable soils results in evident effects on growth and reproduction but not in mortality (Lofs-Holmin, 1982). Benomyl was added as the formulated compound (Benlate) dissolved in water. After 24 h equilibration of the test soil, the earthworms were added to the pots.

To avoid systematic errors in distributing the worms among the containers, 40 groups of 3 clitellate *A. caliginosa* were blotted with filter paper and their fresh weight determined with analytical scales (i.e. total fresh weight of 3 worms). The groups of earthworms were temporarily placed in Petri dishes and arranged in order of fresh weight. The experimental units (i.e. three worms per pot) were allocated at random to the eight treatments (two negative controls, one positive control and five concentrations of *Bt*-corn), ensuring nearly equal average initial fresh weight within each treatment group. The pots were incubated in darkness at  $15 (\pm 1)\text{ }^{\circ}\text{C}$  for 28 days. The contents were then placed in a tray and the surviving adults were removed, washed, blotted with filter paper and weighed. The test soil, containing any cocoons that had been produced, was incubated in darkness at  $15\text{ }^{\circ}\text{C}$  for 4 additional weeks. At the end of this period, the contents were wet sieved through a 1-mm mesh recovering cocoons. The number of cocoons produced over the 4-week reproduction period was determined.

Table 1

Characterisation of the corn cultivars in terms of mean [95% C.L.] fodder constituents followed by a reference to the method

Cultivar	MEB307		Isogenic to MEB307		Conventional variety	
D.M. (%)	23.3 <sup>a</sup>	[22.6–23.9]	22.7 <sup>a</sup>	[20.8–24.6]	24.6 <sup>b</sup>	[24.0–25.2]
Yield (DM t ha <sup>-1</sup> )	12.3	[11.8–12.8]	11.9	[11.6–12.3]	12.9	[12.1–13.7]
Ash (%)	3.9	[3.7–4.1]	3.8	[3.5–4.1]	4.0	[3.7–4.3]
Total N (%) (Hansen, 1989)	1.58 <sup>a</sup>	[1.55–1.60]	1.54 <sup>ab</sup>	[1.47–1.60]	1.52 <sup>b</sup>	[1.47–1.57]
P (%)	0.18	[0.17–0.19]	0.17	[0.16–0.19]	0.17	[0.15–0.19]
K (%)	1.48	[1.40–1.56]	1.42	[1.22–1.61]	1.51	[1.40–1.61]
Crude fibre (%)	23.6 <sup>a</sup>	[23.3–24.0]	23.8 <sup>a</sup>	[22.6–25.0]	22.0 <sup>b</sup>	[21.4–22.5]
Enzyme digestible organic matter (Danish Plant Directorate, 1999)	66.9 <sup>a</sup>	[66.2–67.6]	67.1 <sup>ab</sup>	[65.5–68.7]	68.0 <sup>b</sup>	[66.9–69.1]

Different superscript letters (a and b) indicate a significant difference by two-way ANOVA,  $P < 0.05\%$ , Tukey’s test.

The cocoons were then placed on moist filter paper in Petri dishes and incubated at 20 °C until all viable cocoons had hatched.

### 2.5. Growth experiment with finely ground corn leaves

For the growth test, 160-ml containers were filled with 64.5 g of mixed moist soil that was prepared as described above. To avoid systematic errors in distributing the worms to the containers, 120 juvenile (2–3 weeks old) *A. caliginosa* were blotted with filter paper and their fresh weight determined with analytical scales. Then, 15 juveniles were randomly selected and randomly allocated to each of the eight treatments.

After 24 h equilibration of the test soil, one juvenile was added to each of the containers, which were then closed with perforated lids allowing ventilation. The containers were incubated in darkness at 15 °C. At 14-day intervals, the soil was gently searched to recover the earthworm without causing mechanical injury. The worms were gently rinsed in tap water, blotted with filter paper and weighed to determine fresh weight (including gut contents). It was noted if worms had reached sexual maturity. Every 28 day, the soil was replaced with freshly prepared test soil. Water content of the test soil was checked weekly and maintained at 27% of dry mass by control weighing of containers and replenishment with tap water if necessary. The experiment was terminated after 14 weeks.

### 2.6. Juvenile growth in pots with corn plants

The effects of growing *Bt*-corn plants (MEB307) on juvenile growth was studied and compared with two control corn cultivars (the near-isogen to MEB307 and the conventional variety). In addition, a control treatment without plants and a positive control with benomyl (0.5 mg a.i. kg<sup>-1</sup> dry soil; no plant) were tested. For each treatment, five replicate plastic pots (height, 135 mm; top diameter, 120 mm; bottom diameter, 95 mm) were used. Test soil, water and earthworm food were mixed as described in previous sections.

For the reference test (benomyl) and for the treatment without plants, the test containers were filled with 605 g test soil. For the treatments with growing

plants, the test containers were filled with 968 g of test soil. In these pots, one seed was planted in each container and the corn plants were grown for 14 days in a plant-growth room (25 °C and 12-h light:12-h dark cycle). After this germination and growth period, five juvenile worms were placed in each replicate. It was ensured that the average fresh weight of the five worms was nearly equal in all treatments as described in previous sections. The containers were incubated for 28 days at 20 °C and 12-h light:12-h dark cycle. In order to reduce water loss from the pots and prevent escape of earthworms, a lid with a small hole was fitted to the corn plant stem. The water content of test soil was checked every day and maintained at the desired level.

After the 28-day period, the plants were removed. The soil was placed on a plate and the surviving earthworms were collected, washed, blotted with filter paper and weighed to determine fresh weight (including gut contents). The corn plants were recovered and adhering soil gently removed from roots before measuring the total length of each plant.

### 2.7. Statistical analysis

A significance level of 5% was used throughout. Statistical analyses were performed using procedures of SAS/STAT (SAS Institute Inc., 1999). Prior to analysis, the data were checked for need of transformation and variance inhomogeneity by SAS/LAB (SAS Institute Inc., 1992). Body weight and cocoon production of adults in the reproduction experiment and juvenile growth with plants were subject to ANCOVA with initial weight as the covariate. Tukey's test was used for pairwise comparisons of means. Comparison of treatments of cocoon hatchability was carried out by means of a generalized linear model (GLZ) with a binomial probability distribution. To estimate the EC<sub>10</sub> for hatchability (*h*) dose–response was modelled by fitting the second-degree polynomial  $h = h_0 + \alpha c^2$ , where  $h_0$  is the hatchability at a *Bt* concentration of 0, i.e. the control,  $\alpha$  the slope and  $c$  is the concentration of g *Bt* kg<sup>-1</sup> soil. The normal logistic model could not be used because a full dose–response curve (including 0% hatchability) was not available. However, the second-degree polynomial and the logistic curve are similar in the first part of the curve where only a small

effect is seen. Growth of earthworms was analysed as a repeated measures mixed model on log transformed fresh weights with the starting weight of the juveniles as a covariate.

### 3. Results

#### 3.1. Reproduction experiment with finely ground corn leaves

No mortality of adults occurred in any of the treatments. There was a significant decrease of 18% in worm fresh weight in the positive control treatment (benomyl) when compared with the near-isogenic variety to MEB307 control, but not when compared with the conventional variety (Fig. 1). A significantly higher body weight was attained in the 4 g *Bt* kg<sup>-1</sup> treatment in comparison with each of the treatments 1 and 5 g *Bt* kg<sup>-1</sup>, the conventional variety and benomyl.

Neither the *Bt*-corn treatments nor the benomyl treatment was significantly different from the near-isogen to MEB307 or the conventional variety with respect to cocoon production (Fig. 2). However, there was a weak significant difference ( $P < 0.057\%$ ) between the highest cocoon production in the ‘Bt 1’ treatment

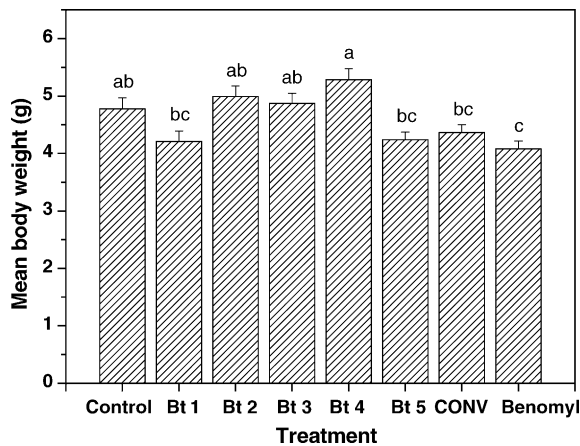


Fig. 1. The mean ( $\pm$ S.E.M.,  $n = 5$ ) fresh body weight of three *A. caliginosa* specimens after 28 days of incubation with finely ground corn leaves at a total concentration of 5 g kg<sup>-1</sup> dry soil. Control: near-isogen to MEB307; Bt 1–5: *Bt*-corn (MEB307 cultivar) in concentrations from 1 to 5 g kg<sup>-1</sup> dry soil; CONV: conventional variety control; benomyl: reference compound (0.5 mg a.i. kg<sup>-1</sup> dry soil). Different letters indicate significant differences.

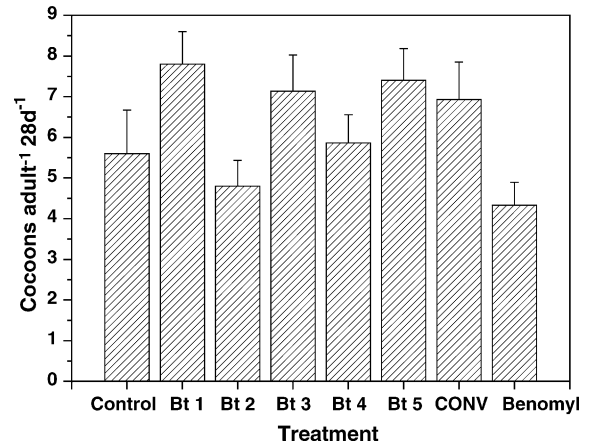


Fig. 2. The mean ( $\pm$ S.E.M.,  $n = 5$ ) cocoon production of each adult *A. caliginosa* after 28 days of incubation with finely ground corn leaves at a total concentration of 5 g kg<sup>-1</sup> dry soil. Control: near-isogen to MEB307; Bt 1–5: *Bt*-corn (MEB307 cultivar) in concentrations from 1 to 5 g kg<sup>-1</sup> dry soil; CONV: conventional variety control; benomyl: reference compound (0.5 mg a.i. kg<sup>-1</sup> dry soil).

and the benomyl treatment. Cocoon production was on average 6.2 [5.6–6.9] cocoons adult<sup>-1</sup> (28 day)<sup>-1</sup>. The lowest cocoon production rate (4.3 adult<sup>-1</sup> (28 day)<sup>-1</sup>) was observed in the benomyl treatment.

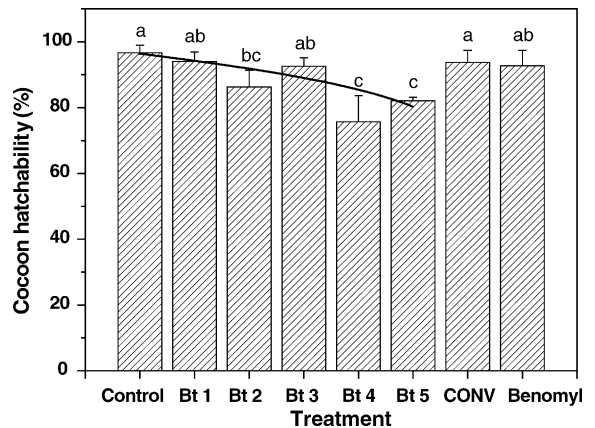


Fig. 3. The mean ( $\pm$ S.E.M.,  $n = 5$ ) hatchability of *A. caliginosa* cocoons produced during 28 days of incubation with finely ground corn leaves at a total concentration of 5 g kg<sup>-1</sup> dry soil. The cocoons were further incubated for 4 weeks in the same soil (see text for details). Each replicate consisted of 16–24 cocoons. Control: near-isogen to MEB307; *Bt*-corn: MEB307 cultivar, 5 g kg<sup>-1</sup> dry soil; CONV: conventional variety control; benomyl: reference compound (0.5 mg a.i. kg<sup>-1</sup> dry soil). Different letters indicate significant differences. The curve is a second-degree polynomial fitted to the hatchability percentage at increasing concentrations of *Bt*-corn.

Cocoon hatchability ranged from 75% in the “Bt 4” treatment to 97% in the control treatment (Fig. 3). With increasing *Bt*-corn concentration there was a significant decrease in hatchability rate ( $t = -3.36$ ,  $P = 0.0024$  for the slope of the polynomial model) and the GLZ analysis comparing the treatments gave an overall  $\chi^2 = 23.2$  and  $P = 0.0016$ . A hatchability  $EC_{10}$  of 4.2 [3.0–5.4] was estimated by fitting a non-linear regression curve to the dose–response data.

### 3.2. Growth experiment with finely ground corn leaves

The juveniles had an average fresh weight of 32.1 mg [30.7–33.5] ( $n = 120$ , range 15–49 mg) at the outset of the experiment. There were no significant differences between juvenile growth curves at the various finely ground plant materials during 14 weeks (Fig. 4). In contrast, growth was drastically reduced in the benomyl treatment. No mortality occurred during the experiment, and from 6 to 14 worms (out of 15) were adult at the last sampling after 14 weeks. There were no significant effects on the number of adults after 14 weeks of exposure to genetically modified plant material (Steel’s test;  $P > 0.05$ ; data not shown). In fact, at the highest dose of *Bt*-corn ( $5 \text{ g kg}^{-1}$  dry

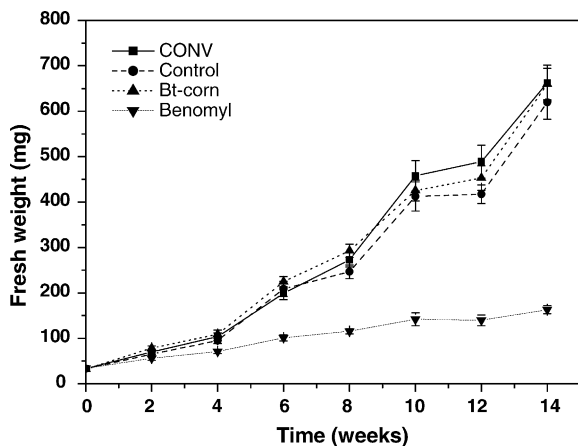


Fig. 4. Growth of individual juvenile *A. caliginosa* incubated with finely ground corn leaves at a total concentration of  $5 \text{ g kg}^{-1}$  dry soil over a period of 14 weeks. Only data for the highest *Bt*-corn concentration are shown. Control: near-isogen to MEB307; *Bt*-corn: MEB307 cultivar,  $5 \text{ g kg}^{-1}$  dry soil; CONV: conventional variety control; benomyl: reference compound ( $0.5 \text{ mg a.i. kg}^{-1}$  dry soil). Values are shown as mean  $\pm$  S.E.M. ( $n = 15$ ).

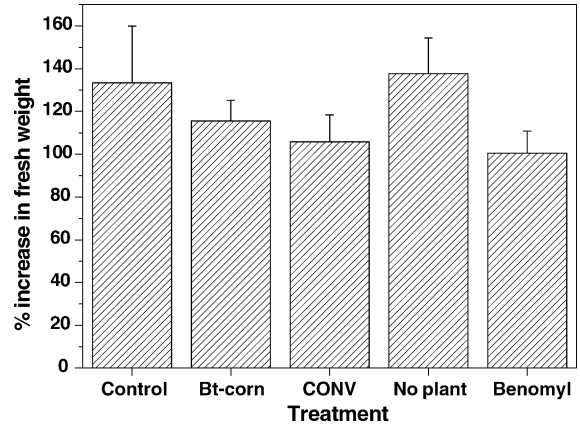


Fig. 5. Relative increase in fresh weight (percent of initial weight) of juvenile *A. caliginosa* kept in pots with growing corn plants for 4 weeks. Control: near-isogen to MEB307; *Bt*-corn: MEB307 cultivar; CONV: conventional variety control; benomyl: reference compound ( $0.5 \text{ mg a.i. kg}^{-1}$  dry soil); no plant: only earthworm food added (see text for details). Values are shown as mean  $\pm$  S.E.M. ( $n = 5$ ).

soil), 14 out of 15 worms were adult after 14 weeks. In contrast, no worms became adult during the 14 weeks in benomyl treatments (Steel’s test;  $P < 0.001$ ; data not shown). Thus, time to maturation was not longer in *Bt* treatments than in controls.

### 3.3. Juvenile growth in pots with corn plants

At the time when juvenile worms were added to the pots, the corn plants had an average shoot length of 36 cm (conventional variety), 41 cm (the near-isogen to MEB307) and 45 cm (MEB307). The initial average fresh weight of the added worm groups (five specimens) was 182 mg [177–186] ( $n = 25$ ) per replicate. At the end of the experiment (28 days), the worms had increased their fresh weight by 100–135% of the initial fresh weight (Fig. 5). There were no significant differences ( $P > 0.05$ ) among any of the treatments. Growth also occurred in the benomyl treatment even though the weight increase was lowest here.

## 4. Discussion

This is the first study to evaluate effects of *Bt*-corn on the complete set of life-history traits (reproduction, cocoon hatchability and growth to maturity) in an

earthworm species. The species we used for the study, *A. caliginosa*, is abundant in agricultural soils and therefore a relevant test organism when considering the side effects of *Bt*-corn on soil organisms.

*Bt*-corn residues had no detrimental effects on growth or development in *A. caliginosa* (Fig. 4). Moreover, the growth rate was comparable between two non-*Bt*-corn cultivars and the *Bt*-corn cultivar, demonstrating that growth was a robust parameter in the present study. The growth rate and time taken to reach maturity observed in the experiments were similar to other published studies (Lofs-Holmin, 1983; Holmstrup et al., 2001). On the other hand, growth was retarded in soil containing benomyl as a positive control, showing that the test system had a sufficient sensitivity to detect possible negative effects on growth. Juvenile earthworms kept in soil with a growing *Bt*-corn plant also had the same growth rate as control worms (Fig. 5). The absence of *Bt*-corn litter effects on growth are comparable with results of Zwahlen et al. (2003b) and Saxena and Stotzky (2001) who observed that juvenile *L. terrestris* had the same weight gain during 200 days irrespective of whether they were fed *Bt* or non-*Bt*-corn leaves.

The cocoon production and cocoon hatchability rate we observed in this study are similar to other published laboratory studies (Lofs-Holmin, 1983; Boström and Lofs-Holmin, 1996; Holmstrup et al., 1991) and can therefore be considered as representative for the species. Cocoon production was unaffected by *Bt*-corn litter (Fig. 2), whereas a small but statistically significant negative effect was seen on cocoon hatching (Fig. 3). Hatching success was reduced from about 95 to 75% at one of the highest concentrations of *Bt*-corn litter. At present, it is difficult to explain why this particular parameter is affected by *Bt* toxin when other parameters like growth and development are not. Abiotic factors like moisture and pH can influence hatching success of cocoons (Bengtsson et al., 1986; Holmstrup, 2001); however, we have no reason to believe that these factors were different in the treatments that were used.

*A. caliginosa* feeds by ingesting large amounts of soil (Edwards and Bohlen, 1996). Because both earthworm food (cow dung) and *Bt*-corn powder were mixed into the soil, there can be no doubt that the earthworms ingested and were exposed to the *Bt*-proteins in the corn leaf powder. Finely ground corn

leaves were added to soil in concentrations up to 5 g dry mass kg<sup>-1</sup> dry soil. A rough calculation indicates that this would correspond to litter input in the range of 0.7 kg m<sup>-2</sup> under field conditions under the assumption that litter is mixed into the top 10 cm of the soil column. The exposure applied in the present study can therefore be considered as a worst-case scenario because only little aboveground biomass is left on the soil in most corn systems. The application of ground leaves probably resulted in an increased exposure to *Bt*-protein compared with using intact pieces of leaves for earthworm food as in the study of Zwahlen et al. (2003b). Moreover, in the growth experiment of the present study, we added fresh *Bt*-corn material to the soil every 28 day. Under natural conditions, the *Bt*-protein in corn leaves is degraded with a half-life of about 20–40 days.

The mechanisms that underlie the apparent insensitivity of *A. caliginosa* to *Bt* toxin may be due to the lack of activation of the *Bt*-protein or the lack of toxin-binding receptors in the cell membranes of the earthworm gut. *Bt*-protein crystals are activated in the insect gut at high pH (>10) and subsequent modification by proteolytic enzymes (Höfte and Whiteley, 1989). The pH in the earthworm gut is only 6–7 (Laverack, 1963), suggesting that the toxin is never activated in the earthworm gut. Moreover, *Bt* toxins have proven only to bind to very specific receptors, apparently only found within a few insect species (Höfte and Whiteley, 1989).

In conclusion, the present study confirms the findings of other authors for different earthworm species (Ahl Goy et al., 1995; Saxena and Stotzky, 2001; Zwahlen et al., 2003b), namely that the use of *Bt*-corn apparently poses minimal risks to earthworms as far as growth and reproduction is concerned. Particularly, we found that the widespread earthworm *A. caliginosa* was not affected by growing *Bt*-corn plants or finely ground leaves thereof when considering growth, development or cocoon production. We did see a small negative effect on cocoon hatching success, but this was at relatively high concentrations of finely ground *Bt*-corn material and it can be questioned whether this effect would have any ecological significance under field conditions. A sensible way to follow up on the results of this and previous studies, and to bolster a sound risk assessment of *Bt*-corn, would probably be to assess

the effects of *Bt*-corn on earthworm populations in carefully designed field experiments. So far, no published studies of earthworm populations have described the consequences of long-term cultivation of *Bt*-corn.

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