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# Impact of Bt corn on the diplopod *Allajulus latestriatus*

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## Summary

The application of transgenic crops has expanded enormously since their introduction to agriculture in the 1990s. Despite increased research activity in the area of non-target effects, the impact on many groups of soil organisms still remains unclear. In this study, laboratory feeding experiments were conducted to detect possible effects of Bt corn on the detritophagous *Allajulus latestriatus* (Diplopoda, Julidae). We tested the transgenic corn N4640Bt (expressing the Bt protein Cry1Ab), the corresponding non-transgenic isoline, and two further conventional corn varieties. Furthermore, a toxicity test was performed, in which purified Cry1Ab was added to a corn leaf diet in different concentrations. Cry1Ab concentrations in corn leaves, corn leaf diet, faeces and diplopods were quantified, applying an enzyme-linked immunosorbent assay (ELISA). We performed bioassays using the European Corn Borer *Ostrinia nubilalis* to test the insecticidal activity of different samples.

No significant differences in mortality, consumption of corn leaves, or weight gain were found when animals were fed on N4640Bt compared to its isoline and the other two varieties. However, faeces production per diplopod was significantly increased when animals were kept on Bt corn compared to the isoline and to another corn variety. In the toxicity test, no significant differences were found for mortality, consumption, weight gain or faeces production between diets, not even at Cry1Ab concentrations more than 100 times higher than in Bt leaves. Bioassays proved that the Bt protein in transgenic leaves and in faeces of diplopods was still insecticidal. The toxicity test indicated that Cry1Ab excretion in the faeces is linearly correlated to the Cry1Ab uptake through the diet.

Our findings suggest that Bt corn and Cry1Ab will not harm *A. latestriatus*. However, this diplopod excretes considerable amounts of Bt protein with its faeces. The Bt protein is still insecticidally active and, thus, becomes available to other soil organisms.


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## Introduction

Genetic engineering offers the possibility to introduce single and multiple gene traits, for example resistance against pests or herbicides, into plants. The economically most important transgenic crops are soybeans, corn, cotton and canola. About 30% of the aggregate area of these four crops are genetically engineered today. In 2004, about $19.4 \times 10^6$ ha of transgenic corn were grown worldwide; this is 25% more than in 2003 (James, 2004).

Transgenic crops expressing *Bacillus thuringiensis* (Bt) protein are used instead of conventional spray application of Bt formulation and chemical insecticides, which has been used in agriculture since the 1950s (Frankenhuyzen, 1993). Spray applications are limited in reaching endophagous pests. However, transgenic crops express the toxin in all their tissues and can control pests within tissue effectively.

Most of the commercially used Bt corn varieties express the Cry1Ab protein. It is a lepidopteran-specific toxin found originally in the common soil bacterium Bt (Lewellyn et al., 1994). Cry1Ab has a molecular mass of about 68 kDa (Zhipeng et al., 2004). It binds to lepidopteran midgut receptors, forming ion channels and thus leading to lyses of midgut epithelial cells and blockage of digestion (Schnepf et al., 1998). The main target organism of Cry1Ab when used in agriculture is the European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera, Pyralidae). Although the protein has relatively high specificity, there is evidence that it could harm non-target organisms. It has been shown in laboratory studies that Bt pollen on milkweed leaves harms monarch butterfly larvae (Losey et al., 1999; Jesse and Obrycki, 2000). However, these findings were not affirmed by further field investigations (Sears et al., 2001; Jesse and Obrycki, 2003; Anderson et al., 2004; Dively et al., 2004).

An important question is whether Cry1Ab is released into the soil and whether it harms soil organisms. Corn roots exude the protein into the surrounding soil and Cry1Ab bound to soil particles retains insecticidal activity (Saxena et al., 1999; Saxena and Stotzky, 2000). Laboratory and field studies showed that Bt protein remains detectable and insecticidal in the soil for several months (Saxena and Stotzky, 2001a, 2002; Zwahlen et al., 2003a). The degradation of Bt protein may be delayed because Bt plants decompose less in soil than the non-transgenic isolines (Stotzky, 2003; Flores et al., 2005). After 4 years of corn cultivation, traces of Cry1Ab could be detected in the whole unfractionated soil (Hopkins and Gregorich, 2003). In contrast, no detectable Cry1Ab was found in soils from Bt cornfields after 3 years of sustained use (Dubelmann et al., 2005), but as soil subsamples from each field were mixed to create a single composite soil sample per plot, the probability of detecting Cry1Ab was lowered.

Bt proteins appear to have no adverse effect on springtails and mites in laboratory experiments (Sims and Martin, 1997; Yu et al., 1997). The incidence of cutworm (Noctuidae) and wireworms (Elateridae) in cornfields is not affected by Bt corn (Pons et al., 2005). The finding of Saxena and Stotzky (2003) that Cry1Ab has no consistent effect on earthworms (Oligochaeta) and threadworms (Nematoda), points in the same direction. In a laboratory study, the woodlouse *Porcellio scaber* consumed significantly less of the transgenic corn N4640Bt than of the isogenic control line, whereas the transgenic Bt corn variety Max88 belonged to the most consumed varieties (Wandeler et al., 2002). Bacterial growth on the faeces of *P. scaber* fed on transgenic corn was up to 60% lower than that on the faeces of non-transgenic fed woodlice (Escher et al., 2000). However, the bacterial community structure in fields seems to be more influenced by other environmental factors than by exuded proteins from Bt corn (Baumgartner and Tebbe, 2005). Consequently, it is rather unclear today to what extent the soil fauna is affected by Bt corn.

Diplopods are important decomposers at certain sites (Anderson and Bignell, 1980; Hanlon, 1981; Anderson and Leonard, 1988) but so far, no study has analyzed the impact of Bt corn on diplopods. As we found *Allajulus latestriatus* (Curtis, 1844) (Diplopoda; Julidae) several times in soil samples from cornfields (personal observation), we used this species as a model organism to investigate the effect of Bt corn on diplopods. *A. latestriatus* is a detritophagous species, feeding on dead plant material, mainly on senescent grasses (Cotton and Miller, 1974). Coprophagy is also reported from diplopods, but the ecological importance is unclear (McBrayer, 1973; Hassal and Rushton, 1982; Crawford, 1987). *A. latestriatus* occurs in the agricultural landscape, in woods, and in sand dunes (Blower and Gabutt, 1964; Cotton and Miller, 1974; Pedroli, 1993). Since *A. latestriatus* is established in the cultural landscape, it was also unintentionally introduced into North America (Shelley, 2002).

In the present study, we performed a variety of laboratory experiments to analyze the effect of Bt corn on mortality, consumption, weight gain, and faeces production of *A. latestriatus*. No significant