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Effect of *Bt* maize on the reproduction and development of saprophagous Diptera over multiple generations

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Abstract

A laboratory experiment was used to quantify the effects of *Bt* maize on *Drosophila melanogaster* and *Megaselia scalaris*, representatives of two saprophagous dipteran families (Drosophilidae, Phoridae). Freshly hatched larvae were reared on a diet containing decaying maize leaves. Two transgenic maize varieties, expressing Cry3Bb1 or Cry1Ab, and their corresponding isolines were tested. In an additional treatment, a solution of pure Cry1Ab was added to the maize diet. According to quantitative ELISA analyses, all *Bt* diets and all larvae feeding on *Bt* maize contained low concentrations of Cry proteins but Cry proteins were not detected in adults, thus, predators of the larvae are exposed to Cry proteins whereas predators of adult flies are not. Highest concentrations were in larvae feeding on a maize diet supplemented with a Cry1Ab protein solution. The developmental time and fertility (offspring/female) were measured over four generations for *D. melanogaster* and over three generations for *M. scalaris*. Only a few significant differences were found between transgenic and non-transgenic treatments but the differences were not consistent and did not indicate any negative effects of *Bt* proteins. We conclude that *D. melanogaster* and *M. scalaris* larvae are not affected in the long term when feeding and developing on decaying Cry1Ab and Cry3Bb1 maize leaves.

Zusammenfassung

Zur Untersuchung möglicher Effekte von *Bt*-Mais auf saprophage Dipteren wurden Laborexperimente mit zwei verschiedenen Dipterenarten durchgeführt, *Drosophila melanogaster* (Drosophilidae) und *Megaselia scalaris* (Phoridae). Dazu erhielten frisch geschlüpfte Larven eine Mischnahrung, welche hauptsächlich aus seneszenten, gemahlenen Maisblättern bestand. Für die Experimente wurden zwei transgene Maissorten verwendet, die Cry3Bb1 bzw. Cry1Ab exprimieren, sowie die isogenen Kontrolllinien. Als zusätzliche Testvariante wurden nicht-transgene Maisblätter angeboten, denen reines Cry1Ab beigemischt wurde. Die quantitativen ELISA-Analysen zeigten, dass alle Bt-Nährmedien und alle Larven, welche mit transgenem Mais gefüttert wurden, geringe Cry-Proteinkonzentrationen enthielten, in adulten Fliegen konnte jedoch nie *Bt*-Protein gefunden werden. Hieraus lässt sich schliessen, dass Räuber dieser Dipterenlarven Cry-Proteinen exponiert sind, die Räuber von adulten Fliegen jedoch nicht. Die höchsten Porteinkonzentrationen wiesen Larven auf, welche nicht-transgene Maisblätter angereichert mit reinem Cry1Ab frassen. Sowohl die Entwicklungszeit wie auch die Fertilität der weiblichen Fliegen wurden bestimmt. Bei *D. melanogaster* erfolgte dies für 4 Generationen, bei *M. scalaris* für 3 Generationen. Es konnten jedoch nur wenige signifikante Unterschiede zwischen transgenen und nicht-transgenen Versuchsserien gefunden werden. Diese waren nicht konsistent und liessen somit keinen relevanten negativen Effekt von *Bt*-Proteinen erkennen. Daraus schlussfolgern wir, dass Larven von

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D. melanogaster und *M. scalaris* durch den Verzehr von Cry1Ab und Cry3Bb1 aus Maisblättern über einen längeren Zeitraum in ihrer Entwicklung nicht negativ beeinflusst werden.

Keywords: *Bacillus thuringiensis*; Transgenic plant; Cry1Ab; Cry3Bb1; Non-target arthropod; *Megaselia scalaris*; *Drosophila melanogaster*; Decomposition

Introduction

For more than 10 years, genetically modified (GM) plants have been cultivated worldwide. Occupying 37.3 million hectares transgenic insect-resistant *Bacillus thuringiensis* (Bt) maize was the second most frequently cropped GM plant in 2008 (James 2008). Different strains of *B. thuringiensis* express different Cry proteins with relatively high target specificity. For example, Cry1 proteins selectively harm herbivorous lepidopteran larvae while Cry3 proteins selectively harm phytophagous Coleoptera, particularly the Western Corn rootworm complex (*Diabrotica* sp., Chrysomelidae).

In addition to controlling target pests, Cry proteins, which are expressed in all plant tissues, could affect non-target organisms and especially those saprophagous organisms that consume plant litter. After harvest, plant residues (i.e., litter: dead or dying leaves, stems, roots, etc.) containing active Cry proteins remain on the soil surface or are incorporated into the soil. In addition to entering the soil as litter, Cry proteins are also released into the soil via root exudates (Saxena, Flores, & Stotzky 2002). Litter bag studies revealed that although Cry1Ab proteins were degraded, small amounts persisted as active toxins and were still detectable after 240 days (Zwahlen, Hillbeck, Guggerli, & Nentwig 2003; Zurbrügg, Hönemann, Meissle, Romeis, & Nentwig 2009). The concentrations in dead plant material are much lower than in fresh leaves but still high enough to harm target species (Wandeler, Bahylova, & Nentwig 2002; Zwahlen, Hillbeck, Guggerli, et al. 2003). In soil, plant litter is ingested by a wide range of invertebrates. If the litter contains *Bt* maize residues, those soil-dwelling invertebrates will also ingest the *Bt* protein. Several studies concluded that *Bt* maize does not negatively affect important soil invertebrates such as mites, collembolans, woodlice, and earthworms (Yu, Berry, & Croft 1997; Sims and Martin 1997; Wandeler et al. 2002; Zwahlen, Hillbeck, Howald, & Nentwig 2003; Icoz & Stotzky 2008), but to the best of our knowledge no study has investigated potentially adverse effects of *Bt* proteins on the reproduction of dipteran larvae. Moreover, no study has analysed fitness effects over several generations.

Dipteran larvae are among the most important decomposers in soil (Speight, Hunter, & Watt 1999). Through their feeding mode, they act mainly as initial decomposers by increasing the surface area of the plant material (Savage 2002). The most abundant saprophagous dipterans in maize fields are members of the Drosophilidae and Phoridae, which can reach densities as high as several thousands of individuals per square metre (Weber & Pescher 1990, 1995; Nielsen & Nielsen 2007). The larvae of these flies are common prey to carabid beetles, spiders, and other polyphagous predators in agroecosystems (Lys 1995). By consuming saprophagous dipterans in *Bt* maize fields, the predators could also be exposed to Cry proteins.

The current study focuses on two species of saprophagous dipterans: *Drosophila melanogaster* (Drosophilidae) and *Megaselia scalaris* (Phoridae). Both occur in various habitats including agricultural fields as polyphagous decomposers but are also frequently used as model species in laboratory experiments. In field studies *M. scalaris* larvae have been collected on a variety of decaying plants including maize (Disney 2008). They have short generation times and are therefore well suited to investigate possible effects of *Bt* proteins on different fitness parameters over multiple generations. As a consequence, it was feasible to examine the effects of *Bt* proteins on all life stages and over many life cycles, in contrast to the vast majority of other risk assessment studies. The aim of this study was to determine whether *Bt* proteins affect the fertility of female flies and the developmental time of the larvae. Furthermore, we determined Cry protein concentrations in various substrates. In particular, we were interested to find out, whether predators of larvae and adult flies would be exposed to different cry protein levels. Based on these data, one can estimate how much protein is transferred to the higher trophic level.

Materials and methods

Plant cultivation

Plants were cultivated in climate chambers with an LD regime 16:8 at 25 and 20 °C, respectively. We used DKC5143Bt (event Mon88017, Monsanto, USA), expressing the Mon88017 Cry3Bb1 protein, and the variety N4640Bt (event Bt11, Syngenta, Switzerland), expressing the Cry1Ab protein, plus the two corresponding non-transgenic isolines, DKC5143 and N4640. The maize was sown in common potting soil (Mioplant, Switzerland) in pots of 30 cm diameter. Each pot contained four maize plants. Initially, 35 g of slow-release fertiliser (14% N, 7% P, 14% K, 1.5% Mg, Hauert, Switzerland) was added to each pot. Once each week, every pot was fertilised with 0.5 L of 0.2% liquid fertiliser (10% N, 10% P, 7.5% K, 1.24% B, Wuxal Universaldünger, Maag