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Ground beetle acquisition of Cry1Ab from plant- and residue-based food webs

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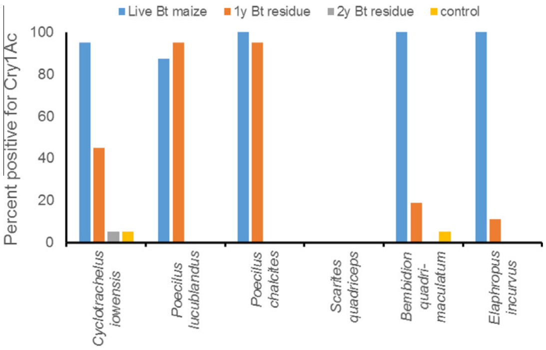
HIGHLIGHTS

- Acquisition of Cry1Ab from live *Bt* maize and *Bt* maize residue in 6 carabids.
- Adult beetles were collected live and analyzed with ELISA.
- Three species participated in both live-plant and residue based food webs.
- Two species appeared to participate only in live-plant based food webs.
- One species did not acquire Cry1Ab and could not be characterized.

GRAPHICAL ABSTRACT



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ABSTRACT

Ground beetles are significant predators in agricultural habitats. While many studies have characterized effects of *Bt* maize on various carabid species, few have examined the potential acquisition of Cry toxins from live plants *versus* plant residue. In this study, we examined how live *Bt* maize and *Bt* maize residue affect acquisition of Cry1Ab in six species. Adult beetles were collected live from fields with either current-year *Bt* maize, one-year-old *Bt* maize residue, two-year-old *Bt* maize residue, or fields without any *Bt* crops or residue for the past two years, and specimens were analyzed using ELISA. Observed Cry1Ab concentrations in the beetles were similar to that reported in previously published studies. Only one specimen of *Cyclotrachelus iowensis* acquired Cry1Ab from two-year-old maize residue. Three species acquired Cry1Ab from fields with either live plants or plant residue (*Cyclotrachelus iowensis, Poecilus lucublandus, Poecilus chalcites*), implying participation in both live-plant and residue-based food webs. Two species acquired toxin from fields with live plants, but not from fields with residue (*Bembidion quadrimaculatum, Elaphropus incurvus*), suggesting participation only in live plant-based food webs. One species did not acquire Cry1Ab from either live-plant or residue (*Scarites quadriceps*), suggesting that its food sources might not contain significant amounts of Cry1Ab. These results revealed significant differentiation among carabid species in their associations with live-plant and residue-based food webs in agricultural fields.

1. Introduction

Transgenic *Bt* (*Bacillus thuringiensis*) crops are grown widely in the USA and several other countries. These crops mainly rely on expression of Cry toxins, which are crystalline proteins from

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B. thuringiensis, to kill target insect pests. There have been many studies investigating potential effects of *Bt*-Cry crops on non-target arthropod species, and most have aimed to measure the effects of the toxins (Andow and Zwahlen, 2006), and have not examined the exposure processes by which they might acquire them (Paula and Andow, 2016).

One of the most important groups of predators in agriculture is carabid beetles (Thiele, 1977; Lövei and Sunderland, 1996). Many carabid species selectively consume soil-associated organisms, such as earthworms, terrestrial gastropods, isopods, nematodes, diplopods, microarthropods (such as Collembola), fungi, seeds, plant matter and arthropod herbivores (e.g., Hengeveld, 1979a,b, 1980; Holopainen and Helenius, 1992; Lövei and Sunderland, 1996; Hartke et al., 1998; Bilde et al., 2000; Symondson et al., 2000). The Cry1Ab protein has been detected in soil-associated non-target organisms in the field (Zwahlen et al., 2003a,b; Harwood et al., 2005, 2006; Zwahlen and Andow, 2005;Harwood and Obrycki, 2006; Zurbrügg and Nentwig, 2009; Arias-Martín et al., 2016). These organisms may acquire the Cry1Ab protein directly from residue for at least 240 days after harvest (Zwahlen et al., 2003a,b), from living maize plants (Harwood et al., 2006), or indirectly through prey that has ingested the *Bt* protein from residues and/or plants. Several authors (Goldschmidt and Toft, 1997; Toft and Bilde, 2002) have investigated the role of carabids as predators associated with live plant-based and residue-based food webs and have suggested that some carabid species may connect the two food webs, while others may not.

While there have been several studies on the effects of *Bt* maize on carabids, they have also focused on effects, without documenting possible routes of acquisition of Cry toxins. Earlier studies focused on laboratory methods for examining toxicity (e.g., Mullin et al., 2005; Duan et al., 2006), and more recent studies focused on estimating changes in activity-density in field (e.g., Farinós et al., 2010; Leslie et al., 2010; Priesnitz et al., 2013). Only Zwahlen and Andow (2005) and Peterson et al. (2009) evaluated potential routes of acquisition of Cry toxins by carabids, focusing on *Bt* maize.

In this paper, we collected carabids exposed in maize fields to Cry1Ab via *Bt* maize residue or live Cry1Ab *Bt* plants and measured Cry1Ab in them to see if they acquired Cry1Ab originating from residues, live plants or both. Many carabids are broadly omnivorous and may acquire Cry1Ab directly from plants or residue or indirectly via prey that themselves had acquired the toxin directly or indirectly from the plants or residue. Carabids may also acquire Cry toxins directed from *B. thuringiensis* bacteria, which have been detected in soils from all over the world (Martin and Travers, 1989). Previous work (Zwahlen and Andow, 2005), however, indicated that either the ELISA antibody used to quantify Cry1Ab from transgenic plants does not detect the Cry proteins from the soil bacterium or that the proteins from *B. thuringiensis* bacteria are not sufficiently abundant to be detected in the soils where our experiments were carried out. Previously, Zwahlen and Andow (2005) found that some carabids acquired Cry1Ab in fields containing only *Bt* maize residue and suggested that these species participated in the residue-based food web. It was unclear, however, whether and to what extent they may also acquire Cry1Ab from live *Bt* maize. Live *Bt* maize has higher Cry1Ab concentrations than maize residue (Zwahlen et al., 2003a,b), and it seems reasonable to hypothesize that beetles could more readily acquire Cry1Ab from the higher concentrations in live *Bt* maize and prey that fed on live plants than from *Bt* residue and prey that fed on residue.

2. Materials and methods

We tested the following four hypotheses: (H1) Beetles acquire Cry1Ab directly or indirectly from live *Bt* maize, (H2) Beetles acquire Cry1Ab directly or indirectly from one-year-old *Bt* maize residue, (H3) Beetles acquire Cry1Ab directly or indirectly from two-year-old *Bt* maize residue, and (H4) Beetles can acquire Cry1Ab by directly consuming residue.

To test the first three hypotheses, carabids were collected from fields with four different cropping histories. Fields were chosen based on their current crop (2005) and the crops that were grown the previous two years (2003 and 2004). Because we found some detectable Cry1Ab in maize residue from two cropping seasons previously, it was important to control for three years of cropping history. The first treatment was live *Bt* maize following a non-*Bt* crop on the field for at least two previous growing seasons (referred to as 'live *Bt* maize' or 'non-*Bt*/non-*Bt*/*Bt*' for the years 2003/2004/2005) and did hence not contain any *Bt* maize residue for at least two consecutive years. Although live *Bt* maize drops some leaves during the growing season, the biomass of all leaves together is usually approximately 10–12% of the weight of the aboveground biomass of the living plant (Pordesimo et al., 2004), and even if a plant sheds several leaves they are unlikely to provide significant quantities of Cry1Ab during the growing season for the decomposer web in live *Bt* maize fields. Thus in these fields, Cry1Ab was available almost entirely from the live *Bt* plant, as its residues were uncommon throughout the collection period.

The second treatment had a non-*Bt* crop in the current year, *Bt* maize planted in the previous year, and a non-*Bt* crop in the year before that ('one-year-old residue' or 'non-*Bt*/*Bt*/non-*Bt*'). The third treatment contained two-year-old *Bt* maize residue and non-*Bt* crops two years in a row after that ('two-year-old residue' or '*Bt*/non-*Bt*/non-*Bt*'. The fourth treatment served as a control and was planted with non-*Bt* crops for at least three consecutive growing seasons ('control' or 'non-*Bt*/non-*Bt*/non-*Bt*'). Care was taken to remove any live volunteer maize from treatments 2–4 prior to the start of the experiment. The first three hypotheses were tested by comparing each of the first three treatments against the control. Thus, if there were a sufficient number of positive samples, the null hypothesis was rejected.

To test the fourth hypothesis, we conducted a no-choice feeding trial to determine if beetles would feed on residue and if Cry1Ab can be acquired directly from feeding on maize residue. Although we believed this to be unlikely, for omnivorous carabids it is a possibility (Toft and Bilde, 2002). This experiment was conducted on the most abundant carabid species in our experimental system, *Cyclotrachelus iowensis* (Freitag).

2.1. Fields

Beetles were collected from ten fields at the University of Minnesota Outreach, Research, and Education Park, Rosemount, Minnesota, USA during 2005. Four fields were non-*Bt* control fields, four fields contained one-year-old *Bt* residue, one field was planted with live *Bt* maize, and one field contained two-year-old *Bt* maize residue. Although the number of fields was not equal for all treatments, this did not influence the analysis since the replicate was the number of carabid samples analyzed. All fields had spring conservation tillage, which leaves ~30% maize residue cover on the soil surface. The non-*Bt* crops were either maize or soybean. Average size of the fields was 18 ha (range: 2.1–37.9 ha). All of the *Bt* maize residues and crops contained Cry1Ab and none of the non-*Bt* residues or crops contained any Cry1Ab, both of which were confirmed using Agdia Cry1Ab/Ac test strips. Consequently, to estimate the concentration of Cry1Ab in maize tissues, four independent samples of stalk and leaf tissue from live plant tissue and residue were collected from different fields, washed to remove adhering particles and ground for quantification by ELISA (Envirologix) as described below. *Bt* maize varieties were DKC44-42 (DeKalb), K4688 (Kaltenberg), and P36N71 (Pioneer).