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

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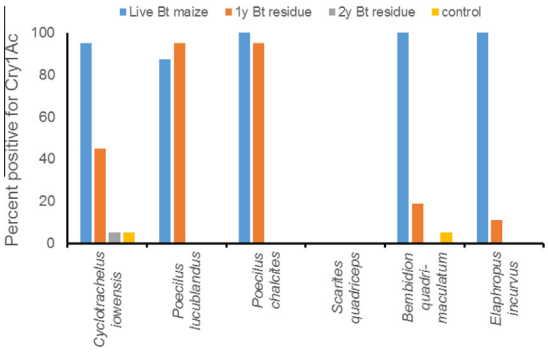
HIGHLIGHTS

- 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).

2.2. Sampling methods

One live inclusion barrier trap was placed in each field (>15 m from field edge). The field adjacent to the trap edge was not maize and had no maize residue. The trap consisted of a 1 m diameter circle of 12.7 cm high plastic edging with the exterior soil surface even with the top of the edging, allowing beetle ingress, and the interior soil surface several centimeters below the top of the edging, preventing beetle egress. The soil inside the traps was not disturbed. Three wood boards (0.3 m × 0.3 m) were placed on the soil surface inside each trap to provide shelter for beetles. Each of the traps contained live plants and crop residue, which allowed the beetles to continuously search for prey even while being inside the trap. The traps were open for 24 h prior to sampling. Adult carabids were caught live *in situ* and immediately placed on dry ice, which ensured that they did not empty their gut. They were stored at −80 °C until they were analyzed using ELISA. Beetles were collected on seven dates in June (22nd, 28th), July (19th, 27th), August (5th, 12th), and September (1st) 2005. Between collection periods, a soil ramp was installed on the interior of the traps to allow organisms to escape. Two traps were 75 m from the nearest field with any maize or maize residue, and most were >100 m from the nearest such field.

2.3. Laboratory feeding trial with Cyclotrachelus iowensis (H4)

A laboratory feeding trial was carried out with adult *C. iowensis*, the most common species trapped in maize fields of this region. This species also contained 34.7 ± 14.6 ng Cry1Ab g^{-1} when found in fields containing *Bt* maize residue (Zwahlen and Andow, 2005). Live specimens were collected from control fields as described above and held individually at the laboratory in a climate chamber at 25 °C and constant humidity for four weeks. They were stored and tested in Petri dishes (100x25 mm) containing a 3 mm layer of moistened plaster of Paris and a source of water. Beetles were fed one laboratory-reared last instar European corn borer (ECB) larva every 48 h until 48 h before the trial began. During one week, sixteen beetles were provided with approximately 1 g of field-collected *Bt* maize residue, and 16 control beetles were fed with approximately 1 g of field-collected non-*Bt* maize residue. The material was provided at the beginning of the experiment and remained in the Petri dishes until the end of the experiment. The residue was kept moist during the feeding trial to encourage consumption. As a positive control, four beetles were fed with one ECB larva every 48 h that had been dipped in a solution of Cry1Ab toxin shortly before using them for the trial. After the experiment, beetles were frozen and stored at −80 °C until they were analyzed using ELISA. Before the experiment, portions of the residue were analyzed using ELISA to determine the Cry1Ab concentration.

2.4. Enzyme-linked immunosorbent assay (ELISA)

The Cry1Ab concentration in beetles, maize residue (stalks and husk leaves), and live maize tissue was determined using an enzyme-linked immunosorbent assay (ELISA) (Howald et al., 2003; Zwahlen et al., 2003a,b; Zwahlen and Andow, 2005). The plant material was collected on 28 June 2005. We analyzed 320 samples of the six carabid species that were found the most frequently in at least two of the treatments, which were *C. iowensis*, *Poecilus lucublandus* (Say), *Poecilus chalcites* (Say), *Scarites quadriceps* Chaudoir, *Bembidion quadrimaculatum* (L.), and *Elaphropus incurvus* (Say). These six species accounted for approximately 80% of all individuals found in pitfall traps in maize fields in this region and included both diurnal (e.g., *B. quadrimaculatum*) and nocturnal species (e.g., *C. iowensis*). Each sample consisted of one individual except for the small *B. quadrimaculatum* and *E. incurvus*, which required 4–5 and 10–15 individuals per sample, respectively. Sample sizes ranged from 25 to 80 samples per species and 2–39 samples per field (average: 22 samples species^{-1} field^{-1}). All carabids were washed thoroughly with deionized water and examined microscopically to remove soil and other particles from the body surface that could potentially influence the ELISA. Beetles were dried at room temperature for one hour and weighed. Each sample was homogenized in 10 μL extraction buffer (see Gugerli, 1986) per mg sample weight for large species (*S. quadriceps, C. iowensis, P. lucublandus*), 15 μL extraction buffer per mg sample weight for the medium-sized species (*P. chalcites*), and 20 μL extraction buffer per mg sample weight for small species (*B. quadrimaculatum*, *E. incurvus*). This method ensured a low detection threshold and the same likelihood of samples from the same species testing positive. Although a higher dilution of smaller species may have decreased the probability of Cry1Ab detection in comparison to larger species, this allowed us to have enough fluid to analyze the smaller carabid species. Using whole-body homogenates instead of gut contents provides the Cry1Ab concentration within the entire beetle, regardless of whether it was in the gut or in any other body tissue. Supernatants of the centrifuged samples were used and each sample was divided into two subsamples. Calibration curves and detection levels for the quantitative analysis were carried out exactly as described by Zwahlen and Andow (2005) following the manufacturer's instructions (Envirologix).

2.4.1. Quantitative analysis

Optical density values (ODs) were log-transformed and a non-linear regression was carried out to calculate the calibration curve for Cry1Ab concentrations for each plate. The equation followed first-order Michaelis-Menten kinetics:

$$\log_{10} Y = B + (T - B)/(1 + \mathrm{EC50}/X),$$

where Y is the optical density, OD, B the estimated bottom asymptote of the curve (limit of detection), T the estimated top asymptote of the curve, EC50 the estimated response halfway between the top and bottom, and X the Cry1Ab concentration.

2.4.2. Detection level (DL)

The threshold value of detectable Cry1Ab was defined as

$$DL_{\mathrm{LogOD}} = B + 3 * \mathrm{SE}_B,$$

where SE is the estimated standard error of B. This is approximately the upper 99% confidence interval of B. The threshold value was calculated separately for each immunoassay plate. This allowed species-specific and ELISA plate-specific evaluation. Beetle samples were considered as positive when both subsamples were above the threshold and as negative when at least one of the subsamples was below the threshold.

2.5. Statistical analyses

Hypotheses 1–3 were tested using a loglinear (g^2) contingency table analysis or Fisher's exact test for small samples. Additionally, we carried out an ANOVA with Cry1Ab concentration (ng sample^{-1}) as dependent variable and species, field type (live *Bt* maize vs. 1-year-old *Bt* residue), and sample weight as independent variables (R version 2.5.1., 2007). The concentration was log-transformed to meet the model assumption of equal variance. Since we found that field type had a significant effect on the Cry1Ab concentration, we additionally carried out ANOVAs with the two field types separately. We did linear regressions on the relationship between sample weight and the log-transformed Cry1Ab concentration (ng.sample^{-1}) for each field type. A linear relation would suggest that larger beetles acquired proportionally

more Cry1Ab than smaller beetles. Hypothesis 4 was tested using a χ^2 test comparing the *Bt* and non-*Bt* residue treatments. Due to low sample sizes for *S. quadriceps* in certain field treatments, only the second hypothesis was tested for *S. quadriceps*.

3. Results

Across all of the collections, the amount of Cry1Ac per sample was related logarithmically with the size of the beetle (Fig 1), implying that larger beetles acquired proportionately more Cry1Ab than smaller beetles. This indicates that when beetles acquire Cry1Ab, it may be detected more readily in larger beetles than smaller ones.

From the control fields containing no live *Bt* plants or *Bt* residue, one individual *C. iowensis* and one sample of *B. quadrimaculatum* had detectable Cry1Ab (Table 1). These two samples had lower concentrations of Cry1Ab compared to other positive samples, ranging from 3.8 to 5.5 ng Cry1Ab g^{-1} beetle sample. These might have been false positives, which considering all of the control samples, would give a false positive rate of <2%. Both samples were collected from traps that were 300 or 550 m away from a field with live *Bt* maize or known *Bt* maize residue, which makes it possible that the beetles or their prey originated from a *Bt* maize field and moved to the trap locations.

Samples from all species found in fields containing live *Bt* maize and samples of five out of six species found in fields containing one-year-old *Bt* residue tested positive for the Cry1Ab (Table 1). The proportion of positive samples and Cry1Ab concentrations were highly variable across treatments and species. Concentrations in positive beetles from these two field types ranged from 8.3 to 35.1 ng Cry1Ab g^{-1} sample.

From fields with two-year-old *Bt* residue, only one individual of *C. iowensis* contained Cry1Ab at a relatively low concentration of 5.1 ng Cry1Ab g^{-1} beetle. This were collected from a trap about 75 m from the nearest field with any history of Cry1Ab, and this field had one-year-old *Bt* maize residue. While Cry1Ab concentration in residue from fields with one-year-old *Bt* residue measured 12.8 ng g^{-1} husk leaf and 38.1 ng g^{-1} stalk, two-year-old residue had a lower concentration, measuring 5.4 ng g^{-1} husk leaf and 17.7 ng Cry1Ab g^{-1} stalk. Although this beetle might be a true positive, it is also possible that it was not.

*Hypothesis 1 (Live plants: non-*Bt/*non-*Bt/Bt *versus control).* Cry1Ab was present in a higher proportion of samples for all species tested (*C. iowensis*, *P. lucublandus*, *P. chalcites*, *B. quadrimaculatum,* and *E. incurvus*) from live *Bt* maize fields than control fields, suggesting that these species were participating in live plant-based food webs and that they acquired Cry1Ab directly and/or indirectly from live maize.

*Hypothesis 2 (1-yr residue: non-*Bt/Bt/*non-*Bt *versus control).* Cry1Ab was present in a significantly higher proportion of individuals of *C. iowensis, P. lucublandus,* and *P. chalcites* from fields with one-year-old *Bt* maize residue than those from control fields. These results indicate that these species were probably participating in a residue-based food web and that they acquired Cry1Ab directly and/or indirectly from the plant residue.

The proportion of samples containing Cry1Ab was not significantly different for *B. quadrimaculatum, E. incurvus,* and *S. quadriceps* from one-year-old *Bt* residue compared to those from control fields, indicating that they either did not participate strongly in residue-based food webs, or their food sources did not contain Cry1Ab. Only a small proportion of *B. quadrimaculatum* and *E. incurvus* from one-year-old residue contained Cry1Ab. In contrast to all the other species, none of the *S. quadriceps* had any Cry1Ab in one-year-old *Bt* residue fields.

Hypothesis 3 (2-yr residue: Bt/*non-*Bt/*non-*Bt *versus control).* Cry1Ab was not detected in carabids from fields with two-year-old *Bt* residue more frequently than from control fields. Although two-year-old stalk residue still contained approximately 50% of the Cry1Ab found in one-year-old *Bt* stalk residue, there was much less *Bt* maize residue remaining on and near the soil surface than in fields with one-year-old residue.

Hypothesis 4 (Direct acquisition from residue). None of the *C. iowensis* adults that were offered either *Bt* or non-*Bt* maize residue contained any Cry1Ab (Fisher's exact test, $p = 1.0$). In contrast, Cry1Ab was detected in all of the beetles that were given ECB larvae dipped in a solution of Cry1Ab (21.2 ± 3.8 ng g^{-1} beetle). Before the trial took place, it was observed that the beetles consumed about one 4th- or early 5th-instar ECB larva per day. Because the trial took place over several of these observed feeding periods, the beetles would be expected to consume considerable food, so the results suggest that under laboratory conditions the beetles did not ingest Cry1Ab by consuming residue.

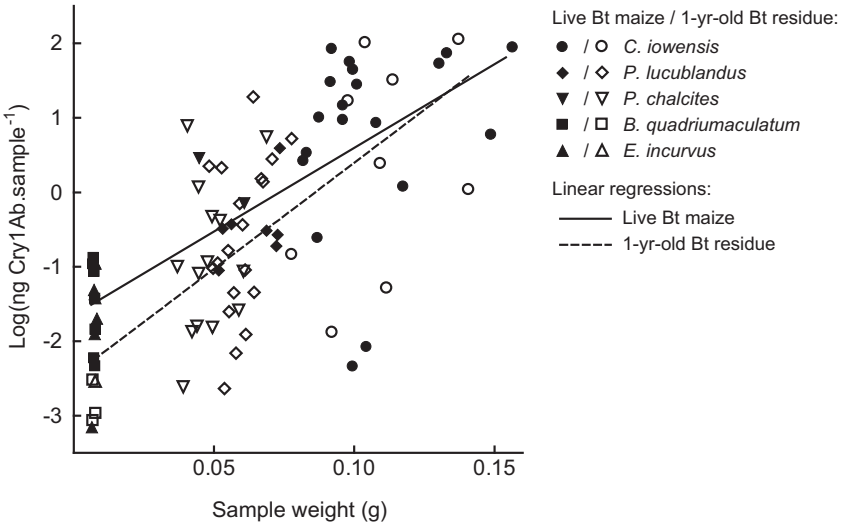

Fig. 1. Sample weight of beetles from live *Bt* maize fields and 1-year-old *Bt* residue plotted against Cry1Ab sample concentration. Lines show the linear regression of samples from live *Bt* maize fields and from 1-year-old *Bt* residue fields. *P*-values for both regressions were <0.0001.

Table 1
ELISA results of adult carabids found in four different field types (treatments 1–4), with mean ± SE Cry1Ab concentration for positive samples, and the number of positive samples of the total samples tested (*n*). Beetle samples were individuals, except *B. quadrimaculatum* and *E. incurvus*, where the total number of individuals is in parentheses. *P*-values for log-linear contingency table analyses or Fisher's exact test (*df* = 1 for all tests) are given for hypothesis H1 (treatment 1 vs. 4), H2 (treatment 2 vs. 4), and H3 (treatment 3 vs. 4).

Species analyzed	Treatments								Hypotheses		
	1: non-*Bt*/non-*Bt*/*Bt*[1]		2: non-*Bt*/*Bt*/non-*Bt*[2]		3: *Bt*/non-*Bt*/non-*Bt*[3]		4: non-*Bt*/non-*Bt*/non-*Bt*[4]		H1	H2	H3
	ng Cry 1Ab g^{-1}	*n*	ng Cry 1Ab g^{-1}	*n*	ng Cry 1Ab g^{-1}	*n*	ng Cry 1Ab g^{-1}	*n*	*P*	*P*	*P*
Maize leaves		4/4	12.8±	4/4	5.4	1/4	–	0/4			
Maize stalks		4/4	38.1 ± 15.8	4/4	17.7	1/4	–	0/4			
Cyclotrachelus iowensis	31.5 ± 4.7	19/20	26.0 ± 8.6	9/20	5.1	1/20	3.8	1/20	<0.0001	0.0020	1
Poecilus lucublandus	11.1 ± 2.4	7/8	14.1 ± 3.1	19/20	–	0/12	–	0/20	<0.0001	<0.0001	1
Poecilus chalcites	24.8 ± 10.6	2/2	13.6 ± 4.2	19/20	–	0/6	–	0/20	0.0003	<0.0001	1
Scarites quadriceps	–	–	–	0/8	–	–	–	0/17	–	1	–
Bembidion quadrimaculatum	35.1 ± 7.2	7/7 (30)	8.3 ± 1.7	3/16 (72)	–	0/2 (7)	5.5	1/20 (92)	<0.0001	0.19	0.66
Elaphropus incurvus	31.5 ± 5.3	8/8 (90)	10.4	1/9 (114)	–	0/1 (15)	–	0/8 (91)	<0.0001	0.25	1

[1] non-*Bt*/non-*Bt*/*Bt* = 2003 and 2004 non-*Bt* residue, 2005 *Bt* live maize.
[2] non-*Bt*/*Bt*/non-*Bt* = 2003 non-*Bt* residue, 2004 *Bt* residue, 2005 non-*Bt* live crop.
[3] *Bt*/non-*Bt*/non-*Bt* = 2003 *Bt* residue, 2004 non-*Bt* residue, 2005 non-*Bt* live crop.
[4] non-*Bt*/non-*Bt*/non-*Bt* = 2003 and 2004 non-*Bt* residue, 2005 non-*Bt* live crop.

4. Discussion

Although the study fields were mostly >100 m from the nearest field with a history of *Bt* maize cultivation during the study period (2003–2005), many carabid species are quite mobile and may disperse into the study fields from neighboring fields. Thus, it is likely that when the proportion of positive samples was very low, such as in the control for *C. iowensis* (1/20) and *B. quadrimaculatum* (1/20), and in the 2-year-old *Bt* residue for *C. iowensis* (1/20), the positive samples may be false positives resulting from immigrants from other fields. With 105 beetle samples from the control this implies a false positive rate of 2%. Using this rate, we can calculate the probability that the observed positives in the other species and treatments are due to false positives. For example, 1/20 *C. iowensis* were positive in the 2-year-old residue fields. This specimen is a false positive with probability 0.272. Similarly, the 1/9 *E. incurvus* positives in the 1-year-old *Bt* residue fields could be a false positive with probability 0.153. However, the 3/16 *B. quadrimaculatum* in 1-year-old *Bt* residue could be false positives with probability 0.0034 and even the 2/2 *P. chalcites* in the live *Bt* maize fields could false positives with probability 0.0004. Consequently, these and all of the other results are unlikely to be due to false positives derived from the immigration of beetles from other fields.

Most ecological studies on *Bt* crops have focused on assessing ecological effects, but similar to Zwahlen and Andow (2005) and Peterson et al. (2009), we focused on the exposure processes by which carabids may acquire Cry toxin from the environment. Despite the detection of Cry1Ab in 2-year old residue, none of the beetles conclusively acquired Cry1Ab toxin from this residue. All beetles except *S. quadriceps* acquired Cry1Ab toxin in fields with either live *Bt* plants or 1-year old *Bt* maize residue. The concentrations detected were similar to those reported by Peterson et al. (2009) and Zwahlen and Andow (2005). From these results, we found three different beetle responses to the residue and plant treatments.

Group 1 species were *C. iowensis*, *P. lucublandus* and *P. chalcites*, which participated in both the live *Bt* maize and *Bt* residue food webs. As the measured Cry1Ab concentrations in these species were similar in both fields types despite the higher environmental availability in the *Bt* maize field, it is possible that these species were more active in the residue based food web than the live-plant based food web. Our laboratory experiment indicated that *C. iowensis* did not acquire Cry1Ab directly from *Bt* maize residue, so it probably acquired the toxin from prey. Zwahlen and Andow (2005) found only 3/7 *C. iowensis* and Peterson et al. (2009) found that only 1/5 *C. sodalis*, a congener of *C. iowensis*, acquired Cry1Ab in live *Bt* maize fields, but it is not clear if these differences from our present results are real or related to the small sample sizes in the previous studies. Peterson et al. (2009) did not find any Cry1Ab in *P. lucublandis* collected in a *Bt* maize field, but they tested only four individuals. Zwahlen and Andow (2005) found about 50% of *P. lucublandus* and *P. chalcites* (10/20 and 7/17) acquired Cry1Ab from live *Bt* maize fields, which was lower than observed here.

Group 2 species were *B. quadrimaculatum*, and *E. incurvus*, which appeared to participate only in the live *Bt* maize food webs. Perhaps these species fed directly on the living plants, or fed on prey that readily acquired toxin from live plants, but did not acquire toxin originating from residue.

Group 3 consisted only of the large *S. quadriceps*, which was not found in live *Bt* maize fields and did not acquire Cry1Ab from the *Bt* residue fields. This parallels the results of Harwood et al. (2006) and Peterson et al. (2009) who did not find Cry toxin acquisition in the related *S. subterraneus*. The absence of Cry1Ab in *S. quadriceps* could have been due to the lack or low level of Cry1Ab in their food source, or that they feed differently from the other carabids in this study. *Scarites* species may be strictly predaceous (McNabb et al., 2001; Toft and Bilde, 2002), and may be a top predator in these invertebrate food webs. As a top predator, the Cry1Ab concentration in its prey may be too dilute to detect, as in some cases, concentration declines up the food web (García et al. (2010), but see Paula and Andow (2016)). Using stable isotope analysis, Wise et al. (2006) suggested that *Scarites* sp. shifted from a live plant-based food webs toward a residue-based food web when the abundance of micro-detritivores increased, which indicates a sensitivity to changing prey abundance that is often characteristic of a top predator.

Our results revealed significant differentiation among carabid species in their associations with live-plant and residue-based food webs in agricultural fields. For some species (Group 1), both the live-plant and the residue-based food webs may have been important food sources, whereas for other species (Group 2), the live plant-based food web might be predominant, or they (Group 3; *S. quadriceps*) might feed on such a high trophic level that their prey does not contain detectable concentrations of Cry1Ab.

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