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Quantification of Bt-endotoxin exposure pathways in carabid food webs across multiple transgenic events

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Abstract

Despite the reported specificity of *Bacillus thuringiensis* proteins against target pests, a number of studies have indicated that the uptake of Bt-endotoxins from bioengineered crops could have negative effects on natural enemies. It is therefore essential to quantify exposure pathways in non-target arthropod food webs across multiple transgenic events. Adult ground beetles (Coleoptera: Carabidae) were collected from transgenic corn fields expressing lepidopteran-specific Cry1Ab, coleopteran-specific Cry3Bb1, and both Cry1Ab and Cry3Bb1 (stacked event), as well as a non-transgenic isoline. Carabid gut-contents were screened for Cry1Ab Bt-endotoxin using enzyme-linked immunosorbent assay. Significant numbers of carabids tested positive for Cry1Ab from the lepidopteran-specific field: *Harpalus pensylvanicus* (39%, 25 of 64), *Stenolophus comma* (4%, 6 of 136), *Cratacanthus dubius* (50%, 1 of 2), *Clivina bipustulata* (50%, 1 of 2), and *Cyclotrachelus sodalis* (20%, 1 of 5). The highest proportion of Bt-endotoxin uptake was 4–6 weeks postanthesis. Only one species, *H. pensylvanicus* (5%, 4 of 75), screened positive for Cry1Ab from the stacked line, despite similar expression of this endotoxin in plant tissue harvested from both lines. This difference in Cry1Ab uptake could be due to changes in the non-target food web or differential rates of Bt-endotoxin decay between genetic events. This study has quantified the differential uptake of Cry1Ab Bt-endotoxin by the carabid community across multiple transgenic events, thus forming the framework for future risk-assessment of transgenic crops.

Keywords: *Bacillus thuringiensis*, non-target effects, Carabidae, transgenic crops, risk-assessment, ELISA

Introduction

Transgenic *Bacillus thuringiensis* (Bt) Berliner (Bacillales: Bacillaceae) corn is genetically engineered to express insecticidal Cry1Ab and Cry3Bb1 proteins. Respectively, these Bt-endotoxins suppress populations of lepidopterous (*Ostrinia nubilalis* Hübner and *Diatraea grandiosella* Dyar (Lepidoptera: Pyralidae)) and, more recently, coleopterous (*Diabrotica* spp. (Coleoptera: Chrysomelidae)) pests. The insecticidal Bt-endotoxins expressed in these crops are coded by *Cry* genes from *B. thuringiensis*, a naturally occurring soil-dwelling bacterium. The planting of transgenic Bt-corn worldwide has increased dramatically since its commercial release in the mid- 1990s (Cannon 2000; Shelton, Zhao, and Roush 2002; Lawrence 2005; James 2006); in the United States it has increased from approximately 1% of corn planted in 1996 to 57% in 2008 (USDA National Agricultural Statistics Service 2008).

Despite the reported specificity of transgenic insecticidal crops (Shelton et al. 2002; Marvier et al. 2007), there is concern associated with the detrimental effect they may have on abundance, diversity, and/or fecundity of some components of the non-target food web (Wolfenbarger and Phifer 2000; Hilbeck 2001; Obrycki, Losey, Taylor, and Jesse 2001; Groot and Dicke 2002; Obrycki, Ruberson, and Losey, 2004; Lövei and Arpaia 2005). Furthermore, O'Callaghan, Glare, Burgess, and Malone (2005) have suggested that the uptake of Bt-endotoxins could negatively affect natural enemies of corn pests. Carabid beetles are important ground-dwelling predators in many agroecosystems (Kromp 1999) and their polyphagous habits allow them to subsist on non-pest prey during periods of pest scarcity (Symondson, Sunderland, and Greenstone 2002). Generalist predators are therefore particularly beneficial in population suppression early in the season during the pests' initial colonization (Settle et al. 1996). Very few carabid species have feeding habits that are limited to one food type or one prey taxon, and may consume the eggs and larvae of Diptera, Coleoptera and Lepidoptera, scavenge on dead invertebrates and/or consume plant material (Holland 2002). Because of their complex food webs and ground-dwelling habits, carabids may be exposed to transgenic Bt-endotoxins through multiple pathways including, but not limited to, root exudates and plant biomass contaminating the soil (Saxena, Flores, and Stotzky 2002), soil-dwelling prey and their carabid predators/scavengers, ingestion of plant material or consumption of Bt-containing herbivores, and pollen feeding or consumption of pollen-dusted material. Bt-endotoxins may persist in the soil for up to 140 days (Koskella and Stotzky 1997), where they can bind to humic acids, organic supplements, or soil particles, protecting the toxins from degradation by microbes and extending the persistence of insecticidal activity in the soil (Glare and O'Callaghan 2000). The presence of Bt-endotoxins in the soil or consumption of Bt-containing vegetation can lead to exposure of earthworms (Zwahlen, Hilbeck, Howald, and Nentwig 2003) and slugs (Harwood

and Obrycki 2006), important components of detrital food webs and major prey items of many carabid species (Symondson, Glen, Erickson, Liddell, and Langdon 2000; McKemey, Symondson, and Glen 2003).

There have been remarkably few studies that accurately quantify the movement of transgenic Bt-endotoxins through arthropod food webs (Harwood, Samson, and Obrycki 2006; Wei, Schuler, Clark, Stewart, and Poppy 2008). Additionally, Sears et al. (2001) pointed out that some risk-assessment studies used unspecified or unrealistically high Bt-endotoxin concentrations, which do not accurately approximate exposure rates in the field. Further study of non-target uptake of Bt-endotoxins is critical to preventing these types of errors in risk-assessment. Molecular techniques, such as enzyme-linked immunosorbent assays (ELISA), allow for the quantification of Bt-endotoxins from field-collected arthropods and can provide clear evidence for their uptake by non-target herbivores and arthropod predators, including Coleoptera (Harwood, Wallin, and Obrycki 2005; Zwahlen and Andow 2005; Obrist, Dutton, Albajes, and Bigler 2006; Harwood, Samson, and Obrycki 2007), Lepidoptera (Torres and Ruberson 2008), Acari (Obrist et al. 2006; Torres and Ruberson 2008), Thysanoptera (Torres and Ruberson 2008), Araneae (Harwood et al. 2005), Hemiptera (Harwood et al. 2005; Obrist et al. 2006; Torres and Ruberson 2008), and Neuroptera (Obrist et al. 2006; Wei et al. 2008). Thus, there is a pressing need to understand levels of connectedness between natural enemies and their prey in transgenic agroecosystems. The identification of Bt-endotoxin movement in the food web forms a critical component of risk-assessment protocols for transgenic crops. This is particularly important as the focus of transgenic development turns towards multiple genetic transformation events, which target a suite of pest species (Johnson 2007).

This study identifies the uptake of Cry1Ab Bt-endotoxin by non-target carabid beetles in the field, in order to test the hypothesis that uptake of lepidopteran-specific Bt-endotoxin will occur at similar rates across single gene and stacked gene transgenic corn fields. We have focused on the lepidopteran-specific Cry1Ab Bt-protein, as it has (somewhat counter-intuitively) been shown to have a more significant impact on nontarget beetles (Coleoptera: Coccinellidae) than the coleopteran-specific Cry3Bb1 (Schmidt, Braun, Whitehouse, and Hilbeck 2009). Given the diverse feeding habits of carabid beetles, ranging from entirely phytophagous seed-feeders to predominantly predatory specialists of Lepidoptera and Mollusca (Thiele 1977), it is predicted that carabid species will be exposed to Bt-endotoxins at differential rates due to their variability in feeding ecology, thus resulting in variation in the proportion screening positive for Cry1Ab Bt-endotoxin. This study specifically examines the uptake of Bt-endotoxins across multiple transgenic events, including a stacked gene variety, thus providing a critical framework for risk-assessment of transgenic crops and helping to identify potential exposure pathways and fate of Bt-endotoxin in the field.

Methods

Field description and transgenic events

Four 2500-m^2 fields (50×50 m) of corn were planted on 17 May 2007, at the University of Kentucky Spindletop Research Station, Lexington, KY, USA in fields that had not been planted with Bt-crops in recent years and maintained under standard agronomic practices for Kentucky with no insecticide applications. Herbicides (Lexar®-Syngenta Crop Protection, Greensboro, NC, USA; Roundup®-Monsanto Company, St. Louis, MO, USA) were applied to all fields on 20 May 2007, followed by ammonium nitrate fertilization on 30 May 2007 (approximately 300 kg/ha). The corn varieties planted were YieldGard Corn Borer™ (Bt-hybrid 4842S) (GPS coordinates at center of field: 38°07.555 N, 84°30.901 W), which expresses lepidopteran-specific Cry1Ab protein, YieldGard Rootworm™ (Bt-hybrid 4843X) (38°07.667 N, 84°30.636 W), which expresses coleopteran-specific Cry3Bb1 protein, YieldGard Plus™ (Bt-hybrid 4846T) (38°07.703 N, 84°30.440 W), which expresses both Cry1Ab and Cry3Bb1, and a non-transgenic isoline (isoline 4847) (38°08.141 N, 84°30.206 W) (Monsanto Company). Distances between fields ranged from 150 to 800 m and non-Bt crops, including soybean, alfalfa, and sweet pepper, surrounded the corn.

Carabid collection

Twenty wooden board refuge traps (size 25×46 cm, 2.5 cm thick) were aligned in transects between rows of corn (five refuge traps spaced 8 m apart in four rows 4 m apart) in each field. Adult ground beetles (Coleoptera: Carabidae) were collected weekly from each refuge trap in all fields between 4 June and 30 September 2007. Carabids were collected by hand and stored in 7 or 30 mL (depending on specimen size) Sterilin® plastic containers (Dynalab Corporation, Rochester, NY, USA). The insects were frozen immediately in a portable Engel MT15 freezer (Engel, Jupiter, FL, USA) and subsequently transferred to a –20°C freezer until sample preparation for ELISA screening (below).

Quantification of Cry1Ab concentrations in plant tissue

Corn tissue samples (n = 10), mean weight = 10.02 ± SE 0.02 mg, were taken from each of the four corn varieties (YieldGard Corn Borer™, YieldGard Rootworm™, YieldGard Plus™, and a non-transgenic isoline) at seven tissue locations: topmost leaf, basal leaf, stem, nodal root, seminal root (from 8-week-old plants, growth stage V5); seedling leaf, seedling root (from 7-day-old plants, growth stage V1).

ELISA screening protocols

Sample preparation: carabid beetles

Protocols for the detection of Cry1Ab in non-target invertebrates followed those described in detail by Harwood et al. (2005). In summary, beetles were thawed at room temperature and the foregut removed by carefully separating the thorax and abdomen and extracting the crop with sterile forceps. Where size prohibited gut extraction (*Stenolophus comma* (Fabricius), *Agonum* spp., *Clivina bipustulata* Fabricius, and *Poecilus lucublandus* (Say)), whole-body homogenates were prepared. The foregut or body was weighed, placed in a 1.5-mL microcentrifuge tube, and diluted 1:100 (mg mL^{-1}) in Abraxis LLC Extraction/Dilution Buffer. The sample solution was then homogenized using a disposable polypropylene Kontes™ Pellet Pestle™ (Fisher Scientific Company LLC, Pittsburgh, PA, USA), mixed on a vortex for 10 s and centrifuged at 5000×*g* for 5 min. The resulting supernatant was removed to a clean microcentrifuge tube and added into antibody-coated ELISA plate wells (see below).

Sample preparation: corn tissue

Preparation of corn tissue followed manufacturer's guidelines for plant tissue screening with the Abraxis Bt Cry1Ab/Cry1Ac Microtiter Plate Kit (Abraxis LLC, Warminster, PA, USA). Fresh plant tissue was weighed, placed in a 1.5 mL microcentrifuge tube, and homogenized using a disposable polypropylene Kontes™ Pellet Pestle™. The corn tissue was then diluted 1:200 (mg mL^{-1}) or 1:50 (mg mL^{-1}) (stem only) in Abraxis LLC Extraction/Dilution Buffer and homogenized as above. The particulate matter was separated and the supernatant removed into clean microcentrifuge tubes prior to ELISA analysis.

Negative controls

Harpalus pensylvanicus DeGeer (Coleoptera: Carabidae) were collected by dry pitfall trapping from fields of non-transgenic corn and maintained in the laboratory on a diet of *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) at 21°C on a 16 h L:8 h D cycle. In addition, non-transgenic corn tissue was grown in the greenhouse (22 ± 2°C, 16 h L:8 h D cycle) for corn tissue negative controls. Sample preparation for these negative controls followed protocols described above.

ELISA screening

All samples were screened for Cry1Ab Bt-endotoxin by double antibody sandwich ELISA using an Abraxis Bt Cry1Ab/Cry1Ac Microtiter Plate Kit. The sample supernatants were coated into two ELISA plate wells, at 100 µL per well. On each plate, calibrators of known Cry1Ab concentration (0.00, 0.25, 0.50, 1.00, 2.00, and 4.00 ng mL^{-1}) were loaded, at 100 µL per well, into two wells per calibrator. Positive (1.5 ng mL^{-1} Cry1Ab concentration) and negative (0 ng mL^{-1} Cry1Ab concentration) controls were also loaded into 8 wells, at 100 µL per well. The ELISA plates were carefully rotated in a circular motion for 30 s to ensure mixing of samples within wells and the plate covered with an acetate sheet. The solutions were incubated at room temperature for 30 min, ejected and all wells washed three times with Abraxis wash solution. To each well, 100 µL Abraxis Cry1Ab/Ac endotoxin specific rabbit polyclonal antiserum was added and plates rotated as above. After 30 min incubation at room temperature, the wells were ejected, washed as above and 100 µL horseradish peroxidase-labelled goat anti-rabbit enzyme conjugate (100× dilution) was added, mixed as above and incubated for a further 30 min. The wells were ejected and washed once more before adding 100 µL color solution (3,3′, 5,5′-tetramethyl benzidine in an organic base) to each well, and incubated for 20 min. After incubation, 50 µL of dilute acid stopping solution was added to each well and the absorbance recorded at 450 nm using a Thermo Labsystems Multiskan Plus® spectrophotometer (Fisher Scientific Company LLC).

Calculation of Cry1Ab Bt-endotoxin concentrations

For each plate, the absorbance values of the calibrators of known Bt-endotoxin concentration were calculated and a linear regression fitted to these values. The regression equation was used to extrapolate Cry1Ab endotoxin concentrations in carabid and corn tissue samples using the OD_{450} value for each sample. A positive threshold for each ELISA plate was determined by the mean absorbance of the eight negative control samples plus three standard deviations.

Statistical analysis

Prior to analysis using SAS® statistical software (SAS® Institute Inc., Cary, NC, USA), all Bt-endotoxin concentrations were log10 transformed. The proportions of *H. pensylvanicus* and *S. comma* screening positive for Cry1Ab from the lepidopteran-specific field were compared using a χ^2 analysis and the concentrations of Cry1Ab were compared using a two-sample *t*-test. A χ^2 analysis was also used to compare proportions of *H. pensylvanicus* screening

positive from the lepidopteran-specific and stacked fields. In order to temporally compare the proportions of beetles screening positive, the growing season was split into four time periods based on corn phenology (after Harwood et al. 2007): pre-anthesis (4 June–8 July), anthesis (9 July–23 July), post-anthesis (24 July–28 August), and late season (29 August–30 September). The frequencies of *H. pensylvanicus* testing positive for Cry1Ab from the lepidopteran-specific field during the four phenological periods were compared with a χ^2 analysis. The Bt-endotoxin concentrations from the lepidopteran-specific and stacked genetic events were compared using a two sample *t*-test for each tissue location. An ANOVA was used to compare Cry1Ab concentrations for lepidopteran-specific and stacked events among plant tissues.

Results

Carabid collection

In 2007, 806 adult carabids belonging to 11 species were collected: *Stenolophus comma* (n = 359), *Harpalus pensylvanicus* (n = 331), *Agonum* spp. (n = 50), *Scarites subterraneus* Fabricius (n = 37), *Cyclotrachelus sodalis* (LeConte) (n = 11), *Cratacanthus dubius* (Beauvois) (n = 5), *Poecilus lucublandus* (n = 4), *Chlaenius tricolor* Dejean (n = 3), *Harpalus caliginosus* (Fabricius) (n = 2), *Clivina bipustulata* (n = 2) and *Anisodactylus sanctaecrucis* (Fabricius) (n = 2). Carabid species were classified as demonstrating significant herbivory in their dietary breadth: *S. comma*, *H. pensylvanicus*, *Agonum* spp., *H. caliginosus*, *C. bipustulata*, and *A. sanctaecrucis*; or demonstrating little to no herbivory: *S. subterraneus*, *C. sodalis*, *C. dubius*, *P. lucublandus*, and *C. tricolor* (Allen 1979; Kegel 1994; Larochelle and Larivière 2003).

Bt-endotoxin uptake by carabid beetles

Five species of carabid beetles collected from the lepidopteran-specific field screened positive for Cry1Ab Bt-endotoxins, but there was no evidence for uptake in six other species (Figure 1). Concentrations as high as 0.1349 µg g^{-1} Cry1Ab Bt-endotoxin were found in beetles screening positive, although mean concentrations in species collected in greater numbers were considerably lower (Figure 2). Interestingly, there was no significant difference between the concentration of Bt-endotoxin in *H. pensylvanicus* and *S. comma* (t_{21} = 1.38, P = 0.182), but more *H. pensylvanicus* screened positive for the presence of the endotoxin (χ^2 = 39.90, df = 1, P < 0.001). *Harpalus pensylvanicus* from the lepidopteran-specific field also showed high temporal variability in the proportion screening positive for Cry1Ab, peaking during post-anthesis (between 24 July and 28 August), with rates between 68 and 100%

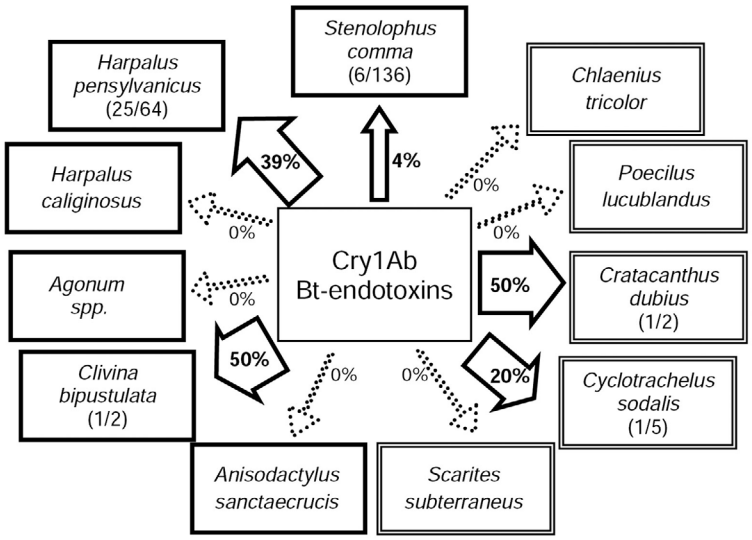

Figure 1. The proportions of beetles screening positive for Cry1Ab Bt-endotoxins for each of 11 species from the lepidopteran-specific transgenic corn field. Number of individuals screening positive out of total collected from the field is given in parentheses for those species with positive results. Species with a significant herbivorous component of their diet are displayed with a solid black frame; those species without a significant herbivorous component of their diet are displayed with a striped frame.

containing Cry1Ab Bt-endotoxins (χ^2 = 29.94, df = 3, P < 0.001). Peak activity-density of both *H. pensylvanicus* and *S. comma* was recorded during the pre-anthesis period and did not correspond with the peak in individuals screening positive for Cry1Ab. In the coleopteran-specific and stacked fields, only *H. pensylvanicus* contained recognizable concentrations of Cry1Ab-endotoxin. However, in both cases, the proportion testing positive was low (Table 1) and significantly more adults tested positive in the lepidopteran-specific field (χ^2 = 23.79, df = 1, P < 0.0001). No adult carabids screened positive for Cry1Ab endotoxins in the nontransgenic isoline field.

Bt-endotoxin concentrations in corn tissue

All plant tissue from the lepidopteran-specific and stacked lines screened positive for Cry1Ab Bt-endotoxin. Bt-endotoxin concentrations were similar across events for all plant tissue locations, except seedling leaves of the stacked (Cry1Ab + Cry3Bb1) line, which contained significantly higher concentrations of Cry1Ab than the lepidopteran-specific line (t_{13} = –2.20, P = 0.047) (Figure 3). Furthermore, seedling leaves had a significantly higher concentration and 8-week-old stems had a significantly lower concentration of Cry1Ab than all other plant tissues ($F_{6,133}$ = 52.46, P < 0.0001).

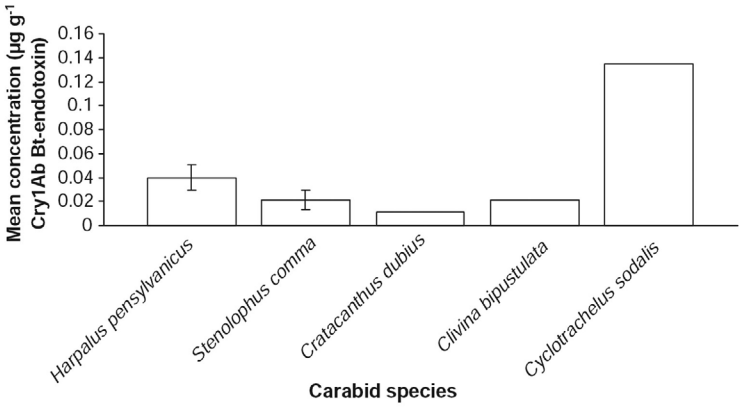

Figure 2. Mean concentration of Cry1Ab Bt-endotoxin in beetles screening positive by ELISA from the lepidopteran-specific field (where error bars not presented, $n \leq 2$).

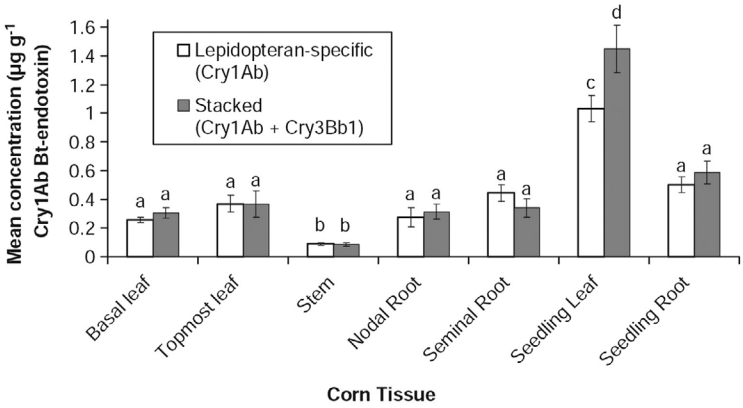

Figure 3. Mean concentration of Cry1Ab Bt-endotoxin in 8-week-old corn plants and 7-day-old seedlings from seven plant tissue locations and four different genetic lines (n = 10 for each).

Table 1. Number of *Harpalus pensylvanicus* collected (*n*), the proportions screening positive for Cry1Ab, and mean concentration (±SE) of Bt-endotoxin from each corn field.

Field	*% Positive*	*Mean concentration (µg g–1 Cry1Ab-endotoxin)*
Lepidopteran-specific (Cry1Ab)	39.1% (25 of 64)	0.0398±0.0104
Coleopteran-specific (Cry3Bb1)	2.0% (3 of 149)	0.0328±0.0267
Stacked (Cry1Ab + Cry3Bb1)	5.3% (4 of 75)	0.0341±0.0125
Isoline	0.0% (0 of 43)	–

Discussion

Foraging behaviors vary among natural enemies and therefore, with the development and commercialization of new transgenic events, exposure pathways in the field are likely to impact non-target species differentially among crops. Thus, to fully understand the impact of Bt crops on the non-target food chain, it is essential that risk-assessment consider this variation in uptake of endotoxins between bioengineered crops which express different endotoxins and/or have different modes of action.

As hypothesized, there was considerable variation among species in the proportion of beetles screening positive for Bt-endotoxin. In the lepidopteran-specific field, large proportions of some species (e.g., *C. dubius*, *C. bipustulata*, and *H. pensylvanicus*) screened positive for Cry1Ab Bt-endotoxin, whereas other species (e.g., *S. subterraneus*, *Agonum* spp., and *H. caliginosus*) tested negative (Figure 1). The lack of evidence for *S. subterraneus* exposure to, and uptake of, Bt-endotoxin is consistent with Harwood et al. (2006), who documented that after consumption of Bt-containing prey, no tri-trophic movement of endotoxins occurred. Despite focusing on single-gene events, Zwahlen and Andow (2005) reported uptake of Cry1Ab Bt-endotoxin by the carabids *H. pensylvanicus*, *Cyclotrachelus iowensis* (Freitag), and *Clivina impressefrons* LeConte. Our research also reports uptake by these species or their congeners, but in contrast to Zwahlen and Andow (2005), who found Cry1Ab uptake in *Agonum placidum* (Say) and *Poecilus lucublandus*, this study reported no uptake by these species or their congeners. These data could be a result of the inherent variation that can occur using gut-content immunoassays (Hagler 1998), regional variability of transgenic protein expression (as seen with Cry1Ac in Bt-cotton (Greenplate 1999)), or the variation in feeding ecology among members of the same genus, as well as potential differences in food web structure between research sites (Kentucky vs. Minnesota). Therefore, levels of connectedness between Bt-containing prey and these predators could vary geographically. Variation in endotoxin uptake across species could also be due to differences in feeding ecology among members of Carabidae. Although most ground beetles can be accurately described as consuming eggs, soft-bodied invertebrates and/or (some) plant material, the foraging behavior of individual species varies significantly, from entirely herbivorous to predominantly predaceous (Holland 2002). For example, the feeding habits of the 11 species collected during this study varied from herbivory of grass seed (*Stenolophus comma*) to predation of lepidopteran larvae and coleopteran eggs (*Poecilus lucublandus*), and orthopteran nymphs, small hemipterans, ants, and small dipterans (*Cyclotrachelus sodalis*) (Allen 1979). The temporal detection of Cry1Ab in *H. pensylvanicus* peaked during the post-anthesis phenological period, 4–6 weeks after the start of anthesis in the lepidopteran-specific field, similar to

the temporal patterns of Bt-endotoxin detection found in adult coccinellids (Harwood et al. 2007), suggesting that tri-trophic interactions or consumption of other plant tissues, rather than direct pollen feeding is contributing to the uptake of Bt-endotoxins in these beetle species.

The uptake of Cry1Ab Bt-endotoxin by *H. pensylvanicus* in the coleopteran-specific field (Table 1) was unexpected, because this event produces only Cry3Bb1 Bt-endotoxin and the ELISA system used in this study does not elicit reactivity to this protein. Movement of adult carabid beetles from the stacked field (which expresses both Cry1Ab and Cry3Bb1 toxins) or lepidopteran-specific field (which expresses Cry1Ab only) could account for these data. Carabid dispersal ability is reportedly high, particularly in those species adapted to agroecosystems (Holland 2002), making the distance of 150 m from the stacked field to the coleopteran-specific field a reasonable distance for *H. pensylvanicus* movement. Additionally, endotoxin-containing prey could have emigrated out of the lepidopteran-specific or stacked fields and into the coleopteran-specific field. Therefore, in the context of risk-assessment of transgenic crops, it is important to consider dispersal patterns and exposure in highly mobile predators and their herbivorous and detritivorous prey.

Similar concentrations of Cry1Ab were found in plant tissue of single and stacked genetic events (except in seedling leaves), in accord with findings by Adamczyk, Adams, and Hardee (2001) that expression of Cry1Ac Bt-endotoxin is not significantly different when expressed alone or in a stacked line with Cry2AB proteins in transgenic cotton. However, further study on the expression profiles of transgenic proteins and the effect of multiple gene insertions is still necessary before eliminating the possibility of differential expression of Cry1Ab in single and stacked lines. There were highly significant differences in the uptake of Cry1Ab endotoxin between the lepidopteran-specific and stacked events. *Harpalus pensylvanicus* was the only carabid species to screen positive for Cry1Ab in the stacked field and the proportion screening positive from the lepidopteran-specific event was significantly higher. Possible reasons for the differential rate of exposure in single and stacked gene events include differences in carabid food webs between the two events, as well as variable rates of Cry1Ab breakdown. Food web differences could include the potential reduction of Western corn rootworm (*Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae)) as potential carabid prey in the stacked event. Differential breakdown of Cry1Ab Bt-endotoxin in the environment or in the gut of carabids and their prey could additionally contribute to the variation in uptake of Cry1Ab in non-target arthropods between a single and a stacked transgenic event.

The effect of this differential uptake of Cry1Ab by carabids between single and stacked corn lines must be further studied. The existing Bt risk-assessment literature includes studies that report no discernable effects of

consumption of transgenic corn or Bt-containing prey by non-target arthropods, such as thrips (e.g., Zwahlen, Nentwig, Bigler, and Hilbeck 2000); mites, collembolans and nematodes (e.g., Al-Deeb, Wilde, Blair, and Todd 2003); lepidopterans (e.g., Anderson, Hellmich, Sears, Sumerford, and Lewis 2004); anthocorids (e.g., Pilcher, Obrycki, Rice, and Lewis 1997; Zwahlen et al. 2000; Al-Deeb, Wilde, and Higgins 2001); lacewings (e.g., Pilcher et al. 1997; Dutton, Romeis, and Bigler 2003); coccinellids (e.g., Pilcher et al. 1997; Lundgren and Wiedenmann 2002; Ferry, Mulligan, Majerus, and Gatehouse 2007); and carabids (e.g., Harwood et al. 2006; Ferry et al. 2007). In contrast, similarly conducted studies have also reported negative effects (e.g., reduced abundance, increased mortality, delay in development, reduction in weight gain, or changes in behavior) on beneficial organisms, such as pollinators (e.g., Ramirez-Romero, Desneux, Decourtye, Chaffiol, and Pham-Delègue 2008), predators (e.g., Hilbeck, Baumgartner, Fried, and Bigler 1998), parasitoids (e.g., Pilcher, Rice, and Obrycki 2005; Ramirez-Romero, Bernal, Chaufaux, and Kaiser 2007) and other nontarget arthropods (e.g., Losey, Rayor, and Carter 1999; Jesse and Obrycki 2000; Zangerl et al. 2001; Zwahlen et al. 2003).

The results of this study have illustrated the need for further elucidation of carabid, and other, non-target food webs in transgenic agroecosystems, to fully understand the exposure pathways and identify routes of transfer of Bt-endotoxins in the field. Accurate knowledge concerning the exposure rates and routes of Bt-endotoxin movement in the field are an essential part of understanding the impact of transgenic crops on agroecosystems. In addition, the feeding ecology of larval carabids is poorly documented. There is significant potential for Bt-endotoxin exposure to this life stage, particularly from components of the below-ground food web, such as earthworms, slugs, and corn rootworm, which are readily fed on by many species of larval carabids (Peterson 1960; Larochelle and Larivière 2003).

This study has documented the exposure of Bt-endotoxin to the carabid community and quantified Cry1Ab concentrations in corn tissue across multiple transgenic events, thus forming the framework for future risk-assessment of transgenic crops. Thus, the risk-assessment of genetically modified crops should be considered on a case-by-case basis because of the potential for variable exposure pathways for transgenic proteins expressed in different genetic events, particularly across single and stacked gene events.

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