

## Effect of Cry3Bb Bt Corn and Tefluthrin on Postdispersal Weed Seed Predation

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Indirect effects of insect control strategies on weed populations are important to consider when developing robust integrated pest management strategies. Weed seed predation rates were investigated in corn managed under three contrasting treatments based on control practices for corn rootworm: (1) the transgenic crop Cry3Bb Bt corn, (2) the broad-spectrum insecticide tefluthrin, and (3) no insecticide control. This 2-yr field study conducted near Ithaca, NY, involved quantifying seed loss from velvetleaf, common lambsquarters, and giant foxtail in arenas with and without vertebrate enclosures. Velvetleaf and giant foxtail were unaffected by the insecticide treatment; however, average seed predation of common lambsquarters was lower in both the Bt corn (11.9%) and insecticide-treated plots (11.8%) compared with control plots (17.5%) that did not receive any insecticide. Seed predation of common lambsquarters was not affected by the vertebrate enclosure. Lower seed predation in the transgenic Bt corn and insecticide treatments was likely due to nontarget effects on carabids (Coleoptera: Carabidae). Although the reduction in seed predation was modest and limited to only one of the three weed species tested, our results highlight the need for greater risk assessment that includes the ecosystem service of weed seed predation when considering insect pest management options.

**Nomenclature:** Common lambsquarters, *Chenopodium album* L. CHEAL; corn rootworm, *Diabrotica* spp.; giant foxtail, *Setaria faberi* Herm. SETFA; velvetleaf, *Abutilon theophrasti* Medik. ABUTH; corn, *Zea mays* L; *Bacillus thuringiensis* var. *kumamotoensis*.

**Key words:** Carabid beetles, integrated pest management, nontarget effects, seed removal, transgenic crops.

Weed and insect pest management is a major production challenge in many cropping systems. Annual agrestal weeds such as velvetleaf, common lambsquarters, and foxtails (*Setaria* spp.) lower yields in annual crops such as corn and soybeans by competing for light, water, nutrients, and space. Herbicides are typically used to control these weeds, but challenges with herbicide-resistant weed populations and environmental concerns over herbicide use have prompted researchers to develop alternative strategies. One approach is to use weed ecology to exploit biotic interactions that limit weed populations, such as weed seed predation by birds, rodents, crickets, and carabid beetles.

The organisms responsible for weed seed predation have been shown to vary temporally and from

site to site. For example, enclosure trials conducted in organic cereal fields in The Netherlands investigated the relative importance of arthropods, small mammals, and birds in weed seed predation and found that vertebrate activity accounted for 30 to 88% of seed loss, whereas invertebrate seed predators accounted for 4 to 38% of seed loss (Westerman et al. 2003). Using video monitoring, birds were found to be the most important weed seed predator of common lambsquarters and Persian speedwell (*Veronica persica* Poir.) in mixed cropping systems in New Zealand (Navntoft et al. 2009). However, a field study in southern Ontario, Canada, reported that ground-dwelling invertebrates, predominantly carabid beetles, were the dominant predators of annual weeds such as common lambsquarters and barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.] (Cromar et al. 1999). Other research from field sites in North America suggests that carabid beetles are widespread and important weed seed predators (Davis et al. 2013; Gallandt et al. 2005; Menalled et al. 2007; Shearin et al. 2007).

Carabid beetles are so ubiquitous that carabid assemblages have been used in Scotland as an alternative to plant communities for classifying habitat types. Unlike plants, carabids have short lifespans and no dormant propagules or seed bank;

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thus, they respond quickly to environmental or management changes (Blake et al. 2003). Carabid diversity and abundance is often quantified in agricultural research because of their responsiveness to cultivation, insecticides, herbicides, and other management techniques that alter plant cover and field microclimate (Holland 2002). For example, Ward et al. (2011) showed that two carabid beetles, *Harpalus pensylvanicus* (DeGeer) and *Amara aenea* (DeGeer), were negatively affected by disturbance from management practices (e.g., tillage, mowing, sowing, harvesting, etc.) and that cover crops increased their activity density.

The use of pesticides can also affect beneficial insects in the corn system. High mortality has been reported resulting from direct exposure to neonicotinoid insecticides (Mullin et al. 2005) in the laboratory and reductions of field densities of coccinellids (Stephens et al. 2012), anthocorids (de la Poza et al. 2005), and staphylinids (Albajes et al. 2003) in plots treated with imidacloprid compared with control plots. Carabids have also been shown to leave insecticide-treated soil, using nearby untreated areas as refugia (Chen and Willson 1996). Prasifka et al. (2008) reported that brief exposure (20 min) of the carabid beetle, *Scarites quadriceps* Chaudoir in the laboratory to one-half the recommended label rate ( $0.18 \text{ kg ai ha}^{-1}$ ) of the soil-incorporated pyrethroid insecticide, tefluthrin increased total distance moved, maximum velocity, and percentage of time spent moving in this beetle.

The introduction of transgenic Cry3Bb Bt [*Bacillus thuringiensis* var. *kumamotoensis*] corn for corn rootworm (CRW) (*Diabrotica* spp.) control has largely replaced broad-spectrum insecticides. Plantings of Bt corn grew from 8% of US corn acreage in 1997 to 76% in 2013 (Fernandez-Cornejo et al. 2014). Recent increases in adoption are largely due to the effective suppression of corn rootworm by Cry3Bb Bt corn varieties. The CRW is more destructive to corn yield than the European corn borer [*Ostrinia nubilalis* (Hübner)], which was the original target pest of Bt corn. Widespread adoption of Bt corn has been attributed to a 41 million kg reduction in insecticide use in the US from 1996 to 2011 (Benbrook 2012). Endogenously expressed Bt can reliably provide excellent control of targeted pests with comparatively low toxicity to natural enemies (Burkness et al. 2001; Lynch et al. 1999; Sorenson and Holloway 1999). Increased use of Bt cotton in China has been associated with decreased use of broad-spectrum insecticides and increases in populations of lady beetles (Coleoptera:

Coccinellidae), lacewings (Neuroptera: Chrysopidae), and spiders (Araneae), as well as biological control services spilling over from Bt cotton fields onto neighboring corn and soybean crops (Lu et al. 2012). In contrast, Cry3Bb Bt corn replaces soil-incorporated granular insecticides that do not affect foliar-foraging predators, while the expressed toxin has a negative effect on lady beetles (Stephens et al. 2012).

Despite the wide adoption of transgenic Cry3Bb Bt corn for corn rootworm control by growers, concerns have also been expressed about the possible effects of Bt on beneficial seed-consuming nontarget invertebrates, including carabid beetles (Coleoptera: Carabidae), which are in the same taxonomic order as CRW. Toxins from Bt in corn root exudates are bound to soil particles for long periods of time (Saxena et al. 1999). Given that Bt is also expressed in leaves, pollen, and silks, the incorporation of these organic materials into the field may also increase soil toxin levels. The potential exposure of seed predators like carabids is heightened by their omnivorous predation on arthropod prey in addition to seeds (Torres and Ruberson 2007). Thus, understanding the overall effect of Cry3Bb Bt protein on arthropod communities and the services they provide (e.g., weed seed predation) is particularly relevant given the likelihood of CRW Bt corn replacing a significant percentage of the corn planted not only in the United States but worldwide. Specifically, understanding how different CRW management strategies affect carabid beetle populations and other weed seed predators could reduce reliance on herbicides while maintaining an economically necessary level of weed suppression.

Agricultural risk assessment has been expanded to include the effect of management tactics on broader ecological functions and ecosystem services such as nutrient cycling, pollination, and integrated pest management (Romeis et al. 2013). Such research is essential to understanding the factors involved in the adoption of a new technology and responsibly increasing environmental stewardship and profitability of crop production systems. The objective of this research was to compare the effect of Cry3Bb Bt corn on weed seed predation to nontransgenic corn grown with and without a standard CRW insecticide. We hypothesized that weed seed predation would be lower in the nontransgenic corn grown with a standard CRW insecticide but that Bt corn on weed seed predation would have no negative effect relative to the nontransgenic corn grown without an insecticide treatment.

## Materials and Methods

**Field Site.** This field study was carried out in 2002 and 2003 in a 12-ha field at Cornell University's Homer C. Thompson Vegetable Farm in Freeville, NY (42.50°N; 76.32°W). The soil at this field is characterized as a Howard gravelly loam (loamy-skeletal, mixed, active, mesic Glossic Hapludalfs; 45.4% sand, 42.2% silt, and 12.4% clay) with a 5.4% organic matter content and pH 6.9.

**Experimental Design.** The experiment was set up as a completely randomized split plot design with three main plot types and three subplot types. The main plot treatments were (1) transgenic Bt corn (DKC4623) resistant to corn rootworm (Bt), (2) the nontransgenic isoline (DKC4626) (NoBt), and (3) the nontransgenic isoline with the insecticide tefluthrin (Force 3G<sup>®</sup>-Zeneca, Wilmington, DE) applied to the soil at a rate of 0.372 g m<sup>-1</sup> row in the seed furrows at planting (NoBt+I). A total of four replicate plots were established for the Bt treatment and three replicate plots for the NoBt and NoBt+I treatments. All corn seeds were supplied by Monsanto (St. Louis, MO) and had been treated with the fungicide Captan (*N*-trichloromethylthio-4-cyclohexene-1,2-dicarboximide) at the label rate of 2.3 ml kg<sup>-1</sup> of seed (Drexel Chemical Co., Memphis, TN) and the insecticide imidacloprid (0.165 mg per seed) (Gaucho<sup>®</sup>, Gustafson, Shakopee, MN). Gaucho protects seeds from several important insect pests including wireworms [Coleoptera: Elateridae], seedcorn maggot [*Delia platura* (Meigen)], and seedlings from damage by flea beetles [Coleoptera: Chrysomelidae] and white grubs (*Phyllophaga* spp.).

Main treatment plots measured 30 by 132 m (36 corn rows planted at 76-cm spacing), although data were only collected from the middle 20 corn rows. Within each main plot, three randomly assigned weed seed predator enclosure treatments were established in subplots: (1) open seed arenas without an enclosure, (2) seed arenas covered with a 30 by 30 by 10-cm enclosure made of 0.5-cm wire mesh allowing easy passage of most soil arthropods but not mammals or birds, and (3) a modified enclosure to determine whether the presence of the enclosures alone indirectly inhibited ground-foraging insect activity. This treatment simply elevated the enclosures 10 cm off the ground using polyvinyl chloride (PVC) stilts to allow for unobstructed movement of ground-foraging insects. In each of the 10 main plots there were two replicates each of the open,

vertebrate enclosure, and control enclosure treatments for a total of 60 arenas per sample date.

**Data Collection.** Seed predation rates were determined for three common annual weeds: common lambsquarters, velvetleaf, and giant foxtail (2003 only). These are three of the most costly and ubiquitous weed species in corn production in the United States. Seed predation was assessed by placing the weed seeds on 2-cm-deep 0.5-mm nylon mesh bottom 20 by 20-cm PVC frames (arenas) filled with soil and buried flush with the surrounding soil surface. The soil was collected from a dredged river bottom and did not contain seeds of the three target weed species. Extra care was taken to ensure a continuous substrate surface between these soil-containing PVC arenas and the surrounding field. Forty seeds of each species were spread as evenly as possible by hand on the soil surface of these arenas laid between the corn rows. This 3,000 seeds m<sup>-2</sup> weed seed density is typical of temperate cropping systems with moderate weed seed production. Arenas were installed in random locations and seeded in the morning on the day that pitfall traps were installed to quantify invertebrate activity density. To avoid seed loss during precipitation, sampling was carried out when the forecast called for no to low probability of precipitation. Seed arenas were left in the field for 48 h and then the soil was carefully removed and placed in paper bags. Bags were immediately transported to the laboratory and dried at 40 C for at least 24 h. This procedure ensured that none of the target weed seeds germinated in the paper bags. Weed seeds present in the dried soil were extracted using a high-volume hydraulic elutriator and then dried and sorted by species, and viable seed were counted to determine the percentage lost to predation in the field. Seed viability was determined using the seed crush test (Sawma and Mohler 2002). Seed predation rates were assessed once monthly from June to September in 2002 and 2003. Weed seed predation data were expressed as percentage of seeds consumed after 48 h, which was calculated by subtracting the number of seeds recovered from the number of seeds offered, dividing by seeds offered, and multiplying by 100.

**Statistical Analyses.** We used a mixed effect model in SAS (SAS v9.3, SAS Institute Inc., Cary, NC) to analyze weed seed predation. Data and treatment means were compared using Tukey-Kramer with  $P < 0.05$  to determine significance. We first fit a

Table 1. Results from mixed effects ANOVA on weed seed predation data for each weed species.

Effect	Common lambsquarters		Velvetleaf		Giant foxtail <sup>a</sup>	
	P value					
Insecticide	0.017		0.597		0.873	
Exclosure	0.257		0.363		0.017	
Insecticide × exclosure	0.271		0.134		0.240	
Month	0.014		< 0.001		< 0.001	
Month × insecticide	0.175		0.298		0.860	
Month × exclosure	0.423		0.289		0.269	
Month × insecticide × exclosure	0.362		0.961		0.927	
Year	< 0.001		< 0.001		—	
Insecticide × year	0.992		0.867		—	
Exclosure × year	0.173		0.004		—	
Insecticide × exclosure × year	0.596		0.251		—	
Month × year	0.006		< 0.001		—	
Month × insecticide × year	0.399		0.714		—	
Month × exclosure × year	0.182		0.642		—	
Month × insecticide × exclosure × year	0.856		0.870		—	

<sup>a</sup> Only data from 2003 were included in the analysis of giant foxtail seed predation.

mixed model with all possible factors and interactions and found that weed species and several higher order interactions were significant. We then reanalyzed the data for each weed species separately. Weed seed predation data were square root-transformed before conducting an ANOVA to reduce heteroscedasticity and better meet the assumptions of the ANOVA. Back-transformed means from each analysis are presented in the text, tables, and figure.

## Results and Discussion

Weed seed predation tended to be lower in the exclosure treatment and greater in the open treatments; however, mean seed predation in the

open and exclosure treatments were not different within years (Tables 1 and 2). Weed seed predation varied by month in 2003, but not 2002 (Tables 1 and 2). Although vertebrate access tended to increase weed seed predation, the relatively small difference between these treatments shows that invertebrates consumed the majority of weed seeds in this experiment. Three species of carabid beetles, *Agonum muelleri* (Herbst), *Poecilus lucublandus* (= *Pterostichus lucublandus*) (Say), and *Pterostichus melanarius* (Illiger), accounted for > 70% of all beetles captured by pitfall traps in our experiment, which has been described previously (Stephens et al. 2012). Although strong correlations between carabid beetle activity density and weed seed predation have been found in some field experiments

Table 2. Mean weed seed predation showing the interaction between exclosure and year and the interaction between month and year for each weed species.<sup>a</sup>

	Common lambsquarters				Velvetleaf				Giant foxtail <sup>b</sup>	
	2002		2003		2002		2003		2003	
Exclosure × year										
Open	13	AB	17	A	10	bc	22	a	30	AB
Control	09	B	19	A	04	c	27	a	35	A
Exclosure	09	B	15	AB	08	c	17	ab	23	B
Month × year										
June	11	bc	10	c	10	B	07	B	08	c
July	10	bc	25	a	08	B	37	A	44	a
August	10	bc	19	ab	04	B	49	A	49	a
September	10	bc	17	abc	07	B	08	B	26	b

<sup>a</sup> Within each weed species and interaction type, similar letters indicate no significant difference in weed seed predation according to the Tukey–Kramer test with significance set at P = 0.05.

<sup>b</sup> Only data from 2003 were included in the analysis of giant foxtail seed predation; thus, only main effects of exclosure and month in 2003 are presented.



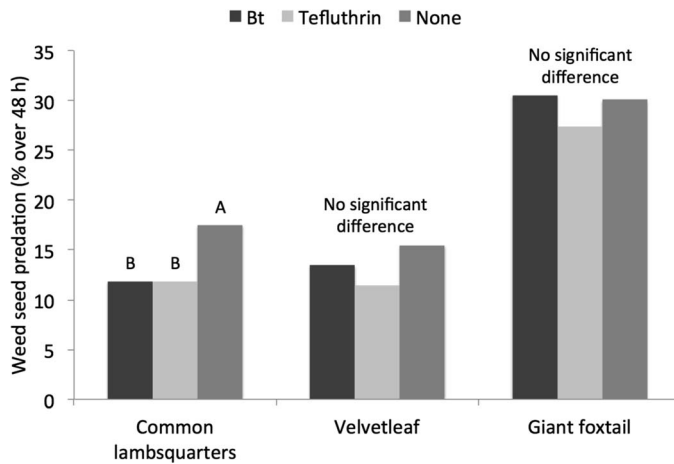


Figure 1. Mean weed seed predation (% removal over 48 h) across insecticide treatments (Bt, Bt corn; tefluthrin, noBt+I; none, NoBt) for each of the three weed species tested. Similar letters above bars indicate no significant difference (Tukey–Kramer test with significance set at  $P = 0.05$ ).

(Menalled et al. 2007), weed seed predation appears to be highly site specific (Davis et al. 2013), depending largely on vegetation cover and habitat complexity (Fox et al. 2013). In Pennsylvania, activity density of *H. pensylvanicus* was positively correlated with weed seed predation but only explained 29 to 33% of the variation (Ward et al. 2011). In this research carabid activity density and weed seed predation sampling were not synchronized in time, and an earlier analysis showed no correlation (data not shown).

**Effects of Insecticide Treatments.** Insecticide treatments did not affect velvetleaf or giant foxtail seed predation (Table 1). Compared with the NoBt treatment (17.5%), common lambsquarters seed predation was lower in the Bt (11.9%) and NoBt+I (11.8%) treatments (Table 1; Figure 1). This suggests that weed suppression benefits conferred by the community of seed predators could be reduced in systems where growers apply some form of insecticide to control CRW. In a related parallel study at the same field site and experimental setup as our study, Stephens et al. (2012) reported substantial reductions in carabid abundance in both the Bt corn and insecticide-treated plots relative to the non-Bt control plots, suggesting that ecosystem services such as weed seed predation may be adversely affected. Our results contradict a recent report, which suggested that insecticidal activity of the Cry3Bb protein is limited to species belonging to the coleopteran family of Chrysomelidae and that the use of genetically modified Cry3Bb Bt corn does not cause adverse effects to nontarget organisms and

the ecosystem services they provide (Devos et al. 2012).

Bt corn is a valuable tool, and the widespread adoption of Bt corn has reduced insecticide use and increased area-wide suppression of insect pests (Hutchison et al. 2010). However, more long-term field studies in different regions are needed to better understand the effects of Bt corn on nontarget organisms, such as carabids, and the ecosystem services they provide. While Bt corn might be better than broad-spectrum insecticides for most arthropods, stewardship is needed to limit nontarget effects and to avoid the development of populations of resistant insect pests. Our research is important in the context of understanding the effect of our current technologies on nontarget communities and ecosystem services. Because CRW is typically only a problem in crop rotations where corn follows corn, diversifying crop rotations could help maintain the pest and weed control services of arthropod communities and provide other well-documented agroecological benefits (Davis et al. 2012; Norris and Kogan 2005).

Our research also contributes to a body of literature on the environmental and farm management practices that affect the diversity and abundances of seed predators and seed predation (Davis et al. 2013; Menalled et al. 2007; Westerman et al. 2003). Such research is necessary to understand the externalized costs associated with agricultural practices and to help farmers reduce purchased inputs on their farm as well as the costs to society associated with agroecosystem simplification and degradation. Future research should integrate multitrophic effects into pest management decision support tools, which could help farmers optimize their cropping systems by maximizing biological control and reducing purchased inputs.

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