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Volunteer Corn Presents New Challenges for Insect Resistance Management

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ABSTRACT

Genetically-modified (GM) corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] dominate the North American agricultural landscape and are becoming increasingly important as biofuels. However, as herbicide-tolerance and insecticidal traits are often simultaneously expressed by individual plants, glyphosate [*N*-(phosphonomethyl) glycine]-resistant (GR) volunteer corn is becoming a widespread problem as a weed in corn-soybean rotational systems. We show that these volunteer corn plants not only have herbicide-tolerance genes but also express insecticidal "Bt" protein. We also report high levels of damage to these plants from larvae of the target pest, the western corn rootworm (WCR, *Diabrotica virgifera virgifera* LeConte). This suggests that volunteer herbicide-tolerant Bt corn has the potential to present problems both for weed management and insect resistance management, as it may facilitate more rapid evolution of Bt resistance in corn rootworm populations.

Bacillus thuringiensis corn hybrids are GM plants that produce insecticidal toxins in their tissues and thereby resist feeding by specific insect pests. These hybrids are increasingly being combined, or "stacked," with other transgenic traits such as glyphosate (Roundup; Monsanto Corporation, St. Louis, MO) or glufosinate [Liberty; Bayer CropScience, Research Triangle Park, NC; 2-amino-4-(hydroxymethylphosphinyl)butanoic acid] herbicide-tolerance. As of 2005, over 60% of U.S. corn acreage was annually rotated with soybean (USDA, 2007). Roughly 90% of soybean (26.2 million hectares) and 52% of corn (19.7 million hectares) (NASS, 2007) grown in the United States in 2007 were GR, allowing producers to apply glyphosate-only herbicide treatments to prevent crop yield loss due to weed competition. Adoption of corn hybrids expressing insect-resistance traits (frequently known as "Bt corn") has increased dramatically in the United States in recent years from 29% (9.5 million hectares) in 2004 to 49% (18.6 million hectares) in 2007 (NASS, 2007). Although Bt corn for Lepidopteran pests such as the European corn borer (*Ostrinia nubilalis* Hubner) has been available for over a decade, the recent increase in adoption is mainly due to the release of transgenic traits targeting the WCR, the key insect pest of corn in North America (Metcalf, 1986). Furthermore, 28% of all U.S. corn planted in 2007 expressed multiple transgenic traits, conferring both herbicide tolerance and resistance to insect feeding in the form of Bt-toxin expression (NASS, 2007). The GM corn hybrids discussed in this paper all contain the MON88017 transgenic event, which causes the plant to express both rootworm-resistant Bt traits (the insecticidal Cry3Bb1 toxin) and GR.

The prevalence of corn as a weed (volunteer corn) in soybean in our study area, the state of Indiana, increased from a mean of 3% of all fields sampled in 2003 to 12% of all fields sampled in 2005, and this increase is strongly correlated with the adoption of GR corn (Davis et al., 2008). The long-standing corn-soybean rotation offers many agronomic and economic benefits, including 100% mortality of any corn rootworm neonate larvae that arise from eggs deposited in the field the previous year, when corn would have been present (soybean roots do not support rootworm larvae). However, the presence of volunteer corn among the soybeans will allow some fraction of larvae produced by these eggs to survive to adulthood. Previously, emerging corn seedlings in GR soybeans were easily controlled by early-season glyphosate applications, but volunteer GR corn plants are able to survive these treatments, forcing producers to apply additional herbicide treatments specifically to manage the volunteer corn. Additionally, and perhaps more problematic over the long-term, is the potential for volunteer corn plants expressing the WCR-resistant Bt trait to accelerate the development of resistant populations of WCR.

We wished to test the hypothesis that volunteer corn plants in our study area express the genetic traits of both herbicide tolerance and the Bt toxin Cry3Bb1, thus exposing WCR larvae to the toxin during the soybean crop. Furthermore, we examined whether volunteer corn plants sustained damage due to WCR larval feeding. This research was initiated due to the potential for volunteer corn plants expressing GR and Bt traits to negatively impact current insect resistance management strategies. Current strategies rely on structured refugia (Gould, 2003) to offset the selection pressure presented by

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Abbreviations: Bt, *Bacillus thuringiensis*; GM, genetically modified; GR, glyphosate resistant/resistance; WCR, western corn rootworm.

commercial hybrids expressing Bt toxins to conserve these invaluable tools in production agriculture.

MATERIALS AND METHODS

We surveyed eight soybean fields across northern Indiana where volunteer corn plants were present at estimated densities of 0.1 to 0.3 plants/m^2 and corn hybrids expressing the MON88017 event had been grown the previous season. In June 2007 we collected between 81 and 141 volunteer corn plants/field (mean height of approximately 35 cm) at random by walking transects within each field. Both foliar and root portions of the plants were collected along these transects. Each transect followed a "W" pattern across each field sampled, with each leg of the "W" representing a length of approximately 80 m. We estimated the extent of WCR feeding using the node injury rating system developed by Oleson et al. (2005), who determined that a value of 0.5 is the threshold above which economic damage can be expected to occur. Using this scale, we categorized damage as either absent (no visible feeding), mild (rating ≤ 0.5), or significant (rating > 0.5).

Following collection from the field, the stalks of plants were removed, the roots washed and fully dried, and a 10- by 2-mm section of root tissue was cut from each plant and transferred to a 1.5-mL microcentrifuge tube. Deionized water (0.5 mL) was added to each tube, and root tissue was homogenized using a Branson Sonifier (Model S-250A, Branson Ultrasonics Corporation, Danbury, CT) for 20 s at 40% intensity. Quick-stix lateral flow test strips (Products AS 010 LSS and AS 015 LSS, Envirologix Inc., Portland, ME), were used to test for the presence of GR and the presence of the Bt protein Cry3Bb1, respectively. Following 15 min of exposure to the tissue homogenate, each tube was recorded as positive or negative for GR and Cry3Bb1.

RESULTS AND DISCUSSION

Overall, 86.95 ± 3.36% (SE) of the volunteer corn plants tested positive for GR, 64.93 ± 5.07% tested positive for Cry3Bb1, and 60.28 ± 5.55% tested positive for both traits (n = 733 plants).

We quantified damage to corn roots collected from soybean fields to estimate larval feeding. There were no differences found in feeding damage between roots expressing the Cry3Bb1 toxin and those that did not (Table 1).

Table 1. Mean percentage (±SE) of Cry3Bb1 positive (*n* = 470) and negative (*n* = 263) volunteer corn plants with varying degrees of damage to roots from larval rootworm feeding, as measured using the Oleson root rating system (Oleson et al., 2005). Plants sampled in 2007 were collected from eight soybean fields in northern Indiana. Pearson's chi-square test (α = 0.05) revealed no significant differences between volunteer corn plants expressing the Cry3Bb1 protein and those that did not in each damage category.

Root damage category	% Bt positive ± SE	% Bt negative ± SE	*P* value
No damage	39.17 ± 8.20 *n* = 172	33.94 ± 6.70 *n* = 84	0.4497
Mild (≤0.5)	35.05 ± 4.36 *n* = 167	25.09 ± 6.15 *n* = 87	0.3134
Significant (>0.5)	25.78 ± 10.35 *n* = 131	40.97 ± 8.73 *n* = 92	0.3821

The substantial root injury observed in volunteer plants testing positive for Cry3Bb1 has not been reported previously in refereed publications. In field studies, Bt corn hybrids have shown very little or no feeding damage (Gray et al., 2007; Moellenbeck et al., 2001; Oyediran et al., 2007). The high rate of feeding injury we observed may be due to two factors: (i) intensive pressure from larvae seeking relatively scarce host plants in the soybean fields, or (ii) reduced levels of the insecticidal Cry3Bb1 component in the volunteer plants. The former explanation appears less likely in our case. We would have expected significantly higher rates of damage in volunteer plants that did not test positive for the Cry3Bb1 protein (Table 1) if larval pressure were the primary factor, and the Cry3Bb1 protein was conferring significant insecticidal benefits to the plant. Also, as noted above, other published work (Gray et al., 2007) has demonstrated very little or no damage to commercial Cry3Bb1 hybrids, even under conditions of high larval pressure.

Reduced levels of Cry3Bb1 in plants may be a more likely explanation in the environment of a soybean field. Unlike corn, soybeans fix their own N and do not require additional applications of this nutrient. Volunteer corn plants in this nutrient-deficient environment may be expected to produce reduced levels of protein. The direct relationship between N rates and Bt toxin production has been demonstrated previously in Bt hybrid corn, for a different Bt toxin, Cry1Ab (Bruns and Abel, 2003) and also in another large-acreage Bt crop, cotton (*Gossypium hirsutum* L.) (Pettigrew and Adamczyk, 2006). Because we were supplied with field histories for the fields we sampled, we know that these volunteer plants represent the offspring of commercially-available Bt corn hybrids, planted in fields that also contained 20% refuge (non-Bt) plants. Other investigators measuring Bt concentrations in commercial corn hybrids targeting Lepidopteran insect pests (Chilcutt and Tabashnik, 2004), have demonstrated within-field gene flow between Bt and non-Bt varieties, documenting significant, but diluted levels of toxin in the kernels of refuge corn grown adjacent to Bt corn.

Bacillus thuringiensis corn hybrids registered for commercial use must be planted in accordance with strict requirements based on the high-dose/refuge strategy to manage insect resistance (Gould, 2003). Iterative models that predict the rate of Bt-resistance development in WCR populations indicate that the dose of the toxin (toxicity) is one of the three most important factors determining the evolution of resistance (Onstad et al., 2001). While the initial Bt-resistance allele frequency in WCR populations is not known, and damage due to WCR is typically negligible on Bt corn hybrids, it is has been documented that a low percentage of WCR larvae exposed to the toxin in commercially-available plants do survive to adulthood (Hibbard et al., 2005). This suggests that traits conferring tolerance or resistance to current Bt toxins exist in WCR populations.

Our results suggest that WCR larvae are able to feed on Cry3Bb1-positive volunteer corn plants at levels similar to volunteer plants which test negative for the Cry3Bb1 toxin. If volunteer corn with full or partial expression of the Bt trait becomes a more widespread problem, these plants could allow survival of WCR that are heterozygous for Bt-resistance, while killing susceptible homozygous individuals, thereby increasing the frequency of the resistance allele and allowing resistant populations to evolve faster (Gould, 1998; Chilcutt and Tabashnik, 2004). In fact, modeling results suggest that suboptimal doses of toxin can accelerate evolution of pesticide resistant populations (Tabashnik et al., 2004).

Note that the volunteer corn problem is not confined to corn-soybean rotations and will likely be more prevalent in continuous corn plantings. Since volunteer plants are less visually apparent in corn and would not represent a harvest contaminant (Anderson and Geadelmann, 1982), there would be little impetus for identifying or controlling these plants. The number of corn fields with documented incidence of volunteer corn will likely increase as corn acreage increases to supply the growing demand for grain to feed corn-based ethanol production (Baker and Zahniser, 2006). Volunteer corn plants that may or may not express the Bt toxin will likely occur in fields planted with Bt corn and provide larvae with reduced toxicity or nontoxic feeding options throughout the field. Corn rootworm larvae are capable of moving from one root system to another (Hibbard et al., 2003), meaning that WCR larvae in these fields could consume sublethal doses of Bt toxin by feeding on a combination of volunteer plants and commercial Bt hybrids. The survival of larvae following sublethal exposure could hasten the evolution of resistance to the toxin (Mallet and Porter, 1992; Hoy et al., 1998), and is precisely the situation that structured refuges (where larvae are either exposed to the optimum dose of the toxin in a commercial hybrid, or not exposed at all) were designed to avoid.

Our findings present an example of an unforeseen consequence of stacking multiple transgenic traits within a single plant to facilitate pest management and agronomic practices. Namely, weedy volunteer corn plants stacked with GR and Bt traits may accelerate the development of Bt-resistant WCR populations, circumventing the current management plans.

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