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Competition of Transgenic Volunteer Corn with Soybean and the Effect on Western Corn Rootworm Emergence

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Glyphosate-resistant (GR) volunteer corn has emerged as a problematic weed in corn:soybean rotational systems, partly because of the rapid increase in adoption of corn hybrids that contain traits for both glyphosate and insect resistance. Volunteer GR corn can decrease soybean yields. The objectives of this study were to quantify the impact of volunteer corn on soybean growth and yield and determine how volunteer corn densities affect western corn rootworm (WCR) emergence. Volunteer corn seed was hand-planted at targeted densities of 0.5, 2, 4, 8, 12, and 16 seeds m^{-2} at soybean planting and 21 d after planting to evaluate both early- and late-emerging cohorts. WCR emergence was assessed with the use of field emergence traps placed over individual corn plants in the 0.5- and 16-plants-m^{-2} plots in 2008 and 2009. In 2010, WCR emergence traps were also placed over individual and clumped volunteer corn plants at densities of two and eight plants m^{-2}. Soybean yield reductions ranged from 10 to 41% where early-emerging volunteer corn densities ranged from 0.5 to 16 plants m^{-2}. No soybean yield loss occurred with the late-emerging cohort of volunteer corn. Twice as many adult WCRs emerged from a single volunteer corn plant growing at densities of 8 and 16 plants m^{-2}, compared with plots containing 0.5 and 2 plants m^{-2}. These results demonstrate that controlling volunteer corn will not only prevent soybean yield loss, but also may reduce the risk of WCR larval survival after exposure to Bt (*Bacillus thuringiensis* Berliner derived) corn.
Nomenclature: Glyphosate; western corn rootworm, *Diabrotica virgifera virgifera* LeConte; corn, *Zea mays* L.; soybean, *Glycine max* L. Merr.
Key words: Resistance management, Bt, *Cry3Bb1*.

The adoption of herbicide-resistant (HR) corn and soybean in the United States has increased since the introduction of glyphosate-resistant (GR) soybean varieties in 1996 and GR corn hybrids in 1998 (Castle et al., 2006). In 2010, greater than 93% of the soybean and 70% of the corn planted in the United States was HR [U.S. Department of Agriculture–National Agricultural Statistics Service (USDA-NASS) 2010]. The widespread and rapid adoption of GR technologies has led to the evolution of GR weed biotypes due to selection pressure of glyphosate-only herbicide programs (Johnson et al. 2009). GR crop adoption has also been correlated with increased occurrence of volunteer GR crops such as volunteer corn growing as a weed in soybean (Davis et al. 2008).

Volunteer corn was documented as a weed in soybean before the introduction of GR soybean varieties (Andersen 1976; Andersen and Geadelmann 1982; Beckett and Stoller 1988). The weed has been shown to reduce soybean yield by up to 25% at densities of 5 to 6 plants m^{-2}, and increased densities of volunteer corn reduced soybean yield components such as nodes per plant, plant dry weight, pods, and seeds (Beckett and Stoller 1988). At a density of 10 volunteer corn plants/clump, soybean sustained a yield loss within a 40 cm radius of the clump (Beckett and Stoller 1988).

Volunteer corn is competitive in soybean rows planted 0.76 m apart, yet the competitive effect is not clear in narrow-row (0.19 m row) soybean. Prior to the release of GR soybean, greater than 60% of soybean hectares were planted in $>$ 0.60–m (24 in.) rows (Padgitt et al. 2000). The majority of the soybean currently planted in the upper Midwest is grown in row spacing less than 0.60 m, usually ranging from 0.19-m to 0.38-m rows (USDA-NASS 2007). In Indiana, 87% of soybean planted in 2006 were planted in rows 0.51 m or less (Conley and Santini 2007). Soybean grown in narrow rows could suppress weed competition by shortening the interval between planting to crop-canopy closure (Yelverton and Coble 1991). Previous research did not evaluate soybean yield loss from volunteer corn emerging later than soybean. This aspect may be important information because not all volunteer corn emerges at soybean emergence.

Much of Indiana utilizes a corn:soybean rotational system. The Monsanto Company first registered GR corn in 1998, but the technology was not as rapidly adopted as GR soybean. The introduction of transgenic Bt (*Bacillus thuringiensis* Berliner derived) hybrids to manage western corn rootworm (WCR), and the subsequent addition of GR to these hybrids, has dramatically increased adoption of HR corn. The adoption of HR corn is expected to continue to increase as corn hybrids that express multiple transgenic traits become prevalent. Increased adoption of corn hybrids with multiple resistance traits, in addition to the use of glyphosate as the primary herbicide for POST weed control in soybean, may result in increased occurrences of volunteer corn expressing some or all of the transgenic Bt toxins as the corn hybrids planted in the field the year before. The WCR is a univoltine pest. Eggs are deposited in corn, overwinter in the field, and larvae emerge in late spring, feeding on corn roots. Volunteer corn present another feeding option for developing WCRs. Krupke et al. (2009) hypothesized that Bt volunteer corn expressed the Bt protein at a lower concentration than hybrid corn, and noted that there was no difference in WCR feeding damage between Bt-negative and Bt-positive volunteer corn roots. Exposure of target insects to decreased Bt expression (i.e., sublethal exposure) could increase selection for Bt-resistant insects. In addition, volunteer corn could increase survival of variant WCR females (a biotype that does not lay eggs exclusively in corn), further reducing the utility of crop rotation as a management tool.

Significant research has been directed toward quantifying the efficacy of Bt transgenic corn hybrids, including analyses of WCR adult emergence and the effects on resistance evolution (Hibbard et al., 2009; Lefko et al., 2008; Meihls et al., 2008). Several models have been developed to help predict the evolution of Bt resistance in WCR populations,

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but these do not take into account the effect of volunteer (Bt-positive) corn. Increased prevalence of volunteer corn could alter estimates of the time of evolution of resistance to Bt. This is mainly due to large numbers of WCR larvae potentially being exposed to unknown levels of toxic Bt proteins expressed by volunteer corn. A series of field experiments were conducted to develop baseline data on how volunteer corn may affect soybean yield and WCR populations. The objectives of these studies were to quantify the impact of volunteer corn density and emergence timing on soybean growth and yield, and also to determine how various densities of volunteer corn growing in drilled soybean (0.19-m row spacing) affect WCR emergence.

Materials and Methods

Corn seed was hand harvested in the fall of 2007 and 2008 from DKC 63-42 (Dekalb® Brand, Monsanto Company, 800 North Lindbergh Boulevard, St. Louis, MO 63167) corn hybrids for use in establishing volunteer corn in 2008 and 2009, respectively. This is a transgenic hybrid, expressing proteins for glyphosate resistance, European corn borer resistance, (Bt protein *Cry1A*), and WCR resistance (Bt protein *Cry3Bb1*). Field research was conducted at two locations [Throckmorton Purdue Agricultural Center (TPAC), Lafayette, IN, and Pinney Purdue Agricultural Center (PPAC), Wanatah, IN] in 2008 and 2009. The soil type at TPAC was a Toronto-Milbrook silty loam (fine–silty, mixed, superactive, mesic Udollic Endoaqualfs) with a pH of 6.2 and 2.9% organic matter. The soil type at PPAC was a Pinhook loam (coarse–loamy, mixed, superactive, mesic Mollic Endoaqualfs) with a pH of 6.1 and 2% organic matter. The sites were fall chisel plowed and field cultivated in the spring and fertilized according to Indiana state recommendations. Temperature and weather data for TPAC and PPAC are shown in Table 1. P93M61 GR soybean (Pioneer Hi-Bred, P.O. Box 1000, Johnston, IA 50131) was drilled at a rate of 543,400 seeds ha^{-1} at TPAC (April 23, 2008 and May 12, 2009) and 469,300 seeds ha^{-1} at PPAC (May 9, 2008 and May 7, 2009). The drilled soybean area was divided into plots (3 m by 9 m), and seven targeted densities (a small percentage of seeds did not emerge or were killed by glyphosate applications) of volunteer corn (0, 0.5, 2, 4, 8, 12, 16 seeds m^{-2}) were hand-planted with the use of a spike planter (Hand Jab Planter, Almaco, 99 M Avenue, Nevada, IA 50201) in a randomized complete-block design with six replications. Two volunteer corn planting dates were used (at soybean planting and 21 d after planting). The experimental plots were treated with glyphosate (Roundup® PowerMAX, Monsanto) at a rate of 840 g ae ha^{-1} to keep the plots free of weeds other than volunteer corn.

Volunteer Corn Competition. At the R6 soybean growth stage, volunteer corn and soybean plants in 0.5-m^2 areas were cut off at the soil surface to quantify total aboveground biomass in each plot. The bags containing corn were stored at 0 C. The bags containing soybean were placed into drying ovens for 10 d at 27 C, and then weighed. The biomass samples always contained a corn plant, even in the volunteer corn densities of 0.5, 2, and 4 plants m^{-2} plots. Within 48 h of collection, the volunteer corn leaf area was measured. Leaf area was calculated by removing each leaf at the stem and running the leaves through a leaf area machine (LI-3100 Area Meter, LI-COR Biosciences, 4647 Superior St., Lincoln, NE 68504), which measures leaf area in centimeters squared. After measuring the leaf area, corn biomass was placed into a drying oven for 10 d at 27 C, and then weighed.

Soybean was harvested with a plot combine to calculate the total soybean yield per plot. One-liter subsamples were collected from each plot to calculate actual soybean yield (without harvest contaminants). The subsamples were taken by collecting the harvested material per plot as the plot combine weigh-buckets cycled. Then, 100 g of harvested material was separated from the subsamples. The soybean was separated from the harvest contaminants (e.g., volunteer corn, soil, plant debris) and weighed to calculate the percentage of soybean weight in each plot. This percentage was multiplied by the total harvested material to determine the actual soybean yield in each plot.

WCR Emergence. To quantify WCR emergence, emergence traps were placed over individual volunteer corn plants in all of the 0.5- and 16-plants-m^{-2} plots at each location during the third week in June. The emergence cages were 60 by 60 by 5–cm wood frames covered with a 1-mm aluminum screen with two 5-cm-long by 3.8-cm-diameter polymerized vinyl chloride (PVC) tubes inserted into the screen. The PVC tubes were secured to the screen with aluminum hose clamps, and oriented perpendicular to the screen. One tube allowed for corn plants to grow out of the cage, and the other was used to collect WCR adults with a plastic vial (40-dram plastic vial No. 42460KY, Consolidated Plastics Company, Inc., 8181 Darrow Road, Twinsburg, OH 44087) containing an inverted funnel (58-mm disposable funnel No. 44771L1, Consolidated Plastics). The tubes were sealed to the corn plant and plastic vial with the use of foam tubing. The cages were designed to trap any insects emerging from the root system, while keeping corn plants alive throughout the season to ensure accurate WCR emergence data (Kang and Krupke 2009; Murphy et al. 2010). Before traps were placed over the volunteer corn plants, each plant was tested with qualitative immunoassay test strips (QuickStix™ Kit for Cry3B YieldGard® Rootworm Corn, AQ/AS 015, Envirologix Inc., 500 Riverside Industrial Parkway, Portland, ME 04103) to determine if the plant expressed *Cry3Bb1*. A total of 28 traps were placed at each site. This allowed for 24 traps over volunteer corn plants expressing Bt and four traps placed over volunteer corn plants that did not express Bt. The non-Bt volunteer corn traps were used to estimate the average WCR emergence without Bt toxin; only four traps were used because of the scarcity of volunteer plants that did not express Bt. The traps were checked weekly from the time of placement until the end of August. The number of adult WCRs per plant was measured in each trap.

In 2010, WCR emergence cages were placed over volunteer corn growing in soybean at two densities: two and eight plants m^{-2} in both clumped and unclumped arrangements. The volunteer corn used in the study were naturally emerging plants growing in a bulk soybean field at TPAC and thinned (hand removal) to the desired plant densities. The soil type was a Toronto-Milbrook silty loam (fine–silty, mixed, superactive, mesic Udollic Endoaqualfs) with a pH of 6.2 and 2.9% organic matter. Temperature and weather data for TPAC in 2010 are shown in Table 1. The plants were tested with qualitative immunoassay test strips (QuickStix™ Kit) to

Table 1. Mean monthly air temperature and total precipitation at Throckmorton Purdue Agricultural Center (TPAC) in 2008, 2009, and 2010 and Pinney Purdue Agricultural Center (PPAC) in 2008 and 2009.

	TPAC						PPAC			
	Temperature			Precipitation			Temperature		Precipitation	
Month	2008	2009	2010	2008	2009	2010	2008	2009	2008	2009
	C			cm			C		cm	
April	13	11	15	0.8	5.9	7.4	10	8	8.3	8.7
May	14	17	18	7.0	6.0	6.2	13	15	5.5	11.9
June	23	22	23	10.7	9.3	10.6	21	20	5.2	7.3
July	23	21	24	10.2	3.7	6.5	22	20	5.3	5.7
August	22	21	24	1.5	3.0	4.4	20	20	12.2	10.1
September	20	19	20	8.7	2.8	2.4	18	17	27.3	2.9
October	12	10	14	1.2	8.4	1.2	10	9	8.7	18.1
Mean	18	17	20	–	–	–	16	16	–	–
Total	–	–	–	40.1	39.1	38.7	–	–	72.5	64.7

determine if the plant expressed *Cry3Bb1* before the traps were placed over the plants. Traps were only placed over plants that expressed *Cry3Bb1*. There were a total of four replications in a randomized complete block design. Each replication contained 12 total emergence cages. Individual emergence cages were placed over two and eight individual volunteer corn plants (10 cages) in the nonclumped arrangement, and single emergence cages were placed over two and eight clumped volunteer corn plants (two cages). There were a total of 48 cages in this experiment. The emergence cages were placed in late June and WCR adults were counted weekly through the end of August.

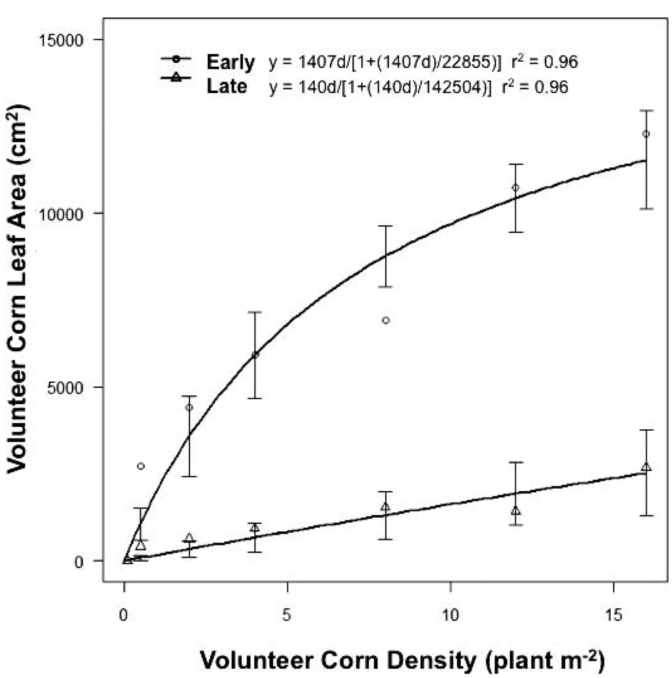

Figure 1. The pooled (site–year) volunteer corn leaf area (cm^2) with volunteer corn ranging from 0 to 16 plants m^{-2}. The Early regression line represents volunteer corn emerging before or at the same time as soybean emergence. The Late regression line represents volunteer corn emerging after soybean emergence. The data were modeled with a three-parameter Michelis-Menton model, $Y_L = Id/[1 + (Id/A)]$. Parameter estimates ($\pm$ standard error) are Early, $A = 22{,}860 \pm 9394.8$, $I = 1406.5 \pm 533.5$; Late, $A = 142{,}504 \pm 2.5 \times 10^6$, $I = 140.8 \pm 71.3$; d represents the volunteer corn density. The error bars represent the standard error for the regression line.

Data Analysis. The data were checked for normality and transformed when necessary as suggested by the Box-Cox procedure in SAS (SAS Software, Version 9.2, 2002–2008, SAS Institute Inc., Cary, NC 27513). The volunteer corn leaf area data were analyzed as a mixed model using the PROC MIXED procedure in SAS (SAS Software). The volunteer corn leaf area data had no interaction with year or location and was pooled into Early and Late planting dates. Harvest contaminants, soybean biomass, and yield data were standardized to the mean value of the weed-free control plots at each site year. This was done to present the data as a percent soybean biomass and yield reduction due to volunteer corn. The harvest contaminants, volunteer corn leaf area, soybean biomass, and yield reduction values were then pooled, respectively, and analyzed with the use of a nonlinear regression model with the drc package in R (drc 1.2, Christian Ritz and Jens Strebig, R 2.12.1, Kurt Hornik). The data were modeled with a three-parameter Michaelis-Menton model (Equation 1).

$$Y_L = Id/[1 + (Id/A)] \quad [1]$$

In this model, Y_L is the leaf area/biomass reduction/yield loss, d is the weed density, I represents the slope of the linear region as d approaches 0, and A is the upper asymptote of the curve as the leaf area/biomass/yield loss approaches its maximum value (Cousens 1985; Harrison et al. 2001). Two-tailed t-tests were used to analyze the WCR emergence data ($\alpha < 0.05$).

Results and Discussion

Volunteer Corn Competition. Much of the impact of volunteer corn on soybean development may be due to direct competition for light, as has been shown with other weed species (Nordby et al. 2007; Page et al. 2010; Raey et al. 2005). There was no interaction with year or location for the volunteer corn leaf area (Early: P = 0.15; Late: P = 0.20). Volunteer corn leaf area in our study was positively correlated to increasing densities of volunteer corn when the corn emerged at or before soybean emergence (Early) (Figure 1). Early volunteer corn leaf area was approximately 11,340 cm^2 at 16 plants m^{-2} (Figure 1). When volunteer corn emerged after soybean emergence (Late), the volunteer corn leaf area was less (Y_L = 2,208 cm^2 at volunteer corn densities of 16 plants m^{-2}) (Figure 1). The reduction in Late leaf area was most likely due to competition with soybean, resulting in smaller corn plants that did not affect soybean growth

Table 2. Late-planted (2 wk after soybean) soybean dry weight per plant, and soybean yield in comparison to volunteer corn density (± standard error).

Volunteer corn density	Soybean biomass	Soybean yield
plant m^{-2}	g $plant^{-1}$	kg ha^{-1}
0	19 ± 1.41	2,805 ± 237
0.5	19 ± 1.46	2,941 ± 237
2	19 ± 1.46	2,861 ± 237
4	18 ± 1.46	2,755 ± 237
8	18 ± 1.46	2,808 ± 237
12	19 ± 1.46	2,746 ± 237
16	19 ± 1.60	2,629 ± 237

(Table 2). For the remainder of the discussion, we will only describe data collected and analyzed from Early planted volunteer corn treatments.

Volunteer corn growing in narrow-row soybean can be a highly competitive weed. Soybean biomass reduction due to competition with volunteer corn ranged from 7 to 39% at volunteer corn densities of 0.5 plants m^{-2} and 16 plants m^{-2} respectively (Figure 2). Volunteer corn decreased soybean yield by 41% at densities of 16 plants m^{-2} (Figure 3), which is a loss of approximately 1,000 kg ha^{-1} (weed-free treatment yield: 2,585 kg ha^{-1}, 16 plants m^2 treatment yield: 1,499 kg ha^{-1}) in this study. Volunteer corn growing in densities of only 0.5 plants m^{-2} reduced soybean yield 10% (Figure 3). Grain contamination due to volunteer corn ranged from 16–46% at densities of 0.5 plants m^{-2} to 16 plants m^{-2}, respectively (Figure 4).

The most economically important measure of weed competition with a crop is the reduction in crop grain yield. Planting soybean in narrowly spaced rows can reduce the competitive ability of weeds (De Bruin and Pedersen 2008), but an aggressive weed can grow above the soybean canopy and compete for light, resulting in soybean yield loss (Page et al. 2010). Our results are consistent with previous work indicating that volunteer corn growing in soybean is a highly competitive weed. Beckett and Stoller (1988) determined that

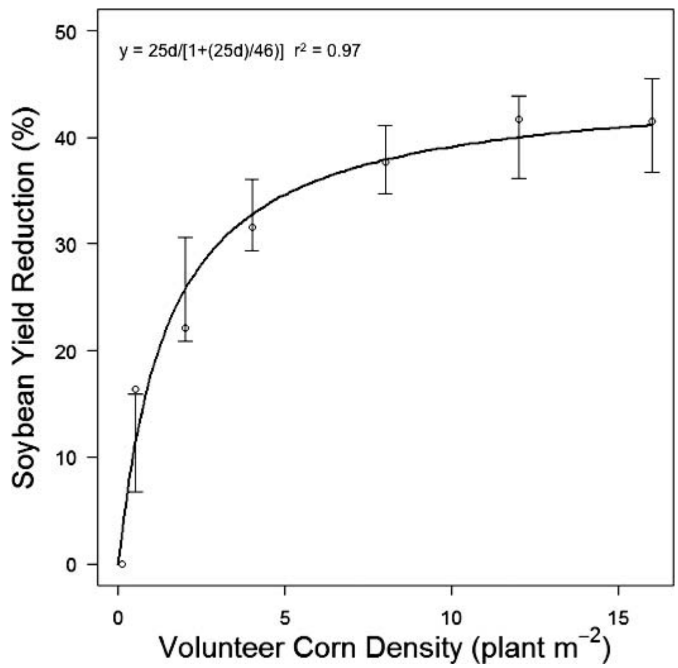

Figure 3. The pooled (site–year) percent yield reduction in soybean due to competition with volunteer corn ranging from 0 to 16 plants m^{-2}. The data were modeled with a three-parameter Michelis-Menton model, $Y_L = Id/[1 + (Id/A)]$. Parameter estimates are $A = 45.9 \pm 4.1$, $I = 25.7 \pm 10.1$; d represents the volunteer corn density. The error bars represent the standard error for the regression line.

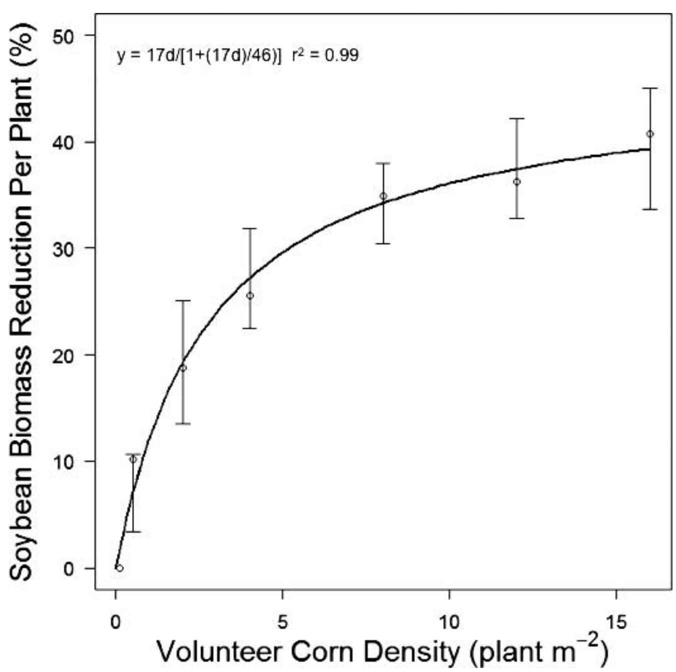

Figure 2. The pooled (site–year) percent soybean biomass reduction due to competition with volunteer corn ranging from 0 to 16 plants m^{-2}. The data were modeled with a three-parameter Michelis-Menton model, $Y_L = Id/[1 + (Id/A)]$. Parameter estimates (± standard error) are $A = 46.2 \pm 6.3$, $I = 16.6 \pm 7.2$; d represents the volunteer corn density. The error bars represent the standard error for the regression line.

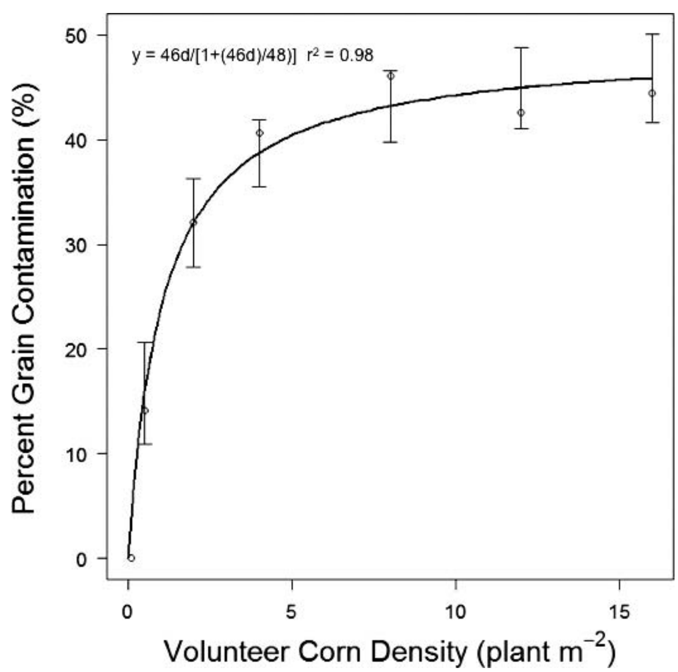

Figure 4. The pooled (site–year) percent grain contaminants due to volunteer corn ranging from 0 to 16 plants m^{-2}. The data were modeled with a three-parameter Michelis-Menton model, $Y_L = Id/[1 + (Id/A)]$. Parameter estimates (± standard error) are $A = 48.8 \pm 2.8$, $I = 46.7 \pm 13.6$. d represents the volunteer corn density. The error bars represent the standard error for the regression line.

Table 3. 2010 mean western corn rootworm (WCR) emergence from low (two plants m^{-2}) and high (eight plants m^{-2}) individual volunteer corn plants, and the clumped (at both densities) versus unclumped (individual plants at both densities) (± standard error). P values from the two-tailed *t* tests are presented ($\alpha = 0.05$).

Volunteer corn density	Mean no. WCR	P value
Two plants m^{-2}	0.50 ± 0.44	0.031
Eight plants m^{-2}	1.47 ± 0.25	
Two plants m^{-2} clumped	0.13 ± 0.40	0.551
Two plants m^{-2} unclumped	0.62 ± 0.29	
Eight plants m^{-2} clumped	0.46 ± 0.75	0.087
Eight plants m^{-2} unclumped	1.18 ± 0.26	

clumps of volunteer corn (10 plants per clump) could reduce soybean yield by 25% at densities of 5,380 clumps ha^{-1} when left untreated throughout the growing season. Our study evaluated similar densities as Beckett and Stoller (1988), with some important differences: We did not incorporate clumped volunteer corn and our soybeans were planted in narrow row spacing.

WCR Emergence from Volunteer Corn. WCR beetles emerged from all volunteer corn plants in soybeans, including those that expressed *Cry3Bb1*. Not surprisingly, more WCRs emerged (mean ± standard error of 2.45 ± 0.43 beetles) from high-density (16 plants m^{-2}) volunteer corn treatments than emerged (mean ± standard error of 0.74 ± 0.27 beetles) from low-density (0.5 plants m^{-2}) treatments ($t = 3.47$; df = 36; P = 0.0014). In 2010, the mean number of WCR emerging from high-density (eight plants m^{-2}) volunteer corn treatments expressing *Cry3Bb1* was nearly triple that of the low-density (two plants m^{-2}) treatments (1.47 vs. 0.5 beetles/cage) (Table 3). There was no effect of volunteer corn clumping at either density on WCR emergence. WCR densities were highly variable, which could be the reason for no differences in WCR emergence between the clumped and unclumped volunteer corn. However, our data illustrate the fact that as volunteer corn density increased, the number of WCRs emerging from the volunteer corn increased.

The objectives of this research were to determine the competitive effects of GR volunteer corn on soybean growth and yield and to quantify the number of WCRs emerging from GR volunteer corn. Our results indicate that GR volunteer corn at densities of 16 plants m^2, when not controlled, can cause up to a 41% soybean yield loss. Because late-emerging volunteer corn had no competitive effect on soybean, early control of GR volunteer corn that emerges at the same time as soybean should be prioritized to avoid yield loss due to weed competition. Early control of GR volunteer corn may also decrease potential selection pressure on WCR populations by killing the plant before insect larval development is complete. Although we did not quantify dose as part of these experiments, reduced toxin expression by volunteer plants could result in sublethal Bt exposure to WCR. Fortunately, herbicide options exist to facilitate management of volunteer corn early in the season (i.e., before mid-June in the upper Midwest), before larvae can complete development. The reason for removing this potential WCR host is twofold: reducing the numbers of viable adults that may mate and lay eggs, and reducing possible exposure to Bt toxins outside of approved Bt corn/refuge environments. Olmer and Hibbard (2008) indicated that non-GR corn treated with glyphosate is no longer a viable food option within 5 d of application. Although treatment of GR volunteer corn would require the use of an Acetyl coenzyme A carboxylase-inhibiting herbicide, similar results may be expected. In most cases, it is likely that the comparatively low numbers of adult WCR that do emerge from GR volunteer corn growing in soybean will be diluted by the much larger numbers of beetles emerging from adjacent cornfields. However, without detailed information about the levels of Bt toxin expressed by volunteer plants, and the number of beetles exposed to these hosts across the corn belt, caution is warranted to minimize the possibility of sublethal exposure. Early control requires timely scouting and management in fields that are rotated from GR corn to GR soybean to decide if control is necessary. Education of growers about the potential problems of GR/Bt volunteer corn is also essential to help optimize soybean yields and maximize the durability of Bt hybrids for rootworm control.

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