RESEARCH

Transgenic Corn Rootworm Protection Increases Grain Yield and Nitrogen Use of Maize

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ABSTRACT

Maize (*Zea mays* L.) hybrids expressing *Bacillus thuringiensis* (Bt) derived resistance to corn rootworm (*Diabrotica* spp.) are widely grown. Our hypothesis was that Bt hybrids exhibit increased N uptake, resulting in greater grain yield and N use efficiency (NUE) relative to their nonprotected counterparts. In 2008 and 2009, two transgenic corn rootworm resistant (Bt) hybrids with VT3 (YieldGard VT Triple) technology along with their near-isogenic non-Bt Roundup Ready Corn 2 (RR2) counterparts were evaluated at Champaign, IL, with supplemental N of 0, 67, 134, 201 or 268 kg N ha^{-1}. Despite minimal corn rootworm feeding pressure on roots, the Bt hybrids produced an average of nearly 1.1 Mg ha^{-1} more grain than their RR2 counterparts. In the comparison of DKC61-72 RR2 and DKC61-69 VT3, Bt protection promoted increased grain yield at low N (+1.0 Mg ha^{-1}; $P \leq 0.01$) and a 31% greater response to fertilizer N. With adequate N, grain yields of the comparison DKC63-45 RR2 and DKC63-42 VT3 did not differ; however, the latter maximized its yield with an average of 38% less fertilizer N. Increases in NUE (+80%; $P \leq 0.10$) and N uptake efficiency (NUpE) (+31%; $P \leq 0.10$) at the N rates required to optimize grain yield of Bt hybrids were detected in 2008, but NUE and NUpE were not significantly different between isolines in 2009. We conclude that transgenic corn rootworm protection has supplemental agronomic benefits, with greater N uptake and NUE in some environments.

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Abbreviations: Bt, *Bacillus thuringiensis*; CRW, corn rootworm; ECB, European corn borer; NUE, N use efficiency; NUpE, N uptake efficiency; RM, relative maturity; RR2, Roundup Ready Corn 2; VT3, YieldGard VT Triple.

Western corn rootworm (*Diabrotica virgifera virgifera* LeConte) is one of the most damaging insect pests of maize with annual production losses due to corn rootworm (CRW) feeding estimated at US$1 billion (Metcalf, 1986). Corn rootworm larvae feed on maize roots, thereby limiting uptake of water and nutrients (Kahler et al., 1985; Riedell, 1990), and promote decreases in CO_2 assimilation, biomass accumulation, and carbohydrate partitioning (Dunn and Frommelt, 1998a, 1998b; Riedell and Reese, 1999; Urías-López et al., 2000). The response of grain yield to N fertilizer was previously shown to be reduced in CRW infested plots, which suggests that injured roots were unable to recover N fertilizer (Spike and Tollefson, 1991). Therefore, cultural, chemical, or genetic strategies for control of CRW may improve N use and yield of maize.

The use of a corn–soybean [*Glycine max* (L.) Merr.] rotation was an effective management strategy for avoiding CRW damage (Gray et al., 2009) previous to the appearance of rotation resistant phenotypes of both Western CRW and Northern CRW (*Diabrotica barberi* Smith and Lawrence), which limited the effectiveness

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of crop rotation in managing these pests (Levine et al., 1992, 2002). Transgenic hybrids expressing proteins from *Bacillus thuringiensis* (Bt) for the control of CRW were first commercialized in 2003 (Vaughn et al., 2005). Although recent reports have suggested that some CRW populations are already developing resistance to transgenic control strategies (Gassmann et al., 2011), the CRW Bt traits have clearly demonstrated their effectiveness for reducing root injury from CRW larval feeding and for improving grain yield (Gray et al., 2007; Ma et al., 2009). The improvement in grain yield observed for CRW resistant hybrids over non-Bt hybrids suggests that a root system with full-season insect protection allows the plant to more fully express its genetic potential. Furthermore, an intact root system and protected yield potential might have a direct impact on N uptake and use. Although the N use characteristics of Bt hybrids possessing resistance to CRW have not yet been reported, Subedi and Ma (2007) compared a Bt hybrid with resistance to European corn borer (ECB) (*Ostrinia nubilalis*) to its isoline and showed that the Bt hybrid accumulated approximately 11% more N. This increase in N uptake was associated with an increase in dry matter accumulation in leaves and grain of the Bt hybrid while root dry matter did not differ. Therefore, a CRW Bt trait that may directly affect root size, distribution, or activity could have an even more dramatic effect on N uptake and, consequently, N use efficiency (NUE).

Grain yield response to insect protection transgenes varies considerably between hybrids and environments. For example, Dillehay et al. (2004) compared near-isoline hybrid pairs for grain yield response to transgenic protection from ECB and showed that responses to Bt protection ranged from 0.6 to 1.5 Mg ha^{-1} depending on isoline pair. The authors theorized that this variability partially resulted from natural ECB resistance and tolerance mechanisms. Similarly, Ma et al. (2009) compared a near-isoline pair for the CRW Bt trait and measured an 11 to 66% grain yield increase in the Bt hybrid, which varied due to year and location. Although some of the variability measured by Dillehay et al. (2004) and Ma et al. (2009) was likely to have resulted from differences in insect pressure, it is also plausible that genetic and physiological characteristics of hybrid genetic backgrounds contribute to differing trait responses. Currently, there is a scarcity of information on variation for grain yield response to the Bt trait in CRW resistant hybrids and how this might impact potential improvements in N use. In particular, it is not known if Bt hybrids require higher inputs of N fertilizer as a result of increased yield or if a reduction in N requirement could occur as a result of improved N uptake.

The primary objective of this study was to measure grain yield, N uptake, and N response characteristics of two high yielding CRW resistant Bt hybrids in comparison to their non-Bt counterpart hybrids. An additional objective was to determine whether CRW Bt hybrids require a different optimum N rate to achieve maximum grain yield compared to non-Bt hybrids. Our hypothesis was that CRW protected transgenic hybrids would exhibit increased N uptake, resulting in higher grain yield and improved NUE relative to their non-Bt counterparts.

MATERIALS AND METHODS

Cultural Practices, Experimental Design, and Treatments

Field experiments were conducted during the 2008 and 2009 growing seasons on the Fisher Farm at the University of Illinois Department of Crop Sciences Research and Education Center near Champaign, IL. The soil at this site is a Drummer-Flanagan soil association (fine-silty, mixed, superactive, mesic Typic Endoaquolls) typical of east-central Illinois with 4.2% organic matter and a pH of 5.8. Plots were mechanically planted on 29 May 2008 and 29 May 2009 at a seeding rate of 84,014 plants ha^{-1} and subsequently thinned to achieve a final plant density of approximately 79,000 plants ha^{-1}. Preemergence weed control consisted of applications of S-metolachlor (2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide), atrazine (6-chloro-*N*-ethyl-*N′*-(1-methylethyl)-1,3,5-triazine-2,4-diamine), and mesotrione ([2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione). Postemergence weed control consisted of mechanical cultivation and glyphosate [*N*-(phosphonomethyl)glycine].

A split-plot arrangement in a randomized complete block design with four replications was used in which hybrids were randomly assigned to the main plots and N rates were randomly assigned to the subplots. Each subplot experimental unit consisted of six rows 11.4 m in length with 76 cm spacing. Four locally adapted DEKALB brand grain corn hybrids were evaluated, which represented two separate comparisons. In each pair, the non-Bt hybrid possessed only glyphosate tolerance (Roundup Ready Corn 2 [RR2]) (Monsanto Company) while the Bt hybrid (YieldGard VT Triple [VT3]) (Monsanto Company) possessed glyphosate tolerance, resistance to European corn borer (Cry1Ab protein from *Bacillus thuringiensis*), and CRW resistance (Cry3Bb1 protein from *Bacillus thuringiensis*). The hybrids included a 111 d relative maturity (RM) pair (DKC61-72 RR2 and DKC61-69 VT3) and a 113 d RM pair (DKC63-45 RR2 and DKC63-42 VT3). Although we cannot conclusively state that the hybrids within each comparison are near-isogenic lines, they are marketed by the seed supplier as representing different trait versions of the same genetic background, with similar phenotypic and agronomic characteristics. The non-Bt hybrids (RR2) received an in-furrow application of tefluthrin [2,3,5,6-tetrafluoro-4-methylphenyl)methyl-(1a,3a)-(*Z*)-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate] at a rate of 0.11 kg a.i. ha^{-1}. Thus, the experiment compared two methods of CRW control: soil-applied insecticide versus transgenic control. Nitrogen was applied and incorporated as granular $(NH_4)_2SO_4$ (21-0-0-24S) in a diffuse band between the rows after emergence during the V2 to V3 growth stages. Five N rates (0 to 268 kg N ha^{-1}) in 67 kg N ha^{-1} increments were included.

Biomass Sampling, Nitrogen Uptake, and Yield Measurements

Plant N uptake was estimated from biomass samples collected at physiological maturity (R6) when at least 50% of the plants exhibited a visible black layer at the base of the kernels. Six representative plants per plot were sampled (aboveground biomass only) and separated into ear (grain plus cob) and stover (leaf plus stem plus husk) fractions. The fresh weight of the stover fraction was determined before shredding using a commercial brush chipper–shredder (Vermeer BC600XL). A representative aliquot of the shredded material was dried to constant weight in a forced-draft oven (75°C) to determine sample moisture concentration and to calculate the per plant stover dry weight. Dried stover aliquots were ground in a Wiley mill to pass a 20-mesh screen and analyzed for total N concentration (g kg^{-1}) using a combustion technique (EA1112 N-Protein; CE Elantech, Inc.). Total stover N content was calculated by multiplying the per plant dry stover biomass by the stover N concentration. The ear samples were shelled to separate the grain and cobs. Grain moisture concentrations were measured using a dielectric (capacitance) type grain moisture meter (SL95; Steinlite Corp.) and used to calculate the dry weight of the R6 grain samples. Grain protein concentration was measured using near-infrared transmittance spectroscopy (Infratec 1241 Grain Analyzer, FOSS). Grain N concentration was estimated from protein concentration using a factor of 6.25. Grain N content was calculated by multiplying the per plant grain weight by the grain N concentration. Total N content (g per plant) was calculated as the sum of the stover and grain N contents. Nitrogen uptake on an area basis was estimated by multiplying per plant N content by the plant density at harvest (79,000 plants ha^{-1}).

For yield and component measurements, the center two rows of each plot were hand harvested. Grain yields are expressed as megagrams per hectare at 0 g kg^{-1} moisture. Individual kernel weights were estimated by counting 300 kernels from a representative grain subsample and are expressed as milligrams per kernel at 0 g kg^{-1} moisture. Kernel number (m^{-2}) was algebraically derived using the total plot grain weight and the estimate of individual kernel weight.

Evaluation of Corn Rootworm Damage

Five random plants per plot were rated in 2008 for damage created by CRW larval feeding. These evaluations occurred on 27 July 2008 at VT to R1. The Iowa State University node-injury scale (0–3 scale) was used in which a rating of 0 corresponds to no feeding damage and a rating of 3 signifies that three or more root nodes are completely damaged (Oleson et al., 2005). The experimental plots were not evaluated for CRW feeding in 2009.

Statistical Analysis and Calculation of Nitrogen Use Efficiency Components

Statistical analysis was accomplished using PROC MIXED in SAS (SAS Institute, 2009). Normality of residuals and potential outliers were assessed using PROC UNIVARIATE in SAS. Year, hybrid, N rate, and their interactions were considered fixed effects while replication and interactions with replication were considered random. The response of grain yield to N rate for each hybrid was described using a quadratic with plateau regression model estimated with PROC NLIN in SAS according to

$$\hat{Y} = \hat{\beta}_0 + \hat{\beta}_1 X + \hat{\beta}_2 X^2 \text{ if } X < X_0 \quad [1]$$

$$\hat{Y} = \hat{\beta}_0 + \hat{\beta}_1 X_0 + \hat{\beta}_2 X_0^2 \text{ if } X \geq X_0 \quad [2]$$

in which $\hat{Y}$ is the predicted value, X is the fertilizer N rate, $\hat{\beta}_0$ is the intercept (predicted grain yield at 0 kg N ha^{-1}), $\hat{\beta}_1$ is the linear coefficient, $\hat{\beta}_2$ is the quadratic coefficient, and X_0 is the fertilizer N rate at which the quadratic and plateau segments of the model join (Cerrato and Blackmer, 1990; Bullock and Bullock, 1994). Similarly, the response of N uptake to N rate was modeled for each hybrid using either a quadratic with plateau regression model Eq. [1] and [2] or a linear with plateau regression model according to

$$\hat{Y} = \hat{\beta}_0 + \hat{\beta}_1 X \text{ if } X < X_0 \quad [3]$$

$$\hat{Y} = \hat{\beta}_0 + \hat{\beta}_1 X_0 \text{ if } X \geq X_0 \quad [4]$$

Using the grain yields and N uptake values predicted by these regression functions, NUE (kg grain kg^{-1} fertilizer N) and N uptake efficiency (NUpE) (kg plant N kg^{-1} fertilizer N) were calculated according to

$$\text{NUE} = (\text{GY}_x - \text{GY}_0)/\text{NR}_x \quad [5]$$

$$\text{NUpE} = (\text{NT}_x - \text{NT}_0)/\text{NR}_x \quad [6]$$

in which GY_x corresponds to the grain yield (kg ha^{-1}) at a level of fertilizer application (>0 kg N ha^{-1}), GY_0 corresponds to the grain yield (kg ha^{-1}) of the unfertilized check plot treatment (0 kg N ha^{-1}), NR_x is the fertilizer N rate (kg N ha^{-1}) at which NUE and NUpE are evaluated, and NT_0 and NT_x represent total aboveground plant N uptake at the 0 and X N rates (kg plant N ha^{-1}).

RESULTS AND DISCUSSION

Growing Conditions

Temperatures and precipitation were generally favorable for high yields during 2008 and 2009. Maximum monthly temperatures were less than the 10-yr average (2000–2009) during silking and grain fill in both years (Table 1). Although total precipitation during the May through August period was similar for both 2008 and 2009, the temporal distribution of precipitation varied between years. During 2008, approximately 41% of the precipitation measured for May through August occurred in July, leading to favorable conditions for pollination and kernel set. Excess precipitation in July 2008 was followed by a negative 7.4 cm deviation from the 10-yr average in August (Table 1). As a result, the grain filling period in 2008 was relatively short and physiological maturity (R6) occurred around 1 Sept. 2008. In contrast, precipitation was more evenly distributed in 2009, with an average of 13.3 cm of precipitation occurring each month during May through August. Minimum and maximum temperatures in 2009 were generally much cooler than average, particularly at flowering and during grain filling. During July and August 2009, maximum and minimum temperatures were approximately 2.8 and 1.8°C less than

Table 1. Average monthly weather data at Champaign, IL, for the period between 1 May and 30 Sept. 2008 and 2009. Tmin and Tmax are the minimum and maximum daily temperatures, respectively. Values in parentheses are the deviations from the 10-yr average (2000–2009) at Champaign, IL.

Year	Month	Tmin	Tmax	Precipitation
		°C		cm
2008	May	9.4 (–1.4)	20.7 (–2.6)	14.9 (+5.3)
	June	18.0 (+1.8)	28.8 (+0.7)	13.0 (+4.0)
	July	18.7 (+0.8)	29.1 (–0.3)	20.2 (+8.1)
	Aug.	17.1 (–0.5)	28.4 (–0.6)	1.7 (–7.4)
	Sept.	14.3 (+1.2)	25.7 (–0.6)	20.2 (+12.6)
2009	May	11.4 (+0.6)	23.1 (–0.3)	13.0 (+3.4)
	June	17.3 (+1.1)	28.4 (+0.3)	10.8 (+1.8)
	July	16.5 (–1.4)	26.6 (–2.8)	15.6 (+3.5)
	Aug.	16.1 (–1.4)	27.2 (–1.8)	13.7 (+4.6)
	Sept.	14.3 (+1.1)	25.0 (–1.3)	1.6 (–6.0)

the 10-yr average. Cool temperatures and adequate rainfall in 2009 resulted in a prolonged grain filling period, which extended into September.

Insect Feeding and Root Damage

Roots were evaluated at the VT to R1 growth stage, a critical time for establishing the number of grain sinks (i.e., number of kernels) and potential size of each sink (i.e., individual kernel weight). As such, insect feeding damage occurring before or at this stage should be indicative of potential yield loss. Root injury was very low in 2008. The mean node-injury rating across all hybrids was 0.11 (approximately one-tenth of a node eaten). There was a significant effect of hybrid (Table 2) resulting from a significant ($P = 0.011$) reduction in root injury for DKC61-69 (mean node-injury rating = 0.04) compared to its non-Bt counterpart (DKC61-72) (mean node-injury rating = 0.15). Mean node-injury ratings for DKC63-45 and DKC63-42 were 0.12 and 0.11, respectively. Based on these low levels of apparent root injury, few differences in grain yield or agronomic performance between non-Bt and Bt hybrids might be expected. Although roots were not evaluated for injury in 2009, University of Illinois corn rootworm research trials located at Urbana, IL, in 2008 and 2009 reported that mean node-injury ratings in 2009 were approximately 28% greater for check treatments (non-Bt hybrids with no soil-applied insecticide) compared to 2008 (Gray et al., 2008; Gray and Estes, 2009). Therefore, CRW pressure in the 2009 trial was expected to have been comparable to or greater than that experienced in 2008.

Responses of Grain Yield and Yield Components to *Bacillus thuringiensis* Corn Rootworm Protection

Hybrid, N rate, and the hybrid × N rate interaction were significant sources of variation for grain yield (Table 2). Year and its interactions with hybrid and N rate were not significant sources of variation for grain yield. As such, quadratic with plateau regression functions were used to describe the response of grain yield to N for each hybrid by combining data across years (Fig. 1). The response of grain yield to N fertilizer was different for isolines within each hybrid comparison. When grown with no supplemental (low) N (0 kg N ha^{-1}), the measured grain yield of DKC61-69 was 1.0 Mg ha^{-1} greater than its non-Bt counterpart ($P \le 0.01$) when averaged across years (Table 3). In addition to an improvement in tolerance to low N, the measured grain yield of DKC61-69 was 2.0 Mg ha^{-1} greater than DKC61-72 when compared at 268 kg N ha^{-1} ($P \le 0.001$). The maximum grain yield of DKC61-69 occurred with 154 kg N ha^{-1} (10.5 Mg ha^{-1}) while DKC61-72 had no further increase in grain yield (8.6 Mg ha^{-1}) when provided with greater than 76 kg N ha^{-1} (Fig. 1).

A significant yield increase of 1.1 Mg ha^{-1} ($P \le 0.01$) occurred for DKC63-42 relative to DKC63-45 at 67 kg N ha^{-1} (Table 3). With adequate fertilizer N supply (≥169 kg N ha^{-1}) grain yields did not differ (10.3 versus 10.5 Mg ha^{-1} for DKC63-45 and DKC63-42, respectively) (Fig. 1). DKC63-42 achieved its predicted maximum grain yield at 104 kg N ha^{-1} versus 169 kg N ha^{-1} for DKC63-45, representing a 38% reduction in N fertilizer application required for maximum yield.

The large increase in grain yield associated with CRW protection in DKC61-69 over DKC61-72 was reflected in both kernel number and kernel weight (Table 3). Significant differences in kernel number between these hybrids occurred at rates of N fertilizer equal to or greater than 134 kg N ha^{-1} ($P \le 0.01$). Averaged across both years and all N rates, CRW protection resulted in a 12% increase in kernel number for the DKC61-72 to DKC61-69 comparison ($P \le 0.001$). Similar to kernel number, individual kernel weights of DKC61-72

Table 2. Tests of fixed sources of variation of year (Y), hybrid (H), N rate, and their interactions on root injury, yield, and yield components for four maize hybrids over 2 yr and five N rates.

Trait	Analysis of variance						
	Year (Y)	Hybrid (H)	Y × H	N rate	Y × N rate	H × N rate	Y × H × N rate
	$P > F$						
Root injury score†	–	0.059	–	0.536	–	0.691	–
Grain yield	0.659	<0.001	0.198	<0.001	0.338	0.030	0.479
Kernel number	0.016	<0.001	0.289	<0.001	<0.001	0.023	0.404
Kernel weight	0.002	<0.001	0.734	<0.001	0.435	0.060	0.551

†Root injury scores were evaluated in 2008 only.

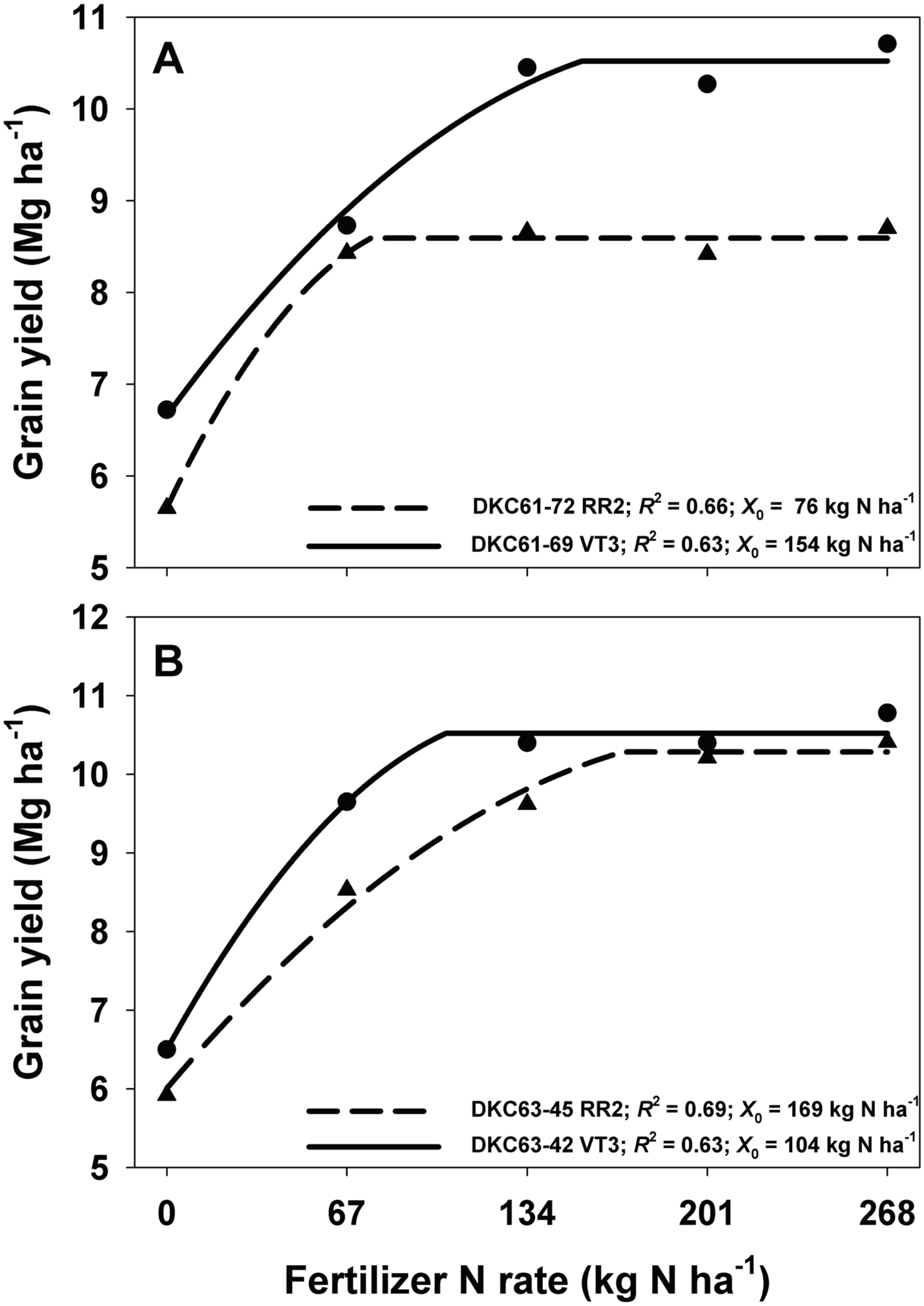

Figure 1. Response of grain yield to N for refuge (Roundup Ready Corn 2 [RR2]) and transgenic rootworm protected (YieldGard VT Triple [VT3]) hybrids. The hybrids included a 111 d relative maturity (RM) pair (A) (DKC61-72 RR2 and DKC61-69 VT3) and a 113 d RM pair (B) (DKC63-45 RR2 and DKC63-42 VT3). Curves were fitted using quadratic with plateau regression functions with data combined across both years of the study. All regression models were significant at $P \leq 0.001$. Predicted maximum grain yields were 8.6 and 10.5 Mg ha^{-1} for DKC61-72 and DKC61-69 and 10.3 and 10.5 Mg ha^{-1} for DKC63-45 and DKC63-42. Individual points represent the grain yield means for each hybrid × N rate treatment combination averaged across both years of the study.

and DKC61-69 were not significantly different at 0 kg N ha^{-1} (mean = 254 mg per kernel) or 67 kg N ha^{-1} (mean = 275 mg per kernel). At N application rates greater than or equal to 134 kg N ha^{-1}, CRW protection in DKC61-69 was associated with an average increase of nearly 17 mg per kernel over its non-Bt counterpart. The corresponding average increase in kernel number at rates greater than or equal to 134 kg N ha^{-1} was 451 kernels m^{-2}. At an average kernel weight for DKC61-72 of 282 mg per kernel, this increase in kernel number would result in a yield increase of 1.3 Mg ha^{-1}. The mean increase in grain yield for DKC61-69 relative to DKC61-72 for N rates between 134 and 268 kg N ha^{-1} was approximately 1.9 Mg ha^{-1}. As such, increased kernel weight accounted for approximately 32% (0.6 Mg ha^{-1}) of the yield increase associated with CRW protection in DKC61-69.

Table 3. Yield and yield components of non-*Bacillus thuringiensis* (Bt) (Roundup Ready Corn 2 [RR2]) and Bt (YieldGard VT Triple [VT3]) hybrid pairs grown at Champaign, IL, in 2008 and 2009. All values are presented on a dry-matter basis (0 g kg^{-1} moisture concentration). Values are the means across both years of the study.

	Hybrid pair 1			Hybrid pair 2		
N rate	DKC61-72 RR2	DKC61-69 VT3	$P > \|t\|$	DKC63-45 RR2	DKC63-42 VT3	$P > \|t\|$
kg N ha^{-1}	Grain yield, Mg ha^{-1}					
0	5.7	6.7	**	5.9	6.5	NS†
67	8.4	8.7	NS	8.5	9.6	**
134	8.7	10.4	***	9.6	10.4	NS
201	8.4	10.3	***	10.2	10.4	NS
268	8.7	10.7	***	10.4	10.8	NS
Mean	8.0	9.4	***	8.9	9.5	**
	Kernel number, m^{-2}					
0	2192	2462	NS	2439	2617	NS
67	3093	3195	NS	3446	3715	*
134	3089	3551	***	3689	3929	NS
201	3049	3474	**	3859	3841	NS
268	3055	3519	***	3854	3852	NS
Mean	2896	3240	***	3457	3591	NS
	Kernel weight, mg per kernel					
0	255	253	NS	241	248	NS
67	276	273	NS	248	259	NS
134	282	295	*	262	266	NS
201	278	296	**	267	272	NS
268	287	306	**	272	280	NS
Mean	276	285	**	258	265	NS

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

†NS, not significant: means not significantly different at $P < 0.05$.

Grain yield of DKC63-42 was statistically greater than DKC63-45 at 67 kg N ha^{-1} ($P \leq 0.01$; Table 3). The greater yield of DKC63-42 (1.1 Mg ha^{-1}) relative to DKC63-45 resulted from a 269 kernel m^{-2} increase in kernel number ($P \leq 0.05$) and kernel weight, which trended 11 mg per kernel higher (not significant).

The increase in kernel number for CRW protected hybrids in this study has also been observed for chemical control of Western CRW (Cox et al., 2008). In that study, comparison of an untreated control to the insecticidal seed treatment clothianidin [(E)-1-(2-cholor-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine] resulted in increased kernel number and grain yield (328 kernels m^{-2} and 0.9 Mg ha^{-1}, respectively). In contrast to our results, Cox et al. (2008) did not find any improvement in kernel weight attributable to CRW control. Similarly, Kahler et al. (1985) and Urías-López and Meinke (2001) showed that kernel weight was unaffected by minor to moderate root damage. Therefore, transgenic CRW protection might have a unique characteristic of promoting increased kernel weight in addition to greater kernel number in some genetic backgrounds. The role of root growth and metabolism in determining yield components in maize is largely unknown; however, cytokinins synthesized in root tissue have been linked to seed number and weight in other cereal crops. For example, Yang et al. (2002) reported that the zeatin and zeatin riboside concentrations of rice (*Oryza sativa* L.) endosperm tissue were positively and significantly correlated ($r = 0.95$) with the rate of endosperm cell division. The concentration of cytokinins in the root tips was also correlated with endosperm cell division rate, leading the authors to conclude that root synthesized cytokinins play a critical role in regulating endosperm development and, consequently, individual seed weight. Similarly, ribonucleic acid interference mediated silencing of cytokinin oxidase expression in barley (*Hordeum vulgare* L.) root tissue (i.e., increased cytokinin concentration) resulted in increased grain yield, number of seeds per plant, and 1000 grain weight (Zalewski et al., 2010). As a result of the apparent link between root cytokinins and yield components, improved root growth and activity resulting from transgenic CRW protection in maize could enhance the synthesis or export of cytokinins from the root, thereby explaining the measured increase in kernel weight. An alternative yet complementary hypothesis is that a CRW protected root system allows the plant to acquire more N after flowering. Increased postflowering N uptake might prolong functional leaf area (stay-green), source activity, and as a result, individual kernel weight (Ma and Dwyer, 1998; Martin et al., 2005).

Table 4. Regression parameter estimates and R^2 values for N uptake and grain yield as a function of fertilizer N rate. Non-*Bacillus thuringiensis* (Bt) (Roundup Ready Corn 2 [RR2]) and Bt (YieldGard VT Triple [VT3]) hybrid pairs were grown at Champaign, IL, in 2008 and 2009. All regression models were significant at $P \leq 0.001$.

				Parameter estimates					
Trait	Year	Hybrid	Model†	β_0‡	β_1‡	β_2‡	X_0§	Plateau	R^2
Grain yield, Mg ha^{-1}	2008	DKC61-72 RR2	QP	6.2	0.046	−0.00022	77	8.4	0.62
		DKC61-69 VT3	QP	6.7	0.043	−0.00011	153	10.8	0.79
		DKC63-45 RR2	QP	6.3	0.036	−0.00008	173	10.2	0.74
		DKC63-42 VT3	QP	6.6	0.078	−0.00033	88	10.9	0.76
	2009	DKC61-72 RR2	QP	5.1	0.078	−0.00039	76	8.8	0.74
		DKC61-69 VT3	QP	6.6	0.037	−0.00009	156	10.2	0.52
		DKC63-45 RR2	QP	5.7	0.045	−0.00010	166	10.4	0.66
		DKC63-42 VT3	QP	6.4	0.044	−0.00012	138	10.2	0.57
R6 N uptake, kg N ha^{-1}	2008	DKC61-72 RR2	LP	88	1.31	–	69	179	0.75
		DKC61-69 VT3	LP	90	0.77	–	165	216	0.82
		DKC63-45 RR2	QP	92	0.81	−0.0013	232	209	0.70
		DKC63-42 VT3	LP	104	0.91	–	111	204	0.67
	2009	DKC61-72 RR2	LP	84	0.78	–	142	195	0.73
		DKC61-69 VT3	QP	95	1.22	−0.0029	160	218	0.86
		DKC63-45 RR2	LP	96	0.73	–	160	212	0.84
		DKC63-42 VT3	L	98	0.48	–	–	–	0.78

†LP, linear with plateau regression model; QP, quadratic with plateau regression model; L, linear regression model.

‡β_0, β_1, and β_2 are the intercept, linear coefficient, and quadratic coefficients, respectively.

§X_0, fertilizer N rate (kg N ha^{-1}) at which the linear or quadratic portions of the response curve join the plateau portion.

Biologically Optimum Nitrogen Fertilizer Rates for Non-*Bacillus thuringiensis* and *Bacillus thuringiensis* Hybrids

Quadratic with plateau regression models were used to describe the response of grain yield to N rate for each hybrid within each year (Table 4). Averaged across hybrids, the N rates required to achieve maximum yield were 123 and 134 kg N ha^{-1} in 2008 and 2009, respectively (Table 4). Despite similar optimum N rates between years, the optimum N rate for each hybrid varied considerably and was impacted differently by CRW protection in each hybrid pair. In 2008, maximum grain yield of DKC61-69 occurred at 153 kg N ha^{-1} while its non-Bt counterpart achieved maximum yield at only 77 kg N ha^{-1}. A similar response was measured in 2009; optimum N rates for DKC61-72 and DKC61-69 were 76 and 156 kg N ha^{-1}, respectively. Therefore, the lower N fertilizer requirement of DKC61-72 could be an indication that N uptake was a yield-limiting factor and that CRW protection in DKC61-69 removed this limitation. In contrast to the increased optimum N rate for maximum yield for the Bt hybrid in the DKC61-72 by DKC61-69 comparison, the optimum N rate for the Bt hybrid in the DKC63-45 by DKC63-42 comparison was decreased relative to the non-Bt hybrid. In 2008, optimum N rates for maximum yield were 173 and 88 kg N ha^{-1} for DKC63-45 and DKC63-42, respectively. Similarly, optimum N rates for this hybrid comparison in 2009 were 166 and 138 kg N ha^{-1} for maximum yield.

The differences in improvement of N response for yield for each non-Bt to Bt comparison could be related to the contrasting yield component and N utilization characteristics of each genetic background. For example, the DKC61-72 and DKC61-69 genetic background exhibited an average kernel number of 3068 kernels m^{-2} and an average kernel weight of 281 mg per kernel (Table 3). In contrast, average kernel number and kernel weight for the DKC63-45 and DKC63-42 genetic background was 3524 kernels m^{-2} and 262 mg per kernel, respectively. Kernel number and kernel weight have different optimum N rates, with kernel number achieving its maximum at a lower rate of fertilizer N compared to either kernel weight or total grain yield (J.W. Haegele and F.E. Below, unpublished data, 2012). Therefore, the greater kernel number phenotype of the DKC63-45 and DKC63-42 genetic background might be expected to respond more to an improvement in N uptake at a reduced rate of N fertilizer application. Furthermore, the DKC63-45 and DKC63-42 genetic background had greater N utilization efficiency (increase in grain yield per increase in plant N uptake over the unfertilized check plot treatment). Averaged across N rates and years, measured N utilization efficiency of DKC63-45 and DKC63-42 was 48.0 kg grain kg^{-1} plant N content while average N utilization efficiency of DKC61-72 and DKC61-69 was 33.7 kg grain kg^{-1} plant N (data not shown). Therefore, for the same increase in N uptake, the DKC63-45 and DKC63-42 genetic background would result in an approximate 43% increase in grain yield relative to the DKC61-72 and DKC61-69 background.

Nitrogen Uptake, Nitrogen Uptake Efficiency, and Nitrogen Use Efficiency

Appropriate regression models were used to describe the response of plant N uptake to fertilizer N rate for each hybrid within each year (Table 4). With the exception

Table 5. Grain yield, N uptake, N use efficiency (NUE), and N uptake efficiency (NUpE) of hybrids evaluated at the N rates required to optimize grain yield for corn rootworm protected *Bacillus thuringiensis* hybrids. Biologically optimum N rates (Opt. N rate, i.e., N rates required to achieve maximum grain yield) for DKC61-69 YieldGard VT Triple (VT3) were 153 and 156 kg N ha^{-1} in 2008 and 2009, respectively. Likewise, optimum N rates for DKC63-42 VT3 were 88 and 138 kg N ha^{-1} in 2008 and 2009, respectively. The values presented for grain yield and N uptake are those predicted by regression ± 90% confidence intervals. Confidence intervals for derived efficiency traits (NUE and NUpE) were constructed by evaluating the values corresponding to the lower and upper limits of the 90% confidence intervals for grain yield and N uptake in Eq. [5] and [6].

Year	Hybrid	Grain yield		N uptake		Efficiencies	
		0 kg N ha^{-1}	Opt. N rate	0 kg N ha^{-1}	Opt. N rate	NUE	NUpE
		Mg ha^{-1}		kg N ha^{-1}		kg grain kg^{-1} N	kg plant N kg^{-1} N
2008	DKC61-72 RR2†	6.2 ± 0.6	8.4 ± 0.4	88 ± 21	179 ± 11	14.4 ± 1.3	0.59 ± 0.07
	DKC61-69 VT3	6.7 ± 0.8	10.8 ± 0.5	90 ± 19	208 ± 13	26.8 ± 2.0	0.77 ± 0.04
	DKC63-45 RR2	6.3 ± 0.8	8.8 ± 0.9	92 ± 25	153 ± 29	28.4 ± 1.1	0.69 ± 0.05
	DKC63-42 VT3	6.6 ± 0.8	10.9 ± 0.9	104 ± 26	184 ± 28	48.9 ± 1.1	0.91 ± 0.02
2009	DKC61-72 RR2	5.1 ± 0.8	8.8 ± 0.5	84 ± 23	195 ± 16	23.7 ± 1.9	0.71 ± 0.04
	DKC61-69 VT3	6.6 ± 1.2	10.2 ± 0.8	95 ± 18	216 ± 12	23.1 ± 2.6	0.78 ± 0.04
	DKC63-45 RR2	5.7 ± 1.2	10.0 ± 0.5	96 ± 22	197 ± 9	31.2 ± 5.1	0.73 ± 0.09
	DKC63-42 VT3	6.4 ± 1.2	10.2 ± 0.5	98 ± 18	164 ± 10	27.5 ± 5.1	0.48 ± 0.06

†RR2, Roundup Ready Corn 2.

of DKC63-42 evaluated in 2009 (linear response; R^2 = 0.78), N uptake curves were best described by linear with plateau or quadratic with plateau regression models (R^2 values ranging from 0.67 to 0.86). Averaged across hybrids, predicted maximum N uptake was 202 kg N ha^{-1} in 2008 and 208 kg N ha^{-1} in 2009. Much like grain yield, plant N uptake was affected differently in each hybrid comparison. Maximum N uptake values predicted by linear with plateau regression models for DKC61-72 and DKC61-69 in 2008 were 179 and 216 kg N ha^{-1}, respectively. These levels of plant N uptake occurred at correspondingly predicted N fertilizer application rates of 69 and 165 kg N ha^{-1}. Predicted maximum plant N uptake values for DKC63-45 and DKC63-42 were similar (mean = 207 kg N ha^{-1}); however, DKC63-42 achieved its maximum plant N uptake with 52% less application of fertilizer N.

Currently, maize producers using single mode-of-action transgenic CRW resistant Bt hybrids are required to plant 20% of their maize production area to a non-Bt hybrid (Environmental Protection Agency, 2011). This structured refuge is intended to slow the development of resistance of CRW to currently available transgenic control options. As such, 80% of a field is planted to the Bt hybrid, and agronomic management practices should be targeted to this hybrid. Differences in optimum N rates for grain yield of non-Bt and Bt hybrids in this study, which were repeatable across years, are evidence that maize hybrids improved through biotechnology may have different agronomic management requirements compared to conventional (non-Bt) hybrids. Furthermore, grain yield and N uptake at the optimum N rates for the Bt hybrids within each comparison are likely closer to the true potential of each genetic background. Therefore, comparisons of N uptake and NUE should be made at the N rate required to achieve maximum yield of the CRW protected Bt hybrids.

A 2.4 Mg ha^{-1} improvement in grain yield over DKC61-72 was predicted at the optimum N rate for yield (153 kg N ha^{-1}) for DKC61-69 in 2008 (Table 5). This remarkable increase in yield was associated with a 16% increase in aboveground plant N uptake ($P \leq 0.10$). Increased responsiveness to fertilizer N for the Bt hybrid (4.1 versus 2.2 Mg ha^{-1}) for this comparison pair resulted in a significant improvement in NUE (increase in grain yield per unit of applied N). At 153 kg N ha^{-1}, calculated NUE values were 14.4 and 26.8 kg grain kg^{-1} N for DKC61-72 and DKC61-69, respectively ($P \leq 0.10$). Similarly, there was a marked increase in NUpE associated with CRW protection; DKC61-69 recovered 77% of applied fertilizer N while DKC61-72 recovered only 59%. Like the DKC61-72 by DKC61-69 comparison, the Bt hybrid in the second comparison (DKC63-45 by DKC63-42) had an N uptake value that was approximately 20% greater than its non-Bt counterpart at 88 kg N ha^{-1}. Significant improvements in NUE (+20.5 kg grain kg^{-1} N) and NUpE (+0.22 kg plant N kg^{-1} N) also occurred at the optimum N rate for yield of DKC63-42 in 2008 ($P \leq 0.10$).

Unlike 2008, no significant improvements in NUE or NUpE occurred in 2009 (Table 5). Nitrogen use efficiency and NUpE are calculated as the difference between a plot receiving some application of N fertilizer and an unfertilized check plot (Eq. [5] and [6]). As such, NUE and NUpE can be decreased by increasing the value of the check plot treatment. Although a significant increase in grain yield (+1.4 Mg ha^{-1}; $P \leq 0.10$) occurred over DKC61-72 at the N rate required to achieve maximum yield for DKC61-69 in 2009 (156 kg N ha^{-1}), a similar increase in grain yield (1.5 Mg ha^{-1}) occurred at 0 kg N ha^{-1}. As such, calculated NUE values were similar for DKC61-72 and DKC61-69 in 2009. Similarly, grain yield of DKC63-42 trended 0.7 Mg ha^{-1} greater at low N in comparison to its non-Bt counterpart DKC63-45 while they had similar yields (mean = 10.1 Mg ha^{-1}) at the optimum N rate for DKC63-42. As a result of greater apparent performance at low N and maximum yield, which occurred at a 20% lower N rate, DKC63-42 had reduced NUE compared to its non-Bt counterpart (Table 5). Therefore, differences in NUE should be carefully interpreted relative to performance at low N.

Low N fertilizer stress tended to be greater in 2009 compared to 2008, particularly for non-Bt hybrids (Table 5). Average predicted grain yields for non-Bt hybrids at low N were 6.3 and 5.4 Mg ha^{-1} in 2008 and 2009, respectively. In contrast, average predicted grain yields for Bt hybrids at low N were 6.7 and 6.5 Mg ha^{-1} in 2008 and 2009, respectively. Although not captured in small research plots, improved stability of grain yield at low N as a result of CRW protection would likely increase average grain yield across commercial scale maize production fields that possess spatial variability for potential soil N supply or risk of fertilizer N loss (Baxter et al., 2003). Therefore, while Bt hybrids did not use fertilizer more efficiently in 2009, better low N tolerance is another indication of the improved agronomic utility of transgenic CRW protected maize.

CONCLUSIONS

Our initial hypothesis was that CRW protected transgenic hybrids would exhibit increased N uptake, resulting in higher grain yield and improved NUE relative to their non-Bt counterparts. This hypothesis is supported by the 2008 data since increases in grain yield and N use occurred even in the absence of severe injury from CRW feeding. In contrast, there were no significant improvements in fertilizer N use attributable to Bt hybrids in 2009 even though grain yield was still increased in one hybrid comparison. Most of the benefit of CRW protection in 2009 occurred at low N, indicating that the utility of transgenic CRW protection traits can vary according to environment. Furthermore, optimum N rates for grain yield were affected differently by CRW protection in each hybrid comparison. In one case, similar yield was achieved at a lower fertilizer N rate suggesting that biotechnological improvements might be used to reduce N inputs in response to environmental or economic concerns. In the second case, an increase in grain yield attributable to CRW protection occurred at a higher rate of fertilizer N relative to the non-Bt hybrid, which is a strategy for protecting yield potential at a predetermined input of fertilizer. Additionally, these contrasting N response types as a result of the Bt trait might help to provide an explanation for the apparent lack of measurable responses to CRW protection traits in some studies. For example, in our evaluation of DKC63-45 and DKC63-42, most of the benefit of CRW protection to grain yield occurred at lower rates of N fertilizer. As such, a trait comparison made at a more typical N fertilizer recommendation for maize production might result in no detectable response. These results also suggest a role for plant breeding in selectively improving genetic backgrounds for the desired agronomic responses to a biotech insect protection trait. Germplasm with an inherently large efficiency of N utilization might be most desirable for magnifying small improvements in N uptake potentially resulting from season long protection of the root system. In conclusion, transgenic corn rootworm protection does have agronomic benefits that extend beyond insect control, and these data support the role of biotechnology in promoting sustainable and resource use efficient maize production to feed a growing world population.

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