Transgenic Corn Rootworm Protection Enhances Uptake and Post-Flowering Mineral Nutrient Accumulation

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ABSTRACT

Although modern maize (*Zea mays* L.) hybrids with transgenic insect protection from corn rootworm (CRW) (*Diabrotica* spp.) demonstrate improved yield and insect control compared to their non-protected (refuge) counterparts, no comprehensive studies have documented the impact of transgenic insect protection on nutrient uptake and partitioning. The objective of this study was to investigate the effect of transgenic protection from CRW on the timing and quantity of uptake for key nutrients such as N, P, K, S, and Zn. Results from two similar experiments across 5 site-years were analyzed and summarized. In the first experiment, transgenic hybrids averaged greater grain yield (10%; 0.9 Mg ha^{-1}), total biomass (7%; 1.2 Mg ha^{-1}), and grain nutrient accumulation of N (8%), P (12%), K (9%), S (9%), and Zn (12%) compared to non-protected hybrids ($P \leq 0.05$). In the second experiment, the yield response associated with transgenic insect protection varied among hybrids. Those hybrids which exhibited a yield response compared to their non-protected counterparts resulted in greater post-flowering acquisition of N (31%), P (24%), and K (38%) ($P \leq 0.05$). The results indicate that in favorable environments, transgenic CRW protected hybrids not only produce more total biomass and yield, but also maintain greater rates of nutrient acquisition during grain-filling.

Corn rootworm is among the most damaging insect pests of maize production in the United States costing farmers an estimated U.S.$1 billion annually as a result of yield loss and chemical control measures (Metcalf, 1986; Agricultural Research Service, 2001). While CRW larvae primarily feed on maize roots (Gray et al., 2009), adult beetles consume silk, pollen, and kernel tissue (Moeser and Vidal, 2005). This damage to above- and belowground tissues reduces grain yield, total dry weight production, and accumulation of key nutrients (Kahler et al., 1985; Godfrey et al., 1993; Rice, 2004). Control of CRW larvae and adult insect populations to mitigate root feeding and yield loss traditionally involved the use of crop rotation and insecticides. The reduced effectiveness of these strategies occurred with the development of insect resistance to seed and foliar-applied insecticides (Meinke et al., 1998), and CRW behavioral changes including extended egg diapause and loss of ovipositional fidelity to maize for Northern CRW (*D. barberi* Smith and Lawrence) and Western CRW (*D. virgifera virgifera* LeConte), respectively (Gray et al., 2009). Transgenic hybrids expressing the *Bacillus thuringiensis* (Bt) toxin, developed for control of CRW, have been rapidly adopted during the past 10 yr in the United States. While the benefits of Bt hybrids include improved consistency of insect control, healthier root systems, greater yields, improved N use, and improved grain quality (Rice, 2004; Folcher et al., 2010; Haegele and Below, 2013), important agronomic questions remain unanswered. Specifically, it is unknown if mineral nutrient uptake patterns, especially in more stable, higher yielding Bt hybrids (Edgerton et al., 2012), differ in comparison to their non-Bt (refuge) counterparts.

While 17 widely accepted plant essential nutrients exist, recent work has documented that N, P, K, S, and Zn are required for maize production in greater quantities, and with the exception of K, these nutrients have relatively high nutrient harvest index (HI) values (Bender et al., 2013). To maximize nutrient use, maize production will require fertilizer availability during key growth stages, especially for these nutrients. Physical transport of soil nutrients is achieved by mass flow and diffusion through soil solutions (Barber, 1962). According to Barber (1994), as much as 93% of P uptake and 80% of K uptake in maize is acquired through diffusion compared to mass flow, which accounts for up to 79 and 100% of N and S accumulation, respectively. The majority of N and K accumulation occurs during vegetative growth, in contrast to P, S, and Zn, which are primarily acquired during grain fill (Sayre, 1948; Hanway, 1962; Karlen et al., 1988; Bender et al., 2013). Total dry weight production after the initiation of reproductive growth (i.e., silk emergence) is partitioned directly into developing grain as opposed to other plant tissues. As a result, it is not surprising that nutrient accumulation during grain fill, especially in nutrients with high harvest index values (e.g., N, P, S, and Zn), is partitioned into maize grain (Bender et al., 2013). Although N is the only nutrient with a relatively high harvest index and relatively low post-flowering uptake, N is rapidly translocated to grain tissues during grain fill.

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Abbreviations: Bt, *Bacillus thuringiensis*; CRW, corn rootworm *Diabrotica* spp.; HI, harvest index; R2, blister stage; R4, dough stage; R6, physiological maturity; RM, relative maturity; RR2, Roundup Ready Corn 2; VT3, YieldGard VT Triple; V6, six leaves with collars visible; V10, 10 leaves with collars visible; V14, 14 leaves with collars visible.

Transgenic protection from CRW larval feeding may increase the soil volume explored by maize root systems, thereby influencing the quantity and duration of total accumulation for nutrients such as P, which are taken up through diffusion and root interception.

A recent study documented that CRW resistant Bt hybrids resulted in greater yield (1.1 Mg ha^{-1}) and overall N uptake by up to 31 kg ha^{-1} compared to non-Bt isolines (Haegele and Below, 2013). Furthermore, some Bt hybrids achieved a greater response to fertilizer N and maximum yield with lower N rates than their non-transgenic counterpart, suggesting that yield improvements associated with transgenic insect protection are sometimes accompanied by better N use efficiency. Ma et al. (2009) documented yield increases of 11 to 66% from CRW resistant hybrids relative to non-Bt counterparts even with minimal CRW feeding. Despite larger root systems with decreased nodal injury and lodging, the effect of Bt insect protection on the quantity and duration of nutrient accumulation was not evaluated.

The objective of this research was to quantify total nutrient uptake and partitioning across several Bt and non-Bt hybrid comparisons. An additional objective was to measure season-long biomass and nutrient accumulation among selected Bt comparisons to more thoroughly understand differences in nutrient uptake and partitioning patterns that might result from transgenic CRW protection. Our hypothesis was that transgenic insect protection would result in greater yield and total nutrient accumulation, and that any differences would occur during grain fill to meet sink demands.

MATERIALS AND METHODS

Cultural Practices, Experimental Design, and Treatments

Field experiments were conducted at the Northern Illinois Agronomy Research Center in DeKalb, IL (2009–2010) and at the Research and Education Center in Champaign, IL (2008–2010). The soil type at DeKalb was a Flanagan silt loam soil (fine, smectitic, mesic Aquic Argiudolls) and nutrient test levels at the 0- to 15-cm depth measured 27 g kg^{-1} organic matter, pH 7.2, 6 mg kg^{-1} NO_3–N, 53 mg kg^{-1} P, 244 mg kg^{-1} K, 823 mg kg^{-1} Mg, and 3051 mg kg^{-1} Ca. The soil at the Champaign site was a Drummer-Flanagan silty clay loam soil (fine-silty, mixed, superactive, mesic Typic Endoaquolls), and nutrient test levels at the 0- to 15-cm depth measured 44 g kg^{-1} organic matter, pH 5.8, 3 mg kg^{-1} NO_3–N, 40 mg kg^{-1} P, 153 mg kg^{-1} K, 491 mg kg^{-1} Mg, and 2936 mg kg^{-1} Ca. The minerals P, K, Mg, and Ca were extracted using Mehlich III solution (Brown, 1998). Soybean [*Glycine max* (L.) Merr.] was the previous crop at each site. Plots were planted between 20 May and 30 May during each year to achieve an approximate final stand of 79,000 plants ha^{-1} (2008 and 2009) and 84,000 plants ha^{-1} (2010). Individual experimental plots consisted of six (2008 and 2009) or four rows (2010), 11.4 m in length with 0.76 m spacing. Weed control consisted of a pre-emergence application 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), and a post-emergence application of glyphosate [*N*-(phosphonomethyl)glycine].

The first experiment was conducted during 2008 (Champaign location only) and 2009 (Champaign and DeKalb) to compare CRW protected Bt hybrids and their near-isogenic non-Bt counterparts for total nutrient uptake and grain nutrient removal. This experiment was part of a larger study designed to evaluate the effect of CRW protected transgenic hybrids on N uptake and utilization across different levels of N (Haegele and Below, 2013). 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. For the nutrient uptake and removal study described here, N rates of 134, 201, and 268 kg N ha^{-1} were selected to represent a range of typical farm N application rates. Nitrogen was applied as granular $(NH_4)_2SO_4$ (21–0–0–24S) in a diffuse band after emergence and incorporated when the seedlings had either two or three visible leaf collars. Four locally adapted maize hybrids were selected to represent two separate comparisons. Each comparison pair contained a hybrid with glyphosate tolerance only (Roundup Ready Corn 2 [RR2], Monsanto Company, St. Louis, MO) and a hybrid with herbicide tolerance plus transgenic insect protection from European corn borer (*Ostrinia nubilalis*) (Cry1Ab protein from *Bacillus thuringiensis*) and CRW (Cry3Bb1 protein from *Bacillus thuringiensis*). Thus, the Bt hybrid was a "triple-stack" hybrid (YieldGard VT Triple [VT3], Monsanto Company, St. Louis, MO). Hybrids DKC61-72 RR2 (Non-Bt) and DKC61-69 VT3 (Bt) represented a 111 d relative maturity (RM) comparison, and DKC63-45 RR2 (Non-Bt) and DKC63-42 VT3 (Bt) represented a 113 d RM comparison. We cannot conclusively state that these hybrid pairs are near-isogenic lines; however, they have been marketed by the seed supplier as representing different trait versions of the same genetic background. Non-Bt hybrids 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-dimethylcyclopropane-carboxylate] at a rate of 0.11 kg a.i. ha^{-1} for control of seedling insect pests.

A second experiment was designed to further investigate the increase in total nutrient uptake associated with transgenic CRW protection that was measured in experiment one. Specifically, in 2010, we investigated the timing of nutrient uptake differences between non-Bt and Bt hybrids. Genetic comparisons were arranged in a randomized complete block design with four replications. Genetic comparisons included a hybrid with herbicide tolerance (RR2) and a hybrid with herbicide tolerance plus transgenic insect protection from certain aboveground insects (Cry1Ab, Cry2Ab2, or Cry1F proteins from *Bacillus thuringiensis*) and CRW (Cry3Bb1, mCry3A, Cry34Ab1, or Cry35Ab1 proteins from *Bacillus thuringiensis*). Hybrid pairs represented a range of maturities (111–114 d RM) and a range of seed brands. The hybrid pairs included DKC61-22 RR2 and DKC61-21 SmartStax (111 d RM), DKC61-72 RR2 and DKC61-69 VT3 (111 d RM), DKC63-45 RR2 and DKC63-42 VT3 (113 d RM), DKC64-27 RR2 and DKC64-24 VT3 (114 d RM) (Monsanto Company, St. Louis, MO); P33W80 RR2 and P33W84 Herculex XTRA (111 d RM) (Pioneer Hi-Bred, Johnston, IA); and Golden Harvest H-9014

GT and H-9014 Agrisure 3000GT (112 d RM; grown at Champaign only) (Syngenta Seeds, Minnetonka, MN).

In contrast to the insect control approach of experiment one where only non-Bt hybrids received a soil-applied insecticide, both non-Bt and Bt hybrids in experiment two received an in-furrow application of tefluthrin at a rate of 0.11 kg a.i. ha^{-1} for control of seedling insect pests. One week before planting, 202 kg N ha^{-1} as urea ammonium nitrate was applied and incorporated by shallow cultivation. At planting, 168 kg P_2O_5 ha^{-1} was applied as MicroEssentials SZ (12–40–0–10S–1Zn) (The Mosaic Company, Plymouth, MN) supplying an additional 50 kg N ha^{-1}, 42 kg S ha^{-1}, and 4.2 kg Zn ha^{-1}. At V6, a side-dress application of 67 kg N ha^{-1} was applied as urea with urease and nitrification inhibitors [$CO(NH_2)_2$ + n-(n-butyl) thiophosphoric triamide + dicyandiamide; 46–0–0] (Agrotain International, Saint Louis, MO). Applications of a soil insecticide and adequate fertilizer to meet the total uptake requirements of N, P, Zn, and S were intended to minimize environmental variation and thereby maximize inherent genetic differences in nutrient uptake potential.

Biomass Sampling, Nutrient Determination, and Yield Measurements

To evaluate biomass and nutrient accumulation, six representative plants per plot were sampled when at least 50% of the plants exhibited grain with a visible black layer (physiological maturity). Plants were sampled at the soil surface, and as a result, dry matter and nutrient measurements represent aboveground accumulation only.

During 2008 and 2009, sampled plants were separated into grain and stover (stalk, leaf, and non-grain reproductive tissue) components. During 2010, plants were sampled at vegetative leaf stage 6 (V6), vegetative leaf stage 10 (V10), vegetative leaf stage 14 (V14), reproductive blister (R2), reproductive dough (R4), and physiological maturity (R6) to evaluate season-long biomass and nutrient accumulation (Hanway, 1963; Ritchie et al., 1997). To more accurately determine the partitioning of nutrient accumulation in 2010, aboveground biomass was partitioned into stalk (stalk and leaf sheaths), leaf (leaf blades), reproductive (tassel, cob, and husk), and grain tissues when present. In all cases, sampled tissues were weighed fresh before shredding with a commercial brush chipper (Vermeer BC600XL, Pella, IA) to obtain a representative subsample. Moisture concentration was determined after subsamples were dried to a constant weight at 75°C and then used to determine partition dry weight. Reproductive tissues were dried whole to a constant weight for dry weight determination. Grain nutrient content at R4 was determined from hand-sampled plants, while grain yield and grain nutrient uptake calculations at R6 were measured using machine-harvested grain.

Dried vegetative and grain tissues were ground to pass through a 2-mm mesh screen and analyzed for N, P, K, S, and Zn (A and L Great Lakes Laboratories, Fort Wayne, IN). Nitrogen was analyzed using a combustion method, and other nutrients analyzed using a two-part process of acid-microwave digestion followed by Inductively Coupled Plasma (ICP) Spectrometry (Latimer and Horwitz, 2011). Total nutrient uptake and removal were determined algebraically from tissue nutrient concentrations and their respective dry weights.

Statistical Analysis

Grain yield, biomass accumulation, and total nutrient uptake and removal were analyzed using PROC MIXED (SAS Institute, 2009), while PROC UNIVARIATE was used to assess the normality of residuals and potential outliers. Site-year, hybrid pair, and level of transgenic protection (i.e., with or without transgenic protection from CRW) were included as fixed effects and replication nested within site-year was included as a random effect. Although yield differences due to N rate were observed during 2008 and 2009, no interactions between other main effects and N rate were apparent. Therefore, the means presented for experiment one were averaged across N levels and N rate was not included in the statistical model.

Biomass and nutrient accumulation figures were prepared with SigmaPlot (SigmaPlot v12.3; Systat Software Inc., San Jose, CA) using the simple spline curve option with smoothed data points. All units are expressed on a dry weight basis (0 g kg^{-1} moisture concentration).

RESULTS

Temperature and Precipitation

Monthly average temperatures during 2008 and 2009 were generally at or below the 20-yr average and were favorable for high yields (Table 1). Season-long precipitation at Champaign during 2008 was well above normal (+18.9 cm between 1 May and 30 September) and evenly distributed except for a rainfall deficit measured during August. The below average temperatures measured at both locations in 2009 were during the critical reproductive growth periods of July and August, improving silk emergence, pollination, and grain-filling duration.

Growing conditions at both locations during 2010 included above average temperatures, especially during grain-fill periods of late July and August. As a result, physiological maturity occurred nearly 2 wk earlier than normal (data not shown). Above average precipitation measured at DeKalb (+13.0 cm between 1 May and 30 September) was distributed throughout vegetative and early reproductive growth. This pattern of precipitation was considerably different than that measured at Champaign, where below average rainfall occurred during 4 mo of the growing season. However, the season-long deficit totaled only 2.8 cm at Champaign in 2010, due to nearly 18 cm of rainfall occurring during a 2-wk period in June.

2008 and 2009: Grain Yield and Biomass Accumulation

Yield and biomass accumulation differed markedly across site-years, by means of the presence of transgenic CRW protection and interactions with genetic background (Table 2). Transgenic protection from CRW increased yield by 0.9 Mg ha^{-1} ($P \leq 0.001$) when averaged across two hybrid comparisons and 3 site-years (Table 3). Yield responses were significant in four of the six comparisons evaluated, and the mean increase in yield of these significant comparisons was 1.3 Mg ha^{-1}.

The magnitude of yield response to Bt protection was hybrid pair specific (Tables 2 and 3). Hybrid DKC61-69 VT3 yielded 1.4 Mg ha^{-1} more ($P \leq 0.001$) than its non-Bt counterpart when averaged across 3 site-years. In contrast, the yield of DKC63-42 VT3 was only greater than its non-Bt counterpart

Table 1. Average monthly weather data at two locations for the period between 1 May and 30 September in 2008, 2009, and 2010. The average daily temperature, Tavg (°C), is for the corresponding month. Precipitation (cm) is the average monthly accumulated rainfall. Values in parentheses are the deviations from the 20-yr average (1991–2010).

	Month				
Location	**May**	**June**	**July**	**August**	**September**
Champaign, 2008					
Tavg, °C	15.1 (–2.1)	23.2 (+0.8)	23.6 (–0.3)	22.5 (–0.6)	19.5 (+0.3)
Precipitation, cm	14.9 (+3.0)	13.0 (+3.0)	20.2 (+8.4)	1.7 (–8.0)	20.2 (+12.5)
DeKalb, 2009					
Tavg, °C	15.1 (–0.7)	20.1 (–0.9)	18.9 (–3.1)	19.1 (–1.8)	16.3 (–0.5)
Precipitation, cm	9.3 (+0.8)	10.5 (+1.2)	6.4 (–2.1)	12.5 (+3.1)	2.8 (–5.2)
Champaign, 2009					
Tavg, °C	17.3 (+0.0)	22.8 (+0.4)	21.3 (–2.5)	21.4 (–1.6)	19.2 (+0.0)
Precipitation, cm	13.0 (+1.1)	10.8 (+0.9)	15.6 (+3.7)	13.7 (+4.0)	1.6 (–6.1)
DeKalb, 2010					
Tavg, °C	16.5 (+0.7)	21.1 (+0.2)	23.1 (+1.1)	22.4 (+1.5)	17.1 (+0.3)
Precipitation, cm	12.3 (+3.9)	17.5 (+8.2)	9.9 (+1.3)	11.9 (+2.6)	5.0 (–3.0)
Champaign, 2010					
Tavg, °C	18.3 (+1.0)	23.8 (+1.4)	25.2 (+1.3)	25.1 (+2.0)	19.7 (+0.6)
Precipitation, cm	7.8 (–4.0)	19.8 (+9.8)	9.0 (–2.8)	4.0 (–5.7)	7.6 (–0.1)

Table 2. Tests of fixed sources of variation on R6 yield parameters and total nutrient uptake for data collected at two locations during 2008 and 2009. Hybrid pair (H) is indicative of the genetic background of a hybrid comparison (i.e., hybrids of the same genetics differing only in the presence or absence of a Bt protection trait), while transgenic protection from CRW (R) is indicative of insect protection within each hybrid pair.

	Biomass parameters			R6 Nutrient uptake				
Source of variation	**Yield**	**Biomass**	**Harvest index**	**N**	**P**	**K**	**S**	**Zn**
	$P > F$							
Location (L)	0.0054	<0.0001	<0.0001	0.0001	<0.0001	0.7895	<0.0001	<0.0001
Hybrid pair (H)	0.0747	0.1334	0.9758	0.1150	0.3824	0.4348	0.3326	0.2992
L × H	0.0001	0.3105	0.0020	0.6662	0.0462	0.0820	0.8270	0.0161
Transgenic protection (R)	<0.0001	<0.0001	0.0004	0.0433	<0.0001	0.2019	0.3665	0.0450
L × R	<0.0001	0.0008	0.0551	0.1083	0.0108	0.1903	0.2251	0.2039
H × R	<0.0001	0.0205	0.0004	0.0370	0.0236	0.1421	0.0131	0.0524
L × P × R	0.0259	0.0003	0.0120	0.0462	0.3214	0.8666	0.0319	0.0217

Table 3. Grain yield, biomass accumulation, and harvest index for non-Bt and Bt maize hybrids evaluated at two locations during 2008 and 2009. Harvest index is the percentage of total aboveground biomass represented by grain. All values are presented on a dry matter basis (0 g kg^{-1} moisture concentration). Pair 1 is represented by DKC61-72 RR2 and DKC61-69 VT3, and pair 2 is represented by DKC63-45 RR2 and DKC63-42 VT3.

Location and comparison	Grain yield			Total biomass			Harvest index		
	Non-Bt	**Bt**	**$P > t$**	**Non-Bt**	**Bt**	**$P > t$**	**Non-Bt**	**Bt**	**$P > t$**
	Mg ha^{-1}						%		
Champaign (2008)									
Pair 1	8.4	10.8	***	15.4	19.1	***	54.7	56.7	ns†
Pair 2	10.0	10.9	**	17.9	19.0	*	56.0	57.5	ns
DeKalb (2009)									
Pair 1	9.4	10.0	*	19.2	18.7	ns	47.8	55.3	***
Pair 2	9.1	9.6	ns	18.7	19.9	ns	49.1	48.5	ns
Champaign (2009)									
Pair 1	8.8	10.1	***	18.1	20.3	***	48.4	50.1	ns
Pair 2	10.4	10.2	ns	20.2	20.1	ns	51.4	50.5	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 $P \leq 0.05$.

Table 4. Total and grain nutrient accumulation of above ground portions of the plant for non-Bt and Bt maize hybrids grown at two locations during 2008 and 2009. The means shown are for Bt hybrids only and values in parentheses indicate the difference relative to its non-Bt counterpart. All values are presented on a dry matter basis (0 g kg^{-1} moisture concentration). Significance levels compare means of Bt vs. non-Bt within a nutrient and plant part.

Nutrient uptake	Champaign, 2008		DeKalb, 2009		Champaign, 2009		Means
	DKC61-69 VT3	DKC63-42 VT3	DKC61-69 VT3	DKC63-42 VT3	DKC61-69 VT3	DKC63-42 VT3	
				kg ha^{-1}			
N							
Total	228 (+41)***	231 (+8)	217 (–5)	232 (+10)	203 (+25)*	195 (–19)	218 (+10)*
Grain	149 (+30)***	144 (+8)	136 (+10)	122 (+2)	131 (+22)***	123 (–9)	134 (+10)***
P_2O_5							
Total	70 (+17)***	68 (+9)*	89 (+5)	81 (+4)	70 (+8)*	59 (–4)	73 (+6)***
Grain	57 (+15)***	54 (+8)**	70 (+8)**	58 (+3)	57 (+8)**	48 (–3)	57 (+6)***
K_2O							
Total	180 (+29)*	175 (+8)	177 (+6)	171 (–1)	184 (+6)	156 (–10)	174 (+6)
Grain	42 (+8)***	43 (+6)***	38 (+3)	37 (+0)	31 (+4)*	32 (–1)	37 (+3)***
S							
Total	22 (+4)***	21 (–1)	23 (+0)	24 (+1)	26 (+2)	25 (–2)*	24 (+0)
Grain	12 (+3)***	12 (+1)*	11 (+0)	11 (+0)	12 (+2)***	13 (+0)	12 (+1)***
				g ha^{-1}			
Zn							
Total	320 (+74)***	311 (+4)	354 (–19)	345 (+20)	429 (+50)*	368 (–22)	354 (+18)*
Grain	194 (+56)***	193 (+15)	198 (+21)	199 (+29)*	195 (+13)	209 (–7)	198 (+21)***

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

in 1 site-year (Champaign 2008), where a significant increase of 0.9 Mg ha^{-1} occurred ($P \leq 0.01$).

Significant increases in grain yield were mirrored by greater total biomass accumulation (Table 3). Relative to DKC61-72 RR2, DKC61-69 VT3 produced approximately 1.8 Mg ha^{-1} ($P \leq 0.001$) more biomass. Much like its lesser yield advantage compared to the other pair, DKC63-42 VT3 increased biomass by only 1.1 Mg ha^{-1} ($P \leq 0.05$) in the one environment where a yield increase occurred. With the exception of a significant increase in harvest index associated with the Bt hybrid of the DKC61-72 RR2 and DKC61-69 VT3 comparison at DeKalb in 2009, harvest indices were generally similar between non-Bt and Bt hybrids (Table 3).

2008 and 2009: Total and Grain Nutrient Accumulation

Total nutrient uptake of N, P, S, and Zn at physiological maturity was subject to site-year effects as well as various interactions with CRW protection (Table 2). As observed with yield, uptake of N, P, and Zn was greater in DKC61-69 VT3 relative to DKC61-72 RR2 at Champaign in 2008 and 2009 (Table 4). Averaged across these 2 site-years, increases in nutrient uptake associated with DKC61-69 VT3 over its associated non-Bt hybrid were 33 kg N ha^{-1}, 13 kg P_2O_5 ha^{-1}, 3 kg S ha^{-1}, and 62 g Zn ha^{-1} (Table 4). Phosphorus was the only nutrient that had increased total accumulation as a result of the yield increase associated with DKC63-42 VT3 at Champaign in 2008. Increases in total nutrient accumulation due to Bt protection were also generally accompanied by increases in grain nutrient accumulation. The Bt hybrid DKC61-69 VT3 increased grain nutrient composition by an average of 26 kg N ha^{-1}, 11.5 kg P_2O_5 ha^{-1}, 2.5 kg S ha^{-1}, and 34.5 g Zn ha^{-1} over its non-Bt counterpart (Table 4). The influence of CRW Bt protection on K uptake and grain accumulation was less consistent. Potassium uptake was increased in DKC61-69 VT3 only at Champaign in 2008 (+29 kg K_2O ha^{-1}; $P \leq 0.05$). Although total uptake of K was not significantly greater due to CRW Bt protection, accumulation of K in the grain was greater for DKC63-42 VT3 at Champaign in 2008 (+6 kg K_2O ha^{-1}; $P \leq 0.001$) and for DKC61-69 VT3 at Champaign in 2009 (+4 kg K_2O ha^{-1}; $P \leq 0.05$).

2010: Grain Yield and Biomass Accumulation

Location, genetic background, and transgenic insect protection were significant sources of variation for grain yield during 2010 (Table 5). Grain yield at DeKalb was 0.6 Mg ha^{-1} greater ($P \leq 0.001$) than that measured at Champaign, and a significant location × transgenic protection interaction indicated that location influenced the yield response to Bt insect protection. The main effect of transgenic Bt protection at Champaign resulted in increased grain yield (+0.7 Mg ha^{-1}; $P \leq 0.001$) and biomass (+1.1 Mg ha^{-1}; $P \leq 0.01$) compared to DeKalb where there was no significant main effect of transgenic insect protection (Table 6). Consistent with experiment one, yield responses to transgenic insect protection varied across genetic backgrounds. Three of the six hybrid pairs resulted in significant increases in yield at Champaign during 2010, compared to only one of the five hybrid comparisons at DeKalb (Table 6). A similar pattern among hybrid comparisons was observed with total biomass production in which the increased biomass due to Bt insect protection typically occurred with hybrids that

Table 5. Tests of fixed sources of variation on yield parameters and R6 total nutrient uptake for non-Bt and Bt maize hybrids evaluated at two locations during 2010. Location (L), hybrid pair nested within location (H), and transgenic protection from CRW (R) served as fixed effects.

Measurement	Source of variation				
	Location (L)	Hybrid pair (H)†	Transgenic protection (R)	L×R	H×R
	$P > F$				
Yield and biomass					
Yield	0.0010	0.0013	0.0095	0.0004	0.0001
Biomass	0.2953	0.0033	0.1109	0.0220	0.0535
Harvest index	<0.0001	0.0071	0.6983	0.6575	0.2647
R6 Nutrient uptake					
N	0.0084	0.0591	0.0400	0.0714	0.0689
P	0.0023	0.0412	0.0266	0.0976	0.0277
K	0.3555	0.5030	0.2319	0.0598	0.1889
S	0.0170	<0.0001	0.8192	0.7616	0.0004
Zn	0.0046	0.0003	0.5709	0.0959	0.1817

† The H-9014 GT (non-Bt) and H-9014 3000GT (Bt) hybrid pair was not included at both locations and as a result, hybrid pair was nested within location.

also possessed significant yield responses. As with the 2008 and 2009 trials, Bt hybrids evaluated in 2010 did not have harvest index values that were significantly different from their non-Bt counterparts (data not shown).

2010: Total and Grain Nutrient Accumulation

Total aboveground nutrient uptake varied according to location, genetic background, and transgenic insect protection (Table 6). Nutrient uptake of Bt hybrids was not consistently greater than their non-Bt counterparts, particularly at DeKalb where yields of non-Bt and Bt hybrids were similar, or even in some examples where yields of non-Bt hybrids were greater than their Bt counterparts. One hybrid comparison (hybrid pair 1, DKC61-22 RR2 and DKC61-21 SmartStax) exhibited a consistent yield response to insect protection across both locations and increased uptake of N (+59 kg ha^{-1}), P (+27 kg P_2O_5 ha^{-1}), K (+41 kg K_2O ha^{-1}), and S (+4 kg ha^{-1}) at Champaign. Similarly, at DeKalb, relative to DKC61-22 RR2, DKC61-21 SmartStax displayed increased accumulation of 29 kg P_2O_5 ha^{-1} and 94 g Zn ha^{-1}.

The Bt hybrids in pairs 1, 2, and 4 achieved significantly greater yield (+1.0 Mg ha^{-1} average) compared to no differences among pairs 3, 5, and 6 at Champaign during 2010 (Table 6). As a result of these differences in response to transgenic insect protection, hybrids were divided into "yield responsive" and "non-responsive" groups and total nutrient accumulation was calculated during vegetative and reproductive growth. Possessing CRW protection did not significantly increase the quantity of biomass or nutrient acquisition during vegetative (pre-flowering) growth (Table 7). However, Bt protection increased biomass production (1.6 Mg ha^{-1}; $P \leq 0.01$) and accumulation of N (30.5 kg N ha^{-1}), P (13.2 kg P_2O_5 ha^{-1}) and K (25.9 kg K_2O ha^{-1}) in yield responsive pairs during grain fill. Greater nutrient accumulation resulting from Bt in non-responsive pairs was not significant and likely a consequence of reduced biomass production during grain fill. To further examine responses to transgenic insect protection, the timing and partitioning of biomass and P accumulation was evaluated (Fig. 1). Hybrids containing CRW protection in yield responsive pairs resulted in greater season-long biomass (+1.6 Mg ha^{-1}) and P (+13.2 kg P_2O_5) accumulation, which primarily occurred from R4 through R6 (Fig. 1). In comparison to non-Bt hybrids of yield responsive pairs, transgenic hybrids sustained relatively greater rates of biomass accumulation after R4 (150 vs. 66 kg ha^{-1} d^{-1}, respectively) (Fig. 1). A similar response was apparent for P, in which the rate of uptake after R4 in Bt-containing hybrids was nearly 70% greater than observed in non-Bt hybrids within the yield responsive pairs. Although the season-long patterns of biomass and nutrient accumulation are representative of six hybrid comparisons at one site, we believe that similar responses are likely across a greater number of locations and weather conditions when differential responses to transgenic insect protection occur.

DISCUSSION

Although we did not measure root injury from CRW, increased biomass accumulation and yield of Bt hybrids relative to non-Bt hybrids suggests that some level of insect feeding occurred. Root node-injury ratings from the 2008 Champaign location indicated that levels of insect feeding were negligible (Haegele and Below, 2013). While the physiological causes of yield responses to Bt protection from CRW when insect feeding is minimal have not been specifically examined, there are other examples in literature which support our results of a hybrid-dependent advantage to transgenic insect protection (Dillehay et al., 2004; Haegele and Below, 2013). Yield increases in Bt hybrids despite negligible root feeding were also observed by Ma et al. (2009), who speculated that these increases were attributable to a more robust fibrous root system of Bt hybrids. Current methods of assessing root injury from CRW feeding attempt to quantify damage to the primary root system (i.e., the proportion of complete root nodes damaged) (Oleson et al., 2005). We speculate that these methods may overlook injuries to secondary roots and root hairs that are more difficult to detect visually, and that injury to root hairs in particular may have a marked influence on nutrient and water uptake of maize plants.

The most significant yield gains were accompanied by increases in biomass (1.1–3.7 Mg ha^{-1}) with negligible contributions from improved harvest index (Table 3). Previous literature has documented the importance of greater total biomass production as a means to further improve maize grain yields and that the current harvest index in maize has likely been maximized (Hay, 1995; Lorenz et al., 2010). Increased productivity of Bt hybrids from CRW protection, therefore, is likely influenced by either greater solar radiation interception or improved efficiency at which light energy is converted into dry matter (Richards, 2000). Although marginal increases in leaf dry weight of Bt hybrids were measured (Fig. 1), it is plausible that healthier, more active roots of Bt hybrids, particularly during reproductive growth, promote greater leaf area duration.

Increased yield of Bt hybrids was variable and influenced by location and genetic background, similar to findings of Edgerton et al. (2012) and Shi et al. (2013). For example, Edgerton et al. (2012) reported that the yield response in so-called "triple-stack" Bt hybrids diminished with increasing control (non-Bt) yields. This pattern was consistent with our 2010 results in which

Table 6. Yield, aboveground biomass, and total nutrient uptake (N, P, K, S, and Zn) of non-Bt and Bt hybrids evaluated at two locations in 2010. Hybrids within each comparison pair share a common genetic background and differ in the presence of CRW Bt protection traits. The means shown are for Bt hybrids only and values in parentheses indicate the difference relative to its non-Bt counterpart. All values are presented on a dry matter basis (0 g kg^{-1} moisture concentration). Significance levels are comparing within a hybrid pair, or for means, the non-Bt to Bt hybrids within a location.

Location and hybrid comparison†	Yield	Biomass	N	P_2O_5	K_2O	S	Zn
	Mg ha^{-1}		kg ha^{-1}				g ha^{-1}
Champaign							
Pair 1	12.5 (+1.0) **	24.3 (+2.6)**	333 (+59)**	134 (+27)**	243 (+41)*	29 (+4)**	561 (+50)
Pair 2	12.4 (+0.9)**	23.8 (+0.7)	296 (+15)	108 (+16)	207 (+14)	26 (+1)	469 (+15)
Pair 3	12.5 (+0.6)	23.9 (+0.6)	295 (–4)	103 (+3)	211 (+10)	26 (+0)	485 (+7)
Pair 4	11.4 (+1.2)**	22.3 (+1.6)	296 (+25)	101 (–1)	202 (+11)	25 (+0)	473 (+9)
Pair 5	10.9 (–0.1)	22.0 (–1.2)	271 (–5)	109 (+7)	185 (–16)	27 (–8)***	497 (+10)
Pair 6	11.6 (+0.6)	24.9 (+2.5)*	302 (+31)	107 (+5)	217 (+38)	25 (+3)	455 (+73)
Means	11.9 (+0.7)***	23.5 (+1.1)**	298 (+20)**	110 (+10)**	211 (+16)*	26 (+0)	490 (+27)
DeKalb							
Pair 1	13.0 (+1.7)***	22.8 (+2.0)*	282 (+33)	134 (+29)**	207 (+16)	26 (+3)	564 (+94)*
Pair 2	12.5 (+0.0)	23.8 (–0.5)	299 (+8)	120 (+3)	192 (+14)	26 (+0)	553 (–36)
Pair 3	11.8 (–0.8)*	22.9 (–0.1)	270 (–4)	109 (–2)	217 (+1)	24 (+0)	491 (+1)
Pair 4	10.9 (–0.7)*	21.0 (–1.1)	258 (+0)	99 (–14)	174 (–15)	23 (–2)	424 (–51)
Pair 5	12.0 (–0.9)*	22.3 (–1.5)	261 (–31)	130 (–8)	178 (–35)*	27 (–2)	495 (–78)
Means	12.1 (–0.1)	22.5 (–0.2)	274 (+1)	118 (–2)	194 (–4)	25 (+0)	505 (–14)

* Significant at the 0.05 probability level

** Significant at the 0.01 probability level

*** Significant at the 0.001 probability level

† Pair 1, DKC61-22 RR2 and DKC61-21 SmartStax; Pair 2, DKC61-72 RR2 and DKC61-69 VT3; Pair 3, DKC63-45 RR2 and DKC63-42 VT3; Pair 4, DKC64-27 RR2 and DKC64-24 VT3; Pair 5, P33W80 RR2 and P33W84 Herculex XTRA; Pair 6, H-9014 GT and H-9014 Agrisure 3000GT (grown at Champaign only).

Table 7. Pre and post-flowering biomass and nutrient accumulation for yield responsive and non-responsive hybrid pairs grown at Champaign, IL, during 2010. Yield responsive hybrids are those pairs with significant yield increases resulting from transgenic insect protection and include pairs 1, 2, and 4 (Table 6). Non-responsive pairs did not measure a significant yield increase due to transgenic insect protection and include pairs 3, 5, and 6. The means are shown for Bt hybrids only and values in parentheses indicate the difference relative to the non-Bt counterparts. All values are presented on a dry matter basis (0 g kg^{-1} moisture concentration).

	Non-responsive hybrid pairs		Yield responsive hybrid pairs	
Measurement	Pre-flowering	Post-flowering	Pre-flowering	Post-flowering
Biomass, Mg ha^{-1}	7.9 (–0.2)	15.6 (+0.8)	8.0 (+0.1)	15.5 (+1.6)**
N, kg ha^{-1}	173.5 (–2.2)	115.7 (+9.7)	177.3 (+2.1)	130.1 (+30.5)*
P_2O_5, kg ha^{-1}	43.6 (+0.9)	62.1 (+6.3)	45.6 (+0.7)	68.8 (+13.2)*
K_2O, kg ha^{-1}	127.1 (+3.3)	77.3 (+7.4)	122.8 (–4.1)	94.4 (+25.9)*
S, kg ha^{-1}	11.7 (–0.2)	14.5 (–0.4)	11.7 (+0.0)	14.9 (+1.7)
Zn, g ha^{-1}	224 (–6)	254 (+36)	254 (+24)	248 (+1)

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

greater yield responses due to insect protection occurred at Champaign (non-Bt yield of 11.2 Mg ha^{-1}) compared to DeKalb (non-Bt yield of 12.2 Mg ha^{-1}) (Table 6). Similarly, Haegele and Below (2013) reported that one genetic background was consistently more responsive to the presence of the Bt trait compared to another genetic background. Collectively, our data and past examples from the literature suggest that differences in nutrient uptake and utilization among Bt hybrid pairs are influenced by a complex interaction of an individual hybrid's genetic potential for CRW damage and the environment.

In general, an individual genetic background's yield response to transgenic insect protection was largely dependent on differences in potential for greater biomass accumulation, which in turn led to greater nutrient accumulation. In maize production, N, P, K, S, and Zn are required in considerable quantities or have relatively high nutrient HI values (Bender et al., 2013); therefore, we focused on these nutrients for this study. Across hybrid pairs and locations, P accumulation appeared to be most consistently influenced by transgenic insect protection from CRW. Phosphorous uptake increased by 6 kg P_2O_5 ha^{-1} ($P \leq 0.001$) during 2008 and 2009 (Table 4), and by 10 kg P_2O_5 ha^{-1} ($P \leq 0.01$) at 2010 Champaign (Table 6). Barber (1994) documented that as much as 93% of total maize P uptake is acquired through diffusion, which is largely influenced by root length and surface area (Anghinoni and Barber, 1980; Schachtman et al., 1998). Increased root mass and length of transgenic Bt hybrids, even documented in minimal root-feeding conditions (Ma et al., 2009), may promote greater interception and accumulation of soil immobile nutrients like P.

The timing and rate of biomass production tended to differ among yield responsive and non-responsive hybrids. Pre-flowering biomass production (i.e., during vegetative growth) was similar for Bt and non-Bt hybrid comparisons, regardless of classification (Table 7). Yield-responsive Bt hybrids, however, produced greater biomass after flowering (Table 7) which primarily occurred between growth stages R4 and R6. Furthermore, the reduction in vegetative biomass (stalk, leaf, and reproductive tissues) of non-Bt hybrids by over 2300 kg ha^{-1} between R4 and R6 suggests that these hybrids were unable to adequately supply developing kernels with current assimilates (Fig. 1). Results also indicated that the greater yields associated with Bt protection were primarily due to greater kernel number

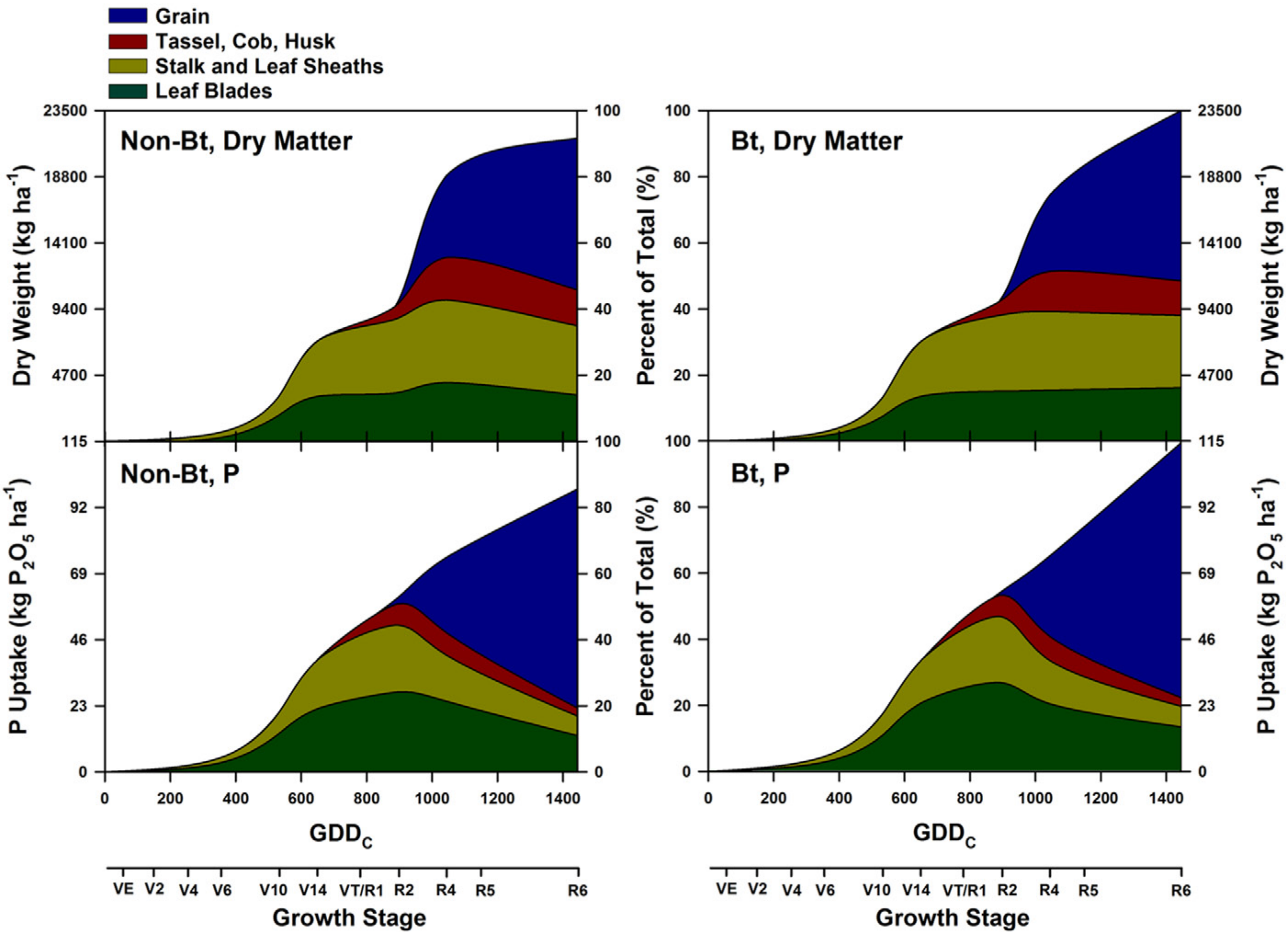

Fig. 1. Seasonal biomass and P accumulation among yield responsive hybrid pairs at Champaign during 2010. Yield responsive hybrids include the non-Bt and Bt comparison of hybrid pairs 1, 2, and 4 (Table 6). All values are presented on a dry matter basis (0 g kg^{-1} moisture concentration). Heat units are quantified with Modified Growing Degree Days (GDDc; minimum and maximum temperature limits of 10 and 30°C, respectively) (Cross and Zuber, 1972).

rather than kernel weight (data not shown). Although the increase in biomass production during grain fill may allow Bt hybrids to more fully achieve their maximum yield potential, it has been well documented that the number of potential ovules on each ear are determined during vegetative growth, and the final kernel number is established in the 2-wk period following pollination. As a result, we hypothesize that certain physiological determinants of yield increases associated with transgenic insect protection occur earlier in the season compared to the post-flowering effects of sustained biomass accumulation that we have documented.

Similar to the patterns of seasonal biomass production, differences in nutrient accumulation were observed only after flowering (Table 7). In yield responsive hybrid pairs, Bt insect protection significantly increased post-flowering uptake of N (31%), P (24%), and K (38%) over non-responsive pairs (Table 7). With respect to P, for example, Bt hybrids accumulated 13.2 and 10.8 kg more P_2O_5 ha^{-1} in total aboveground and grain only tissues, respectively, compared to non-Bt hybrids (Fig. 1). Rates of P acquisition, like biomass production, were greater in Bt than non-Bt hybrids between R4 and R6 (1.0 vs. 0.6 kg P_2O_5 ha^{-1} d^{-1}). Grain P concentration was slightly lower in non-Bt hybrids (7.06 g P_2O_5 kg^{-1} grain; 3.08 g P kg^{-1} grain) compared to Bt hybrids (7.29 g P_2O_5 kg^{-1} grain; 3.18 g P kg^{-1} grain). At a mean yield of 12.1 Mg ha^{-1} for the Bt hybrid pairs represented in Fig. 1, the increase in grain P content due to an increased grain P concentration was approximately 2.7 kg P_2O_5 ha^{-1}. Therefore, the remainder of the difference of grain P, approximately 8.1 kg P_2O_5 ha^{-1} (75%), was associated with the greater yields of Bt hybrids. When supplies of plant and soil P are limited, plants compensate by growing additional roots, translocate P from older leaves, and deplete vacuolar stores of inorganic P, thereby increasing P HI (Schachtman et al., 1998). The similar P HI values and greater rates of P accumulation during grain fill (Fig. 1) in Bt hybrids suggest that more active roots supported increased grain tissue needs, rather than depleting stored P.

CONCLUSIONS

Accurate prediction of nutritional needs in contemporary maize production will allow producers to more efficiently manage season-long nutrient applications, while simultaneously improving nutrient use efficiency and grain yield. We hypothesized that the greater yields associated with transgenic insect protection from *Diabrotica* spp. would result in greater nutrient acquisition. The results obtained across 5 site-years show that increased biomass accumulation and grain yield occurs in some hybrid comparisons and environments. Increases in nutrient uptake occurred for Bt hybrids, particularly for N, P, and K, when accompanied by improved biomass accumulation and grain yield. Furthermore, results from experiment one demonstrated that the grain contents of N, P, K, S, and Zn were greater in transgenic Bt hybrids over their non-Bt counterparts by 8, 12, 9, 9, and 12%, respectively. Yield responsive Bt hybrid pairs acquired greater total amounts of N (31%), P (24%), and K (38%) during grain fill than non-Bt hybrids. The difference primarily occurred after R4, when rates of biomass and P accumulation were approximately twofold greater in insect-protected lines.

Understanding the impact of modern germplasm on maize nutritional needs is critical, especially in hybrids containing transgenic insect protection traits that maintain or increase root size and function. The role of transgenic insect protection in improving yield and yield stability has only recently been considered from an agronomic perspective. Impending efficient and sustainable nutrient management necessitates understanding the complexities of nutrient acquisition in transgenic genotypes. These results suggest that higher-yielding Bt maize hybrids that produce greater biomass and yield also accumulate additional N, P, K, S, and Zn, particularly during late grain fill.

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