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Grain and biomass nutrient uptake of conventional corn and their genetically modified isolines

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ABSTRACT

The adoption of genetically engineered crops in the United States has increased dramatically over the past decade. Differences in agronomic characteristics and protein expression between genetically engineered plants and their naturally recombinant non-genetically modified (GM) counterparts are not well-understood. Experimental field plots were established in the spring of 2005 near Brookings, SD with 18 different commonly used corn hybrids including three conventional hybrids and their corresponding transgenic modifications. Specific research objectives were to evaluate in a side by side comparison, the impact of the genetic modifications on agronomic characteristics. Results show that glyphosate or insect resistance resulting from genetic modification, in the absence of significant insect pest or weed pressure and glyphosate application, were not likely to significantly alter productivity or nutrient composition of corn residue or grain. No significant differences were observed among the hybrids in average grain yield or above-ground biomass over the three years of the experiment.

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Introduction

The use of genetically engineered crops in the United States has increased dramatically over the past decade. The initial adoption coincided with the release of herbicide-tolerant soybean (*Glycine max*) production, which reached an adoption rate of 54% in the United States by 2000 (USDA-ERS, 2011). In contrast, adoption of herbicide-tolerant corn (*Zea mays* L.) was only 6% with an additional 19% for insect-resistant traits during the same time period. By 2011, 88% of corn planted in the US was genetically engineered. Of the total corn planted in 2011, 23% was herbicide resistant, 16% insect-resistant, and 49% had both types of modifications, referred to as ‘stacked’ genes. The rapid and widespread adoption of genetically engineered corn (and other crops) in the US is attributed largely to the perceived economic advantage over conventional crops when and where there is significant insect and weed pressures. However, the potential differences in agronomic characteristics and protein expression between genetically engineered plants and their naturally recombinant non-genetically modified (GM) counterparts may have productivity and environmental impacts that are not well-understood. Full consideration of potential unintended or long-term effects of this technology has not occurred (NRC, 2000, 2002; Rombke et al., 2003; Wolfenbarger and Phifer, 2000) and there is little data for hybrids possessing multiple traits, e.g, insecticidal, herbicide resistance.

Of most interest to producers are whether GM crops differ from conventional varieties in terms of yield, grain quality, residue quantity and quality, and influence on the soil as a resource base. Previous research has shown few consistent, lasting effects of GM crops on soil microorganisms and their

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functions (Icoz and Stotzky, 2008). Despite several reports that some Bt corn hybrids have higher lignin content or different structural carbohydrates than their non-GM counterparts (Fang et al., 2007; Flores et al., 2005; Poerschmann et al., 2005; Saxena and Stotzky, 2001), other compositional evaluations of corn residue have found either slight (Folmer et al., 2002) or no significant (Jung and Sheaffer, 2004; Lehman et al., 2008a, 2008b, 2010; Mungai et al., 2005; et al.,Yanni et al., 2011) differences between Bt and non-Bt hybrids. While differences in yield and biomass production between GM and non-GM hybrids have been measured in some studies, a limited number of hybrids have been examined under a limited number of soil-climatic conditions (Yanni et al., 2010).

Apart from industry-sponsored studies using a finite number of hybrids (Grant et al., 2003), there is little published data on the nutrient uptake into grain and biomass of GM and non-GM corn. Some recent reports suggest nutritional changes in glyphosate resistant (GR) crops, including corn, due to application of glyphosate (Johal and Huber, 2009; Kremer and Means, 2009).

We conducted a three-year field study in the northern US corn belt to evaluate in a side by side comparison, the impact of the GM on plant residue, grain yield, grain nutrient composition and stalk nutrient composition independent of glyphosate use. This study utilized a total of 18 hybrids from three different base genetic platforms with corresponding one-gene, two-gene and three-gene (single-, double-, and triple-stacked) modification for insect (Cry1Ab; Cry3Bb1) and herbicide (GR) resistance.

Materials and methods

Field study

An experiment was established in the spring of 2005 at the Eastern South Dakota Soil and Water Research Farm near Brookings, SD. Soil type was a Doland loam (fine-loamy, mixed, superactive, frigid Calcic Hapludolls). Initial surface (0–15 cm) soil test characteristics showed a soil pH of 7.0 (1:1 soil:water paste), 3.2% organic matter (determined by loss-on-ignition) (Cambardella et al., 2001), extractable phosphorus (P) and exchangeable potassium (K) of 5 and 81 mg kg^{-1}, respectively. Initial subsurface (15–30 cm) soil test characteristics showed a soil pH of 7.4, 2.2% organic matter, extractable P and exchangeable K of 3 and 81 mg kg^{-1}, respectively. Initial soil test characteristics were determined by the South Dakota State Soil Testing Laboratory, Brookings, SD (Gelderman et al., 1995). Extractable P (Olsen P) was determined using the sodium bicarbonate ($NaHCO_3$) method (Olsen et al., 1954). Exchangeable K was determined using the ammonium acetate (NH_4Ac) method (Brown and Warncke, 1988).

The experiment was conducted as a completely randomized design, replicated four times, within a two-year corn/soybean rotation with each phase of the rotation present each year, in two adjacent areas (each 930 m^2). Experimental treatments included a total of 18 different commercial corn hybrids from three unique hybrid families. The corn hybrid families selected for this project were typical hybrids that are appropriate for the region's environmental conditions. Three different base genetic platforms and their GM isoline were selected from two different seed manufacturers. Manufacturers/hybrid families were selected based on the availability of multiple combinations of conventional and GM hybrids within the family. Corn hybrids utilized in the experiment and their genetic modifications (traits) are reported in Table 1. The experiment was conducted under no-tillage soil management beginning in the spring of 2005. Plots were 3 × 3 m, with .78 m row spacing planted utilizing a 2 row ALMACO (Nevada, IA, USA) cone seeder at a target population of 71,000 plants/ha on 16 May 2005, 16 May 2006 and 17 May 2007. The corn was fertilized with 165 kg nitrogen (N) ha^{-1} as ammonium nitrate (34-0-0), 35 kg P ha^{-1} as triple super-phosphate (0-36-0) and 25 kg K ha^{-1} as potassium chloride (KCl) (0-0-60). All fertilizer was surface broadcast prior to planting on 5 May 2005, 10 May 2006 and 15 May 2007. Weeds were controlled in-season for all plots by utilizing a tank mix of 'Callisto' (mesotrione) [2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione] 40% active ingredient and 'Accent' (nicosulfuron) [2-[[(4,6-dimethoxypyrimidin-2-yl) aminocarbonyl] aminosulfonyl]- N, N-dimethyl-3-pyridinecarboxamide)] 75% active ingredient were applied to all plots at a rates of 0.21 and 0.046 liters ha^{-1} respectively. Application dates were, 25 June 2005, 30 June 2006 and 20 June 2007. Environmental conditions were measured with an automated weather station located about 0.4 km east of the experimental plots.

Table 1. Corn hybrids name, suppliers and genetics composition, utilized in the experimental design for the 2005–2007 growing seasons, Brookings, SD.

Hybrid	Supplier	Base Genetics	*Cry*1Ab	*Cry*3Bb1	GR
DKC46-26	DeKalb/Monsanto	X			
DKC46-28	DeKalb/Monsanto				X
DKC47-10	DeKalb/Monsanto		X		X
DKC46-23	DeKalb/Monsanto			X	
DKC46-24	DeKalb/Monsanto			X	X
DKC46-25	DeKalb/Monsanto		X	X	
DKC46-22	DeKalb/Monsanto		X	X	X
DKC51-43	DeKalb/Monsanto	X			
DKC51-45	DeKalb/Monsanto				X
DKC50-18	DeKalb/Monsanto		X		
DKC50-20	DeKalb/Monsanto		X		X
DKC51-41	DeKalb/Monsanto			X	X
DKC51-39	DeKalb/Monsanto		X	X	X
344	Cropland Genetics	X			
344 *Cry*1Ab	Cropland Genetics		X		
344 *Cry*3Bb1	Cropland Genetics			X	
344GR *Cry*1Ab	Cropland Genetics		X		X
344 *Cry*3Bb1,*Cry*1Ab	Cropland Genetics		X	X	

GR - glyphosate resistant.

Plant sampling

At physiologically maturity (black layer), whole plant samples were collected from the two middle rows by collecting 1.5-m of each row on 11 October 2005, 4 October 2006 and 3 October 2007. At the time of whole plant sampling, corn ears within the two rows were separated from the biomass and hand shelled. Grain moisture and test weight were determined. Corn yield was adjusted to 15.5 g kg^{-1} moisture for further analysis. Plant biomass (excluding grain) samples were dried for 120 h in a forced-air oven at 60°C, and weighed. All grain and biomass samples were ground to pass a 2-mm sieve. Subsamples of grain and biomass were digested with nitric acid (70%) and analyzed by inductively coupled plasma atomic emissions for P, K, calcium (Ca), magnesium (Mg), manganese (Mn), sulfur (S), zinc (Zn), and sodium (Na). Additionally all biomass samples were analyzed for acid detergent fiber (ADF), acid detergent lignin (ADL), and neutral detergent fiber (NDF) fractional composition using an Ankom^{200} fiber analyzer (Ankom Technology, Macedon, NY, USA) and AOAC method 973.18 (AOAC, 2003 #2652). Hemicellulose and cellulose composition were determined by subtraction as described by Van Soest (1963). Ground residue and grain samples were milled further (Udy mill, <1 mm) for total carbon (C) and N analysis by dry combustion (LECO TruSpec analyzer, Leco Corp., St Joseph, MI, USA) (Nelson and Sommers, 1996).

Statistical analysis

Biomass and grain compositional analysis and production data were analyzed as dependent variables in a completely randomized block design using the MIXED procedure in SAS (Littell et al., 1996). Specific comparisons between hybrid families and between hybrids with and without the presence of specific genes were performed using ESTIMATE statements utilizing $\alpha = 0.10$.

Results

Environmental conditions

The growing environment during the course of the experiment consisted of temperatures that were relatively close to normal for all years, with a slightly cooler September during the 2006 growing season compared to the 30 year average (Table 2). Rainfall amounts during the three growing seasons were

Table 2. Average monthly temperature, total monthly precipitation and pan evaporation, Brookings, SD 2005–2007.

	2005			2006			2007		
Month	Temp °C	Precipitation mm	Evaporation mm	Temp °C	Precipitation mm	Evaporation mm	Temp °C	Precipitation mm	Evaporation mm
April	8.9 (2.1)	47 (−5)		8.9 (2.1)	67 (16)		5 (−1.8)	92 (40)	
May	12.2 (−1.5)	96 (21)	153	13.9 (0.2)	51 (−24)	145	16 (2.4)	47 (−28)	181
June	20.6 (1.6)	152 (45)	197	19.4 (0.5)	60 (−48)	203	20 (1.1)	76 (−32)	214
July	22.2 (0.7)	88 (9)	221	22.8 (1.3)	6 (−73)	241	22 (0.7)	4 (−75)	210
August	20 (−0.3)	89 (15)	183	20.6 (0.2)	144 (69)	217	20 (−0.3)	164 (89)	147
September	17.8 (2.7)	194 (131)	171	12.8 (−2.3)	162 (99)	102	16 (1.1)	31 (−33)	129
October	8.9 (1.0)	67 (22)	90	6.1 (−1.8)	5 (−40)		10.6 (2.6)	89 (43)	
Total		733	1015		495	908		503	881

() deviations from 30 year average.

drastically different with 238 mm of rainfall above average during the 2005 growing season and average rainfall during the 2006 and 2007 seasons. During the 2006 and 2007 season timing of the rainfall was not ideal for corn production, with deficits in precipitation during critical periods (June and July) and the majority of rain occurring later in the growing season (August and September).

Grain yield and composition

Average grain yields (8585 kg ha^{-1}) were similar to the regional average for corn production in the northern Corn Belt. During the course of the experiment there was no significant infestation of insect pests that limited yields of the non-GM crops. Orthogonal contrasts were utilized to evaluate the impact of the insertion of particular genetic trait(s) on grain yield and composition. There were no significant differences in grain yield, or grain C among the base hybrids and trait combinations, averaged over hybrid families and over all three years of evaluation (Table 3). Grain K levels were significantly lower in triple-stack and Cry3Bb1-GR double-stack hybrids compared to other trait combinations and base hybrids.

Plant residue production and composition

Orthogonal contrasts were used to compare base hybrids and trait combinations across hybrid families and years. There was no significant difference in plant biomass yield, (Table 4) however there was an increased K levels in biomass of triple-stack and Cry3Bb1-GR double-stack hybrids. These same trait combinations also had significantly lower C levels than the other combinations/base hybrids. It is unknown if there is a possible mechanism that caused this difference, or if it is just associated with

Table 3. Mean grain yield, carbon and potassium concentration for the varying genetic traits, Brookings, averaged over three different corn hybrids, Brookings, SD 2005–2007.

Genetic trait	Grain yield kg ha^{-1}	Grain carbon g kg^{-1}	Grain potassium g kg^{-1}
Base	8311	446.3	4.78 A
*Cry*1Ab	8811	445.7	4.89 A
*Cry*3Bb1	8118	446.8	4.94 A
GR	8718	444.0	4.71 A
*Cry*1Ab - *Cry*3Bb1	8436	447.2	4.71 A
*Cry*1Ab -GR	8870	446.4	4.81 A
*Cry*3Bb1-GR	8548	445.4	4.53 B
*Cry*1Ab - *Cry*3Bb1-GR	8878	446.7	4.63 B
Pr > F	0.6391	0.5935	0.0172

GR - glyphosate resistant.
Different letters indicate significant difference (LSD) $\alpha = 0.10$.

Table 4. Mean plant biomass production, carbon and potassium concentration at harvest for the varying genetic traits, averaged over three different corn hybrids, Brookings, SD 2005–2007.

Genetic trait	Biomass yield kg ha^{-1}	Biomass carbon g kg^{-1}	Biomass potassium g kg^{-1}
Base	1006	445.3 A	5.36 B
*Cry*1Ab	1096	444.3 A	5.51 B
*Cry*3Bb1	1021	442.6 A	5.29 B
GR	1026	442.3 A	5.73 B
*Cry*1Ab - *Cry*3Bb1	991	444.1 A	4.95 B
*Cry*1Ab -GR	1075	443.6 A	5.72 B
*Cry*3Bb1-GR	1038	441.8 B	6.55 A
*Cry*1Ab - *Cry*3Bb1-GR	1060	441.7 B	6.10 A
Pr > F	0.6091	0.0422	0.0036

GR - glyphosate resistant.
Different letters indicate significant difference (LSD) $\alpha = 0.10$.

natural genetic variation or perhaps due to variations of gene position within the genome of the base genotype(s).

Results of pair-wise comparisons within hybrid families indicated that there was a significant difference in how certain traits or trait combinations affected biomass nutrient concentration compared to the conventional (non-GM) hybrid. In pair-wise comparisons of biomass C concentration within hybrid families between conventional lines and single-stack hybrids, there was higher C concentration in conventional hybrids compared to the Cry3Bb1 (Figure 1A) or GR (Figure 1B) versions, while the hybrids with the Cry1Ab trait alone were not different from their corresponding conventional line. Two comparisons were available for each single trait utilized (GR, Cry3Bb1, and Cry1Ab). Two comparisons of triple-stack hybrids also showed decreased C concentration compared to the conventional hybrid (Figure 1C). Pair-wise comparisons of biomass-K concentration within families showed significantly higher K in the two single-stack Cry1Ab hybrids compared to their respective conventional base (Figure 2A). Similarly, biomass-Ca was higher in the two single-stack GR lines tested compared to their conventional bases (Figure 2B). All other pair-wise comparisons for Ca or K showed no differences among trait combinations.

Finally, hemicelluloses were shown to be significantly lower for the two GR single stack hybrids as well as the two triple-stack hybrids compared to their respective conventional base hybrids. (Figure 3). The addition of the GR gene resulted in an approximate 7% reduction in hemicellulose content compared to the respective conventional hybrids, while there was a 15% decrease for the triple-stack DKC45 hybrid (Figure 3A) compared to only a 4% reduction for the DKC51 hybrid (Figure 3B).

No other significant differences were observed in Mn, Mg, Na, Zn, or S composition or in ADF, NDF, or ADL and cellulose fractional components relating to digestibility and cell structure among trait combinations, averaged across hybrid families and over years. Similarly, pair-wise comparisons within hybrid families comparing trait packages with the conventional base hybrid showed no significant differences in mineral nutrient content or digestibility/cell structure.

Discussion

Since the development of GM crops, there have been numerous studies conducted to evaluate their direct and indirect impacts on the soil ecosystem and plant composition, including productivity. Past research into the effects of genetic modification on grain yield, biomass yield, and nutrient concentration have been limited in the number of hybrids evaluated within a study. This side by side comparison of 18 different corn hybrids with insecticidal and herbicide resistance traits grown under the same soil, environment and agronomic management did not result in any significant difference in above ground biomass or grain yield among the hybrids evaluated. Our results are similar that of Yanni et al. (2011),

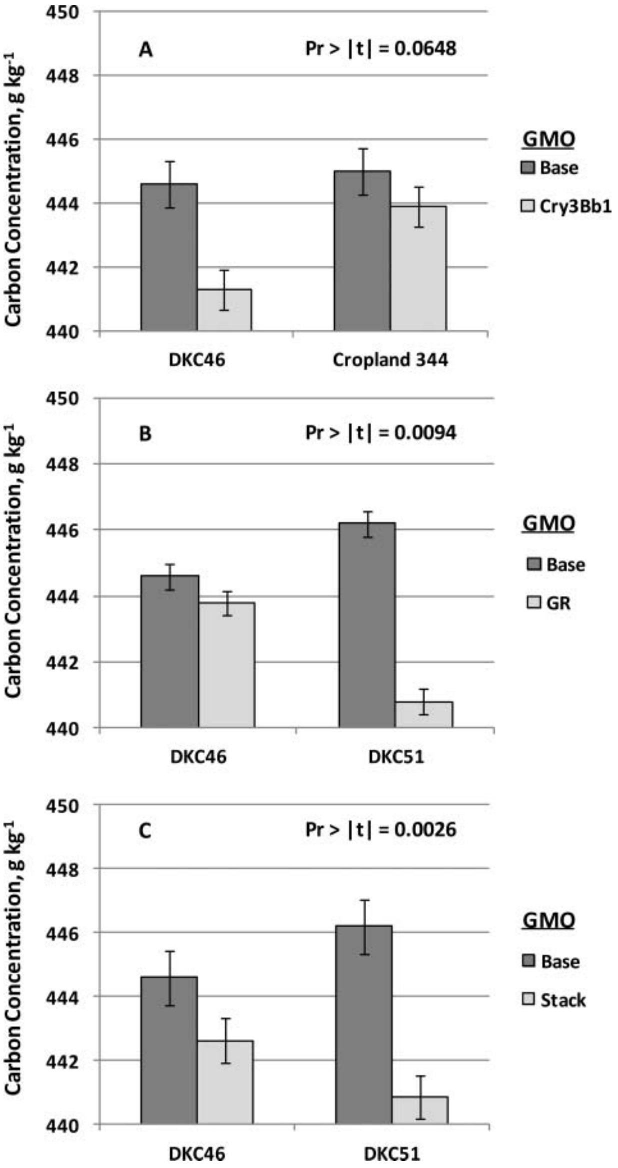

Figure 1. Plant residue carbon concentration as affected by different base genetic compared to the *Cry*3Bb1, GR (glyphosate resistant) and a triple stack hybrid containing *Cry*1Ab, *Cry*3Bb1, and GR, Brookings, SD 2005–2007.

who found no significant difference in corn grain or stover yield in 18 Bt and their non-Bt isolines grown in Quebec, CAN. The average yields observed within our study were typical for the north US corn belt region of the U.S. The lack of any significant insect pest infestation or weed pressure could help explain the lack of any significant difference in yield.

Differences in plant nutrient concentration were limited to significant differences in Ca, K and hemicelluloses. Calcium forms cross-links within the pectin polysaccharide, and is critical to cell wall formation and stalk strength (Marschner, 1995). Potassium is important in several regulatory roles in the plant. It is essential to sustaining plant growth and reproduction including photosynthesis, the translocation of photosynthates, protein synthesis, ionic balance, and regulating plant stomata and water use (Marschner, 1995). Hemicelluloses can be defined as a heterogeneous group of polysaccharides. Their most important of role is in strengthening cell walls (Scheller and Ulvskov, 2010). There was an overall higher level of plant Ca for the conventional hybrids compared to the GR hybrids within the same

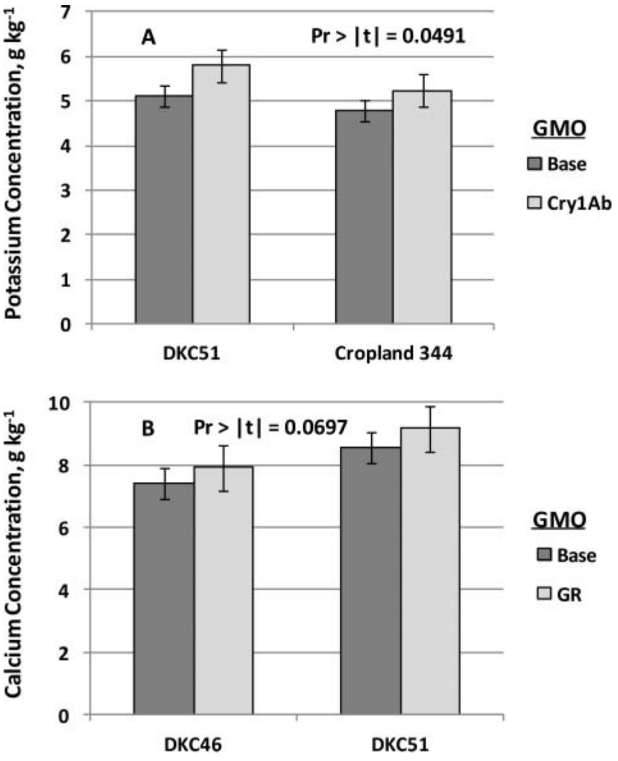

Figure 2. Plant residue potassium and calcium concentration as affected by different base genetic compared to the *Cry*1Ab, and GR (glyphosate resistant), Brookings, SD 2005–2007.

hybrid family, while these same conventional hybrids were higher in hemicelluloses than their GR counterpart. Past research has found mixed results in evaluating plant composition for GM vs. conventional near-isogenic hybrids. Jung and Sheaffer (2004) found no consistent difference in lignin content in Bt and non-Bt corn isolines. Folmer et al. (2002) found higher lignin content for Bt-containing residue compared to their non-Bt isolines. Currently there are no known mechanisms for these differences in plant composition (Saxena and Stotzky, 2001; Stotzky, 2004).

While there were several significant differences observed for certain nutrient/mineral concentrations across the multiple trait combinations, they are not generally considered agronomically important differences. The results detailed here show that glyphosate or insect resistance resulting from genetic modification of adapted germplasm, in the absence of significant insect pest or weed pressure and with no gylphosate applied, are not likely to significantly alter productivity or nutrient composition of corn residue or grain, as a result there should be no differences in mineral component removal/replacement in the soil.

Research conducted to evaluate the impact of GM crops on animal growth found that: feed blends and feed components containing GM (vs. non-GM) material were not significantly different; the variability between products was considered within the range of biological variability; and there was no significant difference in animal health or growth (Aulrich et al., 2001; Flachowsky et al., 2005; Folmer et al., 2002, Grant et al., 2003). Similarly results from this study found that there was no significant difference in feed quality parameters measured (ADF, ADL, and NDF) regardless of the GM trait.

This study could serve as a baseline for comparison with future data from similar experiments conducted under various levels of insect or weed pressures, or following glyphosate applications. The results of this study will allow for stronger inference to be made about the impact of genetic modification on agronomic characteristics. These results are especially valuable because they remove confounding effects of insect and weed pressure, interaction with herbicides, seed-applied insecticides, and other external inputs.

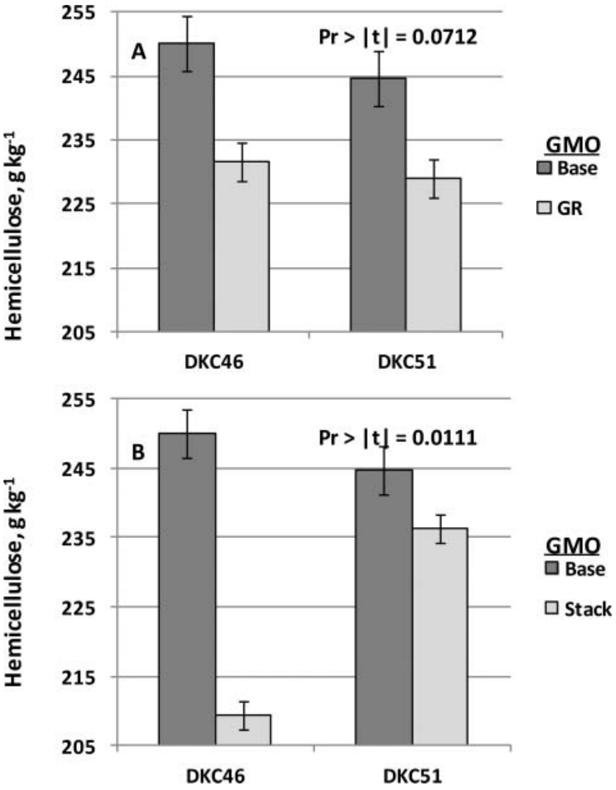

Figure 3. Plant hemicelluloses as affected by different base genetic compared to GR (glyphosate resistant); and triple stack hybrid containing *Cry*1Ab, *Cry*3Bb1, and GR, Brookings, SD 2005–2007.

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