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Agronomic and compositional assessment of genetically modified DP23211 maize for corn rootworm control

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ABSTRACT

DP23211 maize was genetically modified (GM) to express DvSSJ1 double-stranded RNA and the IPD072Aa protein for control of corn rootworm (*Diabrotica* spp.). DP23211 maize also expresses the phosphinothricin acetyltransferase (PAT) protein for tolerance to glufosinate herbicide, and the phosphomannose isomerase (PMI) protein that was used as a selectable marker. A multi-location field trial was conducted during the 2018 growing season at 12 sites selected to be representative of the major maize-growing regions of the U.S. and Canada. Standard agronomic endpoints as well as compositional analytes from grain and forage (e.g., proximates, fibers, minerals, amino acids, fatty acids, vitamins, anti-nutrients, secondary metabolites) were evaluated and compared to non-GM near-isoline control maize (control maize) and non-GM commercial maize (reference maize). A small number of agronomic endpoints were statistically significant compared to the control maize, but were not considered to be biologically relevant when adjusted using the false discovery rate method (FDR) or when compared to the range of natural variation established from in-study reference maize. A small number of composition analytes were statistically significant compared to the control maize. These analytes were not statistically significant when adjusted using FDR, and all analyte values fell within the range of natural variation established from in-study reference range, literature range or tolerance interval, indicating that the composition of DP23211 maize grain and forage is substantially equivalent to conventional maize represented by non-GM near-isoline control maize and non-GM commercial maize.

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Introduction

Western corn rootworm (WCR; *Diabrotica virgifera virgifera* LeConte) is a major insect pest in maize (*Zea mays* L.) production areas within North America and Europe,[1] resulting in over a billion dollars of damage each year.[2–5] WCR have demonstrated a remarkable ability to adapt to many of the existing management practices, and have developed resistance not only to soil and aerially applied insecticides,[6,7] but also to some crystalline (cry) proteins derived from *Bacillus thuringiensis* (*Bt*) that are expressed in genetically modified (GM) maize.[8–10] Corn rootworm have also adapted to crop rotation practices through the selection of rotation-resistant phenotypes.[2] A wide-range of management practices and new modes of action (MOA) will be needed to support a sustainable and durable management plan for this agricultural pest.[11–14]

Event DP-Ø23211-2 (DP23211) maize diversifies the current portfolio of transgenic traits for CRW control, through the expression of two MOAs. DP23211 maize plants express the DvSSJ1 double-stranded RNA (DvSSJ1 dsRNA), which is intended to down-regulate expression of the DvSSJ1 protein in the mid-gut of WCR via RNA interference (RNAi). The DvSSJ1 dsRNA is targeted to match the sequence of the smooth septate junction protein 1 (*dvssj1*) gene from WCR. Smooth septate junctions (SSJ) are unique to invertebrates, and in WCR, the DvSSJ1 protein is important for maintaining the integrity of the paracellular pathway between epithelial cells, which separates the gut lumen from the interstitial space where metabolites and electrolytes are tightly regulated. Reduction in the DvSSJ1 protein in WCR results in the loss of the gut epithelial barrier

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and cellular deformities, which is lethal to WCR.[15,16] DP23211 maize plants also express the IPD072Aa protein, encoded by the *ipd072Aa* gene, which is a non-pore forming protein that targets and disrupts midgut epithelial cells causing the breakdown of the epithelial lining. The IPD072Aa protein has been shown to be specific, with activity limited to within the order Coleoptera.[14,17,18] The *ipd072Aa* gene is derived from *Pseudomonas chlororaphis*,[14] a naturally occurring, ubiquitous bacterium found in the environment that lacks known allergenic or toxic properties and has a history of safe use in agriculture.[19] DP23211 maize plants contain the phosphinothricin acetyltransferase (PAT) protein,[20] encoded by the *mo-pat* gene, which confers tolerance to the herbicidal active ingredient glufosinate ammonium at current labeled rates. The phosphomannose isomerase (PMI) protein,[21] encoded by the *pmi* gene, was used as a selectable marker during the transformation of DP23211 maize.

As part of the regulatory approval process for GM crop cultivation, multi-location field trials are conducted and a standard suite of agronomics endpoints are evaluated and compared between the GM event and a non-GM near-isoline control (control). Compositional analysis studies are also currently required as part of the regulatory approval process for GM crops in many countries. The objective of this study was to fulfill regulatory data requirements and investigate whether the agronomics and composition of grain and forage from DP23211 maize are substantially equivalent to non-modified maize. Publication of these results also adds to the weight of evidence and scientific literature that documents the lack of biologically relevant changes in agronomics or composition of GM plants, including plants expressing dsRNA and non-Bt proteins.

Methods

Field Study

The field study was planted during the 2018 growing season at 12 sites in the United States and Canada (one site in Indiana, Minnesota, Nebraska, Pennsylvania, and Ontario; two sites in Illinois and Texas; and three sites in Iowa), which were selected to represent commercial maize-growing regions of North America. Test entries included one GM maize hybrid (DP23211 maize), one non-modified near-isoline control maize hybrid (control maize), and 14 non-GM commercial maize hybrids (reference maize; P0604, 2R602, 35A52, P0760, BK5883, XL5939, P0928, P0993, XL5828, BK6076, XL6158, P1105, P1151, and P1197). The control maize has the same genetic background as DP23211 maize but does not contain the genetic modification. From the 14 reference maize hybrids, 4 were planted at each site and were selected based on the maturity zone of the site as well as the Comparative Relative Maturity (CRM) of the hybrid. All seeds were analyzed by an event-specific polymerase chain reaction to confirm the presence of the event in the DP23211 maize and absence of the event in the control and reference maize. Each field site employed a randomized complete block design containing four blocks, and DP23211 maize, control maize, and four reference maize hybrids were assigned to a plot within each block. Plots consisted of six rows measuring 6 m in length and 0.76 m in width (with exception of Texas site, where row width was 0.99 m). Each row was planted with 30 seeds. Each block was separated by an alley of at least 0.9 m in width and each plot was bordered on either side by one row of maize. Normal pest control and maintenance practices (irrigation, fertilization, herbicide, and pesticide applications, etc.) were applied as needed. Any applications were consistent with maize production practices in the local region and were applied uniformly to each entire trial area. Planting dates ranged from May 8, 2018 to May 29, 2018. Plants in the first four rows of each plot were allowed to open pollinate and were used for agronomic assessments; self-pollinations were made in the last two rows of each plot and were used for sample collection.

Agronomic Assessment

Agronomic characteristics were collected at each of the 12 sites planted in the field study. Healthy, representative plants from within the first four rows in each plot were selected. Early stand counts, or the number of emerged plants, were determined between the V2 and V4 growth stages.[22] Days to flowering were calculated using the planting date and the date when approximately 50% of plants had begun shedding pollen. Pollen

viability was assessed at 0, 30, 60, and 120 minutes by recording the percentage of grains with collapsed walls and the percentage of grains with yellow color. Plant height was measured in whole centimeters from the soil surface to the collar of the flag leaf (base of the tassel) for five individual plants at the R4 or R5 growth stage, depending on site. Days to maturity were calculated using the planting date and the date when the majority of the plants first reached physiological maturity. Lodging was evaluated at the R6 growth stage and a combined lodging score was calculated from stalk and root lodging values. Stalk lodging was recorded as the number of plants in each plot with stalks broken below the primary ear. Root lodging was recorded as the number of plants in each plot with stalks leaning approximately 45° or more. Final stand count was recorded at the R6 growth stage. The number of dropped ears (ears lying on the ground within each plot) was recorded at the R6 growth stage. Two rows within each plot were used to collect grain weight, harvest grain moisture, and weight of 100-kernels. Grain weight and 100-kernel weight values were adjusted to a standardized moisture content of 15.5%. Yield was calculated in bushels per acre (bu/acre).

Forage and Grain Sample Collection and Processing

From the 12 sites planted in the field study, eight sites (one in Indiana, Iowa, Minnesota, Ontario, Pennsylvania, and Texas, and two in Illinois) were selected for nutrient composition analysis. Plants selected for composition sampling were self-pollinated, healthy, and representative of plants in the plot. One forage sample, which consisted of the aerial portion of three plants at the R4 or early R5 growth stage (depending on site), and one grain (R6) sample, which consisted of grain pooled from five ears, were collected from two rows from each of the DP23211 maize plots (N = 32 total DP23211 forage and grain samples), control plots (N = 32 total control forage and grain samples), and from each of the reference maize plots (N = 32 total forage and grain samples for each reference maize hybrid). Forage and grain samples were collected as described previously[23] and were kept cool using wet ice, artificial ice, or dry ice until placed in a freezer (≤ −10°C). Samples were shipped frozen to EPL Bio Analytical Services (EPL BAS, Niantic, IL, USA) and stored frozen (approximately −20°C) prior to being processed for composition analysis, as described previously.[24]

Composition Analysis

Proximates, fiber, and minerals (crude protein, crude fat, crude fiber, acid detergent fiber (ADF), neutral detergent fiber (NDF), ash, carbohydrates, calcium, and phosphorus) were analyzed in forage samples. Grain samples were analyzed for the same proximates, fiber, and minerals as forage, with the addition of total dietary fiber (TDF), copper, iron, magnesium, manganese, potassium, sodium, and zinc. Grain samples were further analyzed for fatty acids [lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), heptadecanoic acid (C17:0), heptadecenoic acid (C17:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), α-linolenic acid (C18:3), arachidic acid (C20:0), eicosenoic acid (C20:1), eicosadienoic acid (C20:2), behenic acid (C22:0), erucic acid (C22:1), and lignoceric acid (C24:0)], amino acids (alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine), vitamins [β-carotene (pro-vitamin A), vitamin B1 (thiamine), vitamin B2 (riboflavin), vitamin B3 (niacin), vitamin B5 (pantothenic acid), vitamin B6 (pyridoxine), vitamin B9 (folic acid), α-tocopherol (vitamin E), β-tocopherol, γ-tocopherol, and δ-tocopherol], as well as secondary metabolites and anti-nutrients (*p*-coumaric acid, ferulic acid, furfural, inositol, phytic acid, raffinose, and trypsin inhibitor). The analytes included in the compositional assessment were selected based on the OECD consensus document on compositional considerations for new varieties of maize.[25] Composition analysis was performed by EPL BAS using Good Laboratory Practices (GLP) validated methods, as described previously.[23] An additional analyte (total tocopherols) was calculated as the sum of the α-, β-, γ-, and δ-tocopherol values for each sample.

Statistical Analysis: Agronomic Assessment

Statistical analysis was conducted to compare the agronomic endpoints from DP23211 maize and

control maize using SAS software, Version 9.4 (SAS Institute Inc., Cary, NC, USA). Agronomic endpoints were analyzed as follows: if <50% of sites had uniform data values for either GM or the control maize, and <50% of all data across sites for each entry were at a uniform value, then an across-site mixed model analysis was conducted with maize line as a fixed effect and site and the interaction between maize line and site as random effects. If ≥50% of sites had uniform data values for either GM or the control maize, and ≥50% of sites had uniform data values across both maize lines, then statistical analyses were not performed. If the criteria described above were not met, then an across-site analysis using the generalized Cochran-Mantel-Haenszel (CMH) test was conducted. Means were estimated for each maize line and compared to test whether there was a significant difference (raw P-value < 0.05) between the means. The approximate degrees of freedom for the statistical test were derived using the Kenward-Roger method.[26] For each agronomic endpoint, goodness-of-fit of the model was assessed in terms of meeting distributional assumptions of normally, independently distributed errors with homogeneous variance. Deviations from assumptions were addressed using an appropriate transformation or by fitting heterogeneous error variances across sites. The false discovery rate (FDR) method[27,28] was used to control for false-positive outcomes across all agronomic endpoints analyzed using linear-mixed models, and the adjusted P-value was reported for agronomic endpoints with a raw P-value < 0.05. In cases when a raw P-value indicated a significant difference, but the FDR-adjusted P-value was >0.05, it was concluded that the difference was likely a false positive, as described previously.[23] For a given agronomic characteristic, when a statistically significant difference (P-value < 0.05) was identified in the across-site analysis, the respective range of individual values from DP23211 maize (N = 48 for agronomic endpoints) was compared to the in-study reference range comprised all individual values across-sites from all non-GM reference maize lines grown in this study.

Statistical Analysis: Compositional Assessment

Statistical analysis was conducted to compare the composition from DP23211 maize and control maize using SAS software, Version 9.4. Composition analytes were analyzed as follows: if both GM and the control maize had <50% of samples below the lower limit of quantification (LLOQ), then an across-site mixed model analysis was conducted. If either GM or the control maize had ≥50% samples below the LLOQ, but not both entries had 100% of samples below the LLOQ across sites, then Fisher's exact test was conducted. If both GM and the control maize had 100% of samples below the LLOQ, then statistical analyses were not performed. Degrees of freedom were derived using the Kenward-Roger method, and for each analyte, the goodness-of-fit of the model was assessed, as described above for agronomic data. The FDR method was used to control for false positives, as described above for agronomic data. For a given analyte, when a statistically significant difference (P-value < 0.05) was identified in the across-site analysis, the respective range of individual values (N = 32 for composition endpoints) from DP23211 maize was compared to one or more reference ranges (i.e., tolerance intervals, literature ranges, and in-study reference ranges). The tolerance intervals were derived from proprietary accumulated data from 28 multi-site field studies between 2003 and 2017. These studies consisted of a total of 144 non-GM commercial reference maize lines and 148 unique environments representative of commercial maize-growing regions in the United States, Canada, Chile, Brazil, and Argentina. Tolerance intervals are expected to contain at least 99% of the values for corresponding analytes of the conventional maize population with a 95% confidence level.[29] Literature ranges were generated from relevant crop composition data obtained from published literature.[24,25,30-33] The in-study reference range was comprised of all individual values across-sites from all non-GM reference maize lines grown in this study. Collectively, the tolerance intervals, literature ranges, and in-study reference ranges provide context for evaluating natural variation and biological relevance, as described previously.[23]

Results and Discussion

Agronomic Assessment

Three agronomic endpoints (dropped ears, pollen shape at 120 minutes, and pollen color at

120 minutes) were not included in statistical analysis because they did not meet the minimum levels of non-uniformity (Supplemental Information Table 1). The pollen viability observations at 120 minutes indicate that the majority of pollen was non-viable after 2 hours (high percentage of pollen with collapsed walls and yellow in color; Supplemental Information Table 1), which has been observed previously[34] and is unrelated to the GM trait. The number of dropped ears observed in this study was very low (mean of 0.1 for both GM and control maize), which was within the in-study reference range (Supplemental Information Table 1). No statistical differences were identified between DP23211 maize and control maize in the across-site analysis for pollen viability (based on shape and color at 0, 30, and 60 minutes), plant height, days to maturity, lodging, harvest grain moisture, and 100-kernel weight (Supplemental Information Table 1).

A statistically significant difference was detected for yield between DP23211 maize and control maize (*P*-value = 0.0351); however, yield was not statistically significant after FDR adjustment (FDR adjusted *P*-value = 0.132; Table 1), which indicates that this difference in yield is likely a false-positive outcome. All of the yield values were within the in-study reference ranges (Table 1), further indicating that the observed differences are not biologically relevant. A statistically significant difference in early stand counts was observed for DP23211 maize compared to control maize (*P*-value < 0.0001 and FDR-adjusted *P*-value = 0.000566; Table 1). All of the early stand count values were within the in-study reference ranges (Table 1), indicating that the observed differences are not biologically relevant (i.e., within the range of natural variation for commercial non-modified maize). A statistically significant difference in final stand count (*P*-value = 0.000280 and FDR-adjusted *P*-value = 0.00140; Table 1) and days to flowering (*P*-value < 0.0001 and FDR-adjusted *P*-value <0.0001; Table 1) were observed for DP23211 maize compared to control maize. For final stand count, 46 of 47 values for DP23211 maize were within the in-study reference range (with one value below the lower reference range). For days to flowering, 39 of 47 values were within the in-study reference range (with eight values above the upper reference range). The minor differences observed for days to flowering and final stand count are unlikely to result in DP23211 maize plants with increased weediness potential or survivability, compared to conventional maize which is not considered a weedy or invasive plant.[35,36] The results obtained from this field study demonstrate that the agronomic endpoints of DP23211 maize are comparable to those derived from conventional maize.

Composition Assessment

Nutrient composition data were generated for a total of 79 analytes, which includes 9 analytes measured in forage and 70 analytes assessed in grain. No statistically significant differences were observed between DP23211 maize and the control maize for the 9 forage analytes (Supplemental Information Table 2). No statistically significant differences were observed between DP23211 maize and the control maize for 57 of the 63 grain analytes that were subject to either mixed model analysis or Fisher's exact test (Supplemental Information Tables 3–8). Seven of the 70 analytes from grain [myristic acid (C14:0), heptadecenoic acid (C17:1), erucic acid (C22:1), vitamin B2 (riboflavin), β-tocopherol, δ-tocopherol, and furfural] were not statically analyzed because values were all below the LLOQ (Supplemental Information Table 9).

A statistically significant difference between DP23211 maize and the control maize was observed for three grain fatty acids [oleic acid (C18:1, *P*-value = 0.00765), arachidic acid (C20:0, *P*-value= 0.0395), eicosenoic acid (C20:1, *P*-value= 0.0245)] (Table 2 and Supplemental Information Table 4) and one secondary metabolite [*p*-coumaric acid (*P*-value= 0.00244)] (Table 2 and Supplemental Information Table 8). The FDR-adjusted *P*-values for these analytes were not significant, indicating that the observed differences were likely false positives (Table 2). Furthermore, all of the individual values for these analytes were within the tolerance interval, literature range, and in-study reference ranges, indicating DP23211 maize is within the range of natural variation for these analytes and the statistical differences are not biologically meaningful (Table 2).

A statistically significant difference between DP23211 maize and the control maize was observed for two vitamins [α-tocopherol (*P*-value= 0.0243) and vitamin B6 (pyridoxine, *P*-value= 0.0273)] (Table 2 and Supplemental Information Table 7).

Table 1. Mean and range (minimum and maximum individual values) of agronomic characteristics from DP23211 maize and non-modified near-isoline control maize (control) that had statistically significant differences (*P*-value < 0.05). In-study reference ranges were obtained from the four non-modified commercial maize lines grown at each site.

Agronomic Characteristic	Reported Statistics	Control Maize	DP23211 Maize	In-Study Reference Range
Early Stand (count/m^2)	Mean	6.1	5.9	3.8–6.7
	Range	4.3–6.6	4.7–6.5	
	Adj. *P*-value	–	0.000566†	
	P-value	–	<0.0001*	
Days to Flowering (days)	Mean	59.3	60.4	51–65
	Range	53–67	53–67	
	Adj. *P*-value	–	<0.0001†	
	P-value	–	<0.0001*	
Final Stand Count (count/m^2)	Mean	5.9	5.7	4.1–6.6
	Range	4.3–6.6	3.7–6.4	
	Adj. *P*-value	–	0.00140†	
	P-value	–	0.000280*	
Yield (bu/A)	Mean	181.5	172.6	16–275
	Range	28–260	29–255	
	Adj. *P*-value	–	0.132	
	P-value	–	0.0351*	

*A statistically significant difference (*P*-v-value < 0.05) was observed.
†Adj. *P*-value (Adjusted *P*-value) < 0.05 was observed.

The FDR-adjusted *P*-values for these analytes were not significant, indicating that the observed differences were likely false positives (Table 2). Furthermore, all individual values for α-tocopherol were within the tolerance interval, literature range, and in-study reference ranges, and all of the values for vitamin B6 (pyridoxine) were within the literature range. As all values for these two analytes were within at least one of the reference ranges, DP23211 maize is within the range of natural variation for these analytes and the statistical differences are not biologically meaningful.

Similar to conventionally bred crops, GM crops go through an extensive breeding and screening process to incorporate the transgenic trait into elite germplasm and lead events are selected for commercialization if they are shown to have favorable agronomics.[37,38] The agronomic assessment is used to inform the environmental risk assessment for the cultivation of GM crops and assess the potential for increased weediness, gene flow, survival, etc. In this study, there were few statistically significant differences detected between DP23211 maize and control maize for agronomic endpoints. When a statistically significant difference was detected, the agronomic endpoint was further assessed for false-positive outcomes, using the false discovery rate (FDR) method, where non-significance following post-hoc FDR adjustment supports that the difference is a false positive.[23,27,28] Agronomic endpoints that were significantly different than the control were assessed for biological relevance by considering the range of

Table 2. Mean and range (minimum and maximum individual values) of fatty acids (% total fatty acids), vitamins (mg/kg dry weight) and secondary metabolites (% dry weight) in grain from DP23211 maize and non-modified near-isoline control maize (control) that had statistically significant differences (raw P-value < 0.05). Tolerance intervals were derived from Corteva Agriscience's™, proprietary accumulated data from commercial non-modified maize lines, literature ranges were obtained from published literature, and in-study reference ranges were obtained from the four non-modified commercial maize lines grown at each site.

Composition Analyte	DP23211 Mean (Range)	Control Mean (Range)	*P*-value (Adjusted *P*-value)[a]	Tolerance Interval	Literature Range[b]	In-Study Reference Range
Oleic Acid (C18:1)	21.1 (20.2–22.1)	21.5 (20.7–22.3)	0.00765* (0.264)	17.3–38.6	16.38–42.81	20.0–32.8
Arachidic Acid (C20:0)	0.367 (0.337–0.399)	0.361 (0.332–0.399)	0.0395* (0.454)	0.295–0.872	0.267–1.2	0.328–0.539
Eicosenoic Acid (C20:1)	0.313 (0.290–0.331)	0.306 (0.266–0.334)	0.0245* (0.377)	0–0.614	ND – 1.952	0.233–0.425
p-coumaric Acid	0.0198 (0.0159–0.0294)	0.0218 (0.0161–0.0298)	0.00244* (0.168)	0.00742–0.0492	ND – 0.08	0.0132–0.0403
α-Tocopherol	3.00 (<0.500[c] – 7.39)	3.37 (<0.500[c] – 7.22)	0.0243* (0.377)	0–23.5	ND – 68.67	<0.500[c] – 19.3
Vitamin B6 (Pyridoxine)	2.76 (1.40–4.09)	2.99 (2.00–4.65)	0.0273* (0.377)	1.61–8.88	ND – 12.14	1.62–5.26

*A statistically significant difference (*P*-value ≥ 0.05) was observed.
[a]Adjusted *P*-value, which is used to control for false-positive outcomes across all analytes analyzed using linear-mixed models, is reported if the raw *P*-Value was significant.
[b]Literature range.[24,25,30-33] ND (not detectable: one or more assay values in the published literature references were below the lower limit of quantification (LLOQ) and were not quantified.
[c]< LLOQ, one or more sample values were below the assay LLOQ.

natural variation, established using in-study reference ranges from non-GM commercial maize varieties. For days to flowering and final stand counts, a few values for DP23211 maize were outside the in-study reference range, but these minor differences are unlikely to result in DP23211 maize plants with increased weediness potential or survivability, compared to conventional maize which is not considered a weedy or invasive plant.[35,36] These results support the conclusion that the DP23211 maize is agronomically similar to conventional maize, which informs the environmental risk assessment for the cultivation of DP23211 maize.

Compositional analysis studies are currently required as part of the global regulatory approval process for GM crop food and feed safety assessment. In this study, there were few statistically significant differences detected between DP23211 maize and control maize for compositional endpoints. Composition analytes that were significantly different than the control were assessed for biological relevance using tolerance intervals,[29] literature ranges,[24,25,30-33] and in-study reference ranges from non-GM commercial maize varieties.[23] No biologically relevant differences in composition were detected between GM and control maize. These results support the conclusion that DP23211 maize is substantially equivalent in the composition of grain and forage to the non-modified crop. DP23211 maize expresses an insect-specific dsRNA, an insect-active non-Bt protein, the phosphinothricin acetyltransferase (PAT) protein for tolerance to glufosinate herbicide, and the phosphomannose isomerase (PMI) protein that was used as a selectable marker. The PAT and PMI proteins have a history of safe use in agriculture,[20,39-41] and are present in a number of approved events that are currently in commercial use. Based on the history of safe use of the PAT and PMI proteins in agriculture,[20,39-41] and based on the mode of action and specificity of activity of DvSSJ1 dsRNA and IPD072Aa protein,[14–18] there was no *a priori* reason to expect the introduced traits would interact with plant metabolic pathways in such a way as to change the composition. The composition results from this study are in line with results generated from over 25 years GM crop cultivation, which have not identified any biologically relevant changes in composition that are associated with the development of a GM plant.[42] The main objective of this composition study was to fulfill regulatory data requirements, and publication of these results adds to the weight of evidence and scientific literature that documents the lack of biologically relevant changes in the composition of GM plants.

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Disclosure Of Potential Conflicts Of Interest

The authors are employees of Corteva Agriscience™.

Supplemental Information Description

Table summarizing the number of samples that were below the lower limit of detection for various analytes in grain, across-site analysis of agronomic characteristics, and composition analytes.

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References

1. Miller N, Estoup A, Toepfer S, Bourguet D, Lapchin L, Derridj S, Kim KS, Reynaud P, Furlan L, Guillemaud T. Multiple transatlantic introductions of the Western Corn Rootworm. Science. 2005;310(5750):992. doi:10.1126/science.1115871.
2. Gray ME, Sappington TW, Miller NJ, Moeser J, Bohn MO. Adaptation and invasiveness of Western Corn Rootworm: intensifying research on a Worsening Pest. Annu Rev Entomol. 2009;54(1):303–21. doi:10.1146/annurev.ento.54.110807.090434.
3. Metcalf RL. Foreward. In: Krysan JL, Miller TA, editors. Methods for the study of pest *Diabrotica*. New York, USA: Springer-Verlag; 1986. p. vii–xv.
4. PHI. 2010. CropFocus: corn Rootworm. Pioneer Hi-Bred International, Inc. https://www.pioneer.com/CMRoot/Pioneer/US/Non_Searchable/agronomy/cropfocus_pdf/corn_rootworm.pdf
5. Shrestha RB, Dunbar MW, French BW, Gassmann AJ, Riechers DE. Effects of field history on resistance to Bt maize by western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: chrysomelidae). PLoS One. 2018;13(7):e0200156. doi:10.1371/journal.pone.0200156.

6. Meinke LJ, Siegfried BD, Wright RJ, Chandler LD. Adult susceptibility of Nebraska Western Corn Rootworm (Coleoptera: Chrysomelidae) populations to selected insecticides. J Econ Entomol. 1998;91 (3):594–600. doi:10.1093/jee/91.3.594.
7. Souza D, Vieira BC, Fritz BK, Hoffmann WC, Peterson JA, Kruger GR, Meinke LJ. Western corn rootworm pyrethroid resistance confirmed by aerial application simulations of commercial insecticides. Sci Rep. 2019;9(1):6713. doi:10.1038/s41598-019-43202-w.
8. Gassmann AJ, Shrestha RB, Kropf AL, St Clair CR, Brenizer BD. Field-evolved resistance by western corn rootworm to Cry34/35Ab1 and other *Bacillus thuringiensis* traits in transgenic maize. Pest Manag Sci. 2020;76(1):268–76. doi:10.1002/ps.5510.
9. Jakka SRK, Shrestha RB, Gassmann AJ. Broad-spectrum resistance to *Bacillus thuringiensis* toxins by western corn rootworm (*Diabrotica virgifera virgifera*). Sci Rep. 2016;6(1):27860. doi:10.1038/srep27860.
10. Tabashnik BE, Brévault T, Carrière Y. Insect resistance to Bt crops: lessons from the first billion acres. Nat Biotechnol. 2013;31(6):510–21. doi:10.1038/nbt.2597.
11. Gassmann AJ, Petzold-Maxwell JL, Clifton EH, Dunbar MW, Hoffmann AM, Ingber DA, Keweshan RS. Field-evolved resistance by western corn rootworm to multiple *Bacillus thuringiensis* toxins in transgenic maize. Proc Natl Acad Sci. 2014;111 (14):5141–46. doi:10.1073/pnas.1317179111.
12. Gassmann AJ, Petzold-Maxwell JL, Keweshan RS, Dunbar MW, Meyer P. Field-evolved resistance to Bt Maize by Western Corn Rootworm. PLoS One. 2011;6 (7):e22629. doi:10.1371/journal.pone.0022629.
13. Pereira AE, Wang H, Zukoff SN, Meinke LJ, French BW, Siegfried BD, Soberón M. Evidence of field-evolved resistance to Bifenthrin in Western Corn Rootworm (*Diabrotica virgifera virgifera* LeConte) populations in Western Nebraska and Kansas. PLoS One. 2015;10(11): e0142299. doi:10.1371/journal.pone.0142299.
14. Schellenberger U, Oral J, Rosen BA, Wei J-Z, Zhu G, Xie W, McDonald MJ, Cerf DC, Diehn SH, Crane VC, et al. A selective insecticidal protein from *Pseudomonas* for controlling corn rootworms. Science. 2016;354 (6312):634–37. doi:10.1126/science.aaf6056.
15. Hu X, Richtman NM, Zhao J-Z, Duncan KE, Niu X, Procyk LA, Oneal MA, Kernodle BM, Steimel JP, Crane VC, et al. Discovery of midgut genes for the RNA interference control of corn rootworm. Sci Rep. 2016;6(1):30542. doi:10.1038/srep30542.
16. Hu X, Steimel JP, Kapka-Kitzman DM, Davis-Vogel C, Richtman NM, Mathis JP, Nelson ME, Lu AL, Wu G. Molecular characterization of the insecticidal activity of double-stranded RNA targeting the smooth septate junction of western corn rootworm (*Diabrotica virgifera virgifera*). PLoS One. 2019;14(1):e0210491. doi:10.1371/journal.pone.0210491.
17. Boeckman CJ, Huang E, Sturtz K, Walker C, Woods R, Zhang J. Characterization of the spectrum of insecticidal activity for IPD072Aa: a protein derived from *Psuedomonas chlororaphis* with activity against *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). J Econ Entomol. 2019;112(3):1190–96. doi:10.1093/jee/toz029.
18. Carlson AB, Mathesius CA, Ballou S, Boeckman CJ, Gunderson TA, Mirsky HP, Mukerji P, Roe JC, Schmidt JM, Zhang J, et al. Safety assessment of coleopteran active IPD072Aa protein from *Psuedomonas chlororaphis*. Food Chem Toxicol. 2019;129:376–81. doi:10.1016/j.fct.2019.04.055.
19. Anderson JA, Staley J, Challender M, Heuton J. Safety of *Pseudomonas chlororaphis* as a gene source for genetically modified crops. Transgenic Res. 2018;27 (1):103–13. doi:10.1007/s11248-018-0061-6.
20. Hérouet C, Esdaile DJ, Mallyon BA, Debruyne E, Schulz A, Currier T, Hendrickx K, van der Klis R-J, Rouan D. Safety evaluation of the phosphinothricin acetyltransferase proteins encoded by the *pat* and *bar* sequences that confer tolerance to glufosinate-ammonium herbicide in transgenic plants. Regul Toxicol Pharm. 2005;41(2):134–49. doi:10.1016/j.yrtph.2004.11.002.
21. Negrotto D, Jolley M, Beer S, Wenck AR, Hansen G. The use of phosphomannose-isomerase as a selectable marker to recover transgenic maize plants (*Zea mays* L.) via *Agrobacterium* transformation. Plant Cell Rep. 2000;19(8):798–803. doi:10.1007/s002999900187.
22. Abendroth LJ, Elmore RW, Boyer MJ, Marlay SK. Corn growth and development. Iowa (US): Iowa State University Extension; 2011.
23. Anderson JA, Hong B, Moellring E, TeRonde S, Walker C, Wang Y, Maxwell C. Composition of forage and grain from genetically modified DP202216 maize is equivalent to non-modified conventional maize (*Zea mays* L.). GM Crops Food. 2019;10(2):77–89. doi:10.1080/21645698.2019.1609849.
24. Cong B, Maxwell C, Luck S, Vespestad D, Richard K, Mickelson J, Zhong C. Genotypic and environmental impact on natural variation of nutrient composition in 50 non genetically modified commercial maize hybrids in North America. J Agric Food Chem. 2015;63 (22):5321–34. doi:10.1021/acs.jafc.5b01764.
25. OECD. 2002. Consensus document on compositional considerations for new varieties of maize (*Zea Mays*): key food and feed nutrients, anti-nutrients and secondary plant metabolites. Organisation for Economic Co-operation and Development: Paris (France).
26. Kenward MG, Roger JH. An improved approximation to the precision of fixed effects from restricted maximum likelihood. Comput Stat Data Anal. 2009;53 (7):2583–95. doi:10.1016/j.csda.2008.12.013.
27. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc B. 1995;57:289–300. doi: citeulike-article-id:1042553.

28. Westfall PH, Tobias RD, Rom D, Wolfinger RD, Hochberg Y. Concepts and basic methods for multiple comparisons and tests. In: Multiple comparisons and multiple tests using SAS system. Cary: SAS Institute Inc.; 1999. p. 13–40.
29. Hong B, Fisher TL, Sult TS, Maxwell CA, Mickelson JA, Kishino H, Locke MEH. Model-based tolerance intervals derived from cumulative historical composition data: application for substantial equivalence assessment of a genetically modified crop. J Agric Food Chem. 2014;62(40):9916–26. doi:10.1021/jf502158q.
30. Codex Alimentarius Commission. 2013. Codex standard for named vegetable oils. Codex Alimentarius.
31. ILSI. 2019. ILSI crop composition database, version 7.0. International Life Sciences Institute. www.cropcomposition.org
32. Lundry DR, Burns JA, Nemeth MA, Riordan SG. Composition of grain and forage from insect-protected and herbicide-tolerant corn, MON 89034 × TC1507 × MON 88017 × DAS-59122-7 (SmartStax), is equivalent to that of conventional corn (*Zea mays* L.). J Agric Food Chem. 2013;61(8):1991–98. doi:10.1021/jf304005n.
33. Watson SA. Corn: amazing maize. General properties. In: Wolff IA, editor. CRC handbook of processing and utilization in agriculture. Boca Raton (FL): CRC Press; 1982. p. 3–29.
34. Luna VS, Figueroa MJ, Baltazar MB, Gomez LR, Townsend R, Schoper JB. Maize pollen longevity and distance isolation requirements for effective pollen control. Crop Sci. 2001;41(5):1551–57. doi:10.2135/cropsci2001.4151551x.
35. CFIA. The biology of *Zea mays* (L.) (Maize). Ottawa: Canadian Food Inspection Agency; 1994.
36. OECD. 2003. Consensus document on the biology of *Zea mays* subsp. *mays* (Maize). Organisation for Economic Co-operation and Development: Paris (France).
37. Glenn KC, Alsop B, Bell E, Goley M, Jenkinson J, Liu B, Martin C, Parrott W, Souder C, Sparks O, et al. Bringing new plant varieties to market: plant breeding and selection practices advance beneficial characteristics while minimizing unintended changes. Crop Sci. 2017;57(6):2906–21. doi:10.2135/cropsci2017.03.0199.
38. Privalle LS, Chen J, Clapper G, Hunst P, Spiegelhalter F, Zhong CX. Development of an agricultural biotechnology crop product: testing from discovery to commercialization. J Agric Food Chem. 2012;60(41):10179–87. doi:10.1021/jf302706e.
39. CERA - ILSI Research Foundation. 2016. A review of the food and feed safety of the PAT protein. International Life Sciences Institute, Center for Environmental Risk Assessment. http://ilsirf.org/publication/a-review-of-the-food-and-feed-safety-of-the-pat-protein/
40. Vlachos D, Huber SA. 2011. Petition for determination of nonregulated status for Rootworm-resistant event 5307 corn. Submitted to the USDA-APHIS by Syngenta Biotechnology, Inc. https://www.aphis.usda.gov/brs/aphisdocs/10_33601p.pdf
41. Ward DP, Huber SA. 2007. Petition for determination of nonregulated status for insect-resistant MIR162 maize. Submitted to the USDA-APHIS by Syngenta Biotechnology, Inc. https://www.aphis.usda.gov/brs/aphisdocs/07_25301p.pdf
42. Herman RA, Price WD. Unintended compositional changes in genetically modified (GM) crops: 20 years of research. J Agric Food Chem. 2013;61(48):11695–701. doi:10.1021/jf400135r.