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Agronomic Performance and Crop Composition of Genetically Engineered Cotton Tolerant to HPPD Inhibiting Herbicides

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ABSTRACT

Genetically engineered cotton tolerant to two herbicides with unique modes of action has been developed by Bayer CropScience. This cotton event, referred to as HPPDi cotton, was developed through Agrobacterium-mediated transformation to express the modified 5-enolpyruvylshikimate 3-phosphate synthase protein (2mEPSPS) and modified 4-hydroxyphenylpyruvate dioxygenase (HPPD W336) proteins, which respectively confer tolerance to glyphosate and HPPD inhibitor herbicides such as isoxaflutole. The objective of the study was to compare HPPDi cotton (genetic background Coker 312) with non-genetically engineered Coker 312 and commercial reference varieties. Agronomic parameters were collected at 15 field sites including plant population (i.e., stand counts), morphology (i.e., plant mapping) and yield. Lint was analyzed for lint quality via high volume instrument (HVI). Compositional parameters (proximates, anti-nutrients and other components) were analyzed in fuzzy seed produced from eight sites. Statistical analysis was performed to compare HPPDi cotton treated or not treated with isoxaflutole and glyphosate to Coker 312 (the non-genetically engineered conventional counterpart). The results of this comparative assessment indicate that HPPDi cotton is substantially equivalent to its non-genetically engineered conventional counterpart for agronomic parameters, lint quality, and composition as it does not exhibit unexpected agronomic, lint or compositional characteristics.

Weed management is essential for successful crop production and has historically been the most time and labor intensive input (Gianessi and Reigner, 2007). Not surprisingly, herbicides have come to play a vital role in sustaining profitable cotton production operations. While several important weed species have acquired resistance to frequently used classes of herbicides, new biotechnology achievements are producing critically needed new crop-herbicide combinations (Green, 2012). Deployment of novel biotechnology-derived crop traits (events) encoding proteins that inhibit herbicide efficacy in crops (herbicide tolerance traits) are needed to provide growers with another tool to manage weeds in cropping systems, especially weeds resistant to other herbicide classes. Furthermore, the combination of multiple herbicide tolerance traits into a single cotton variety is useful in managing the development of weed resistance. Cotton (Gossypium hirsutum L.) is sensitive to 4-hydroxyphenylpyruvate dioxygenase inhibitor (HPPDi) classes of herbicides. Given the effectiveness and unique mode of action of HPPDi (Green, 2014), cotton tolerance to HPPDi herbicides in combination with tolerance to other classes of herbicides, would provide a valuable new tool for weed management in cotton production systems.

Bayer CropScience developed HPPDi cotton through Agrobacterium-mediated transformation to confer tolerance to two herbicides with unique modes of action. HPPDi cotton contains the modified 5-enolpyruvylshikimate 3-phosphate synthase (2mEPSPS) and the modified 4-hydroxyphenylpyruvate dioxygenase (hpdPfW336-1Pa), which encodes proteins for glyphosate and HPPDi tolerance, respectively. The mode of action of glyphosate is to specifically bind to and block the activity of EPSPS protein, an enzyme essential to the biosynthesis of aromatic amino acids. The 2mepsp gene introduced into HPPDi cotton was generated by introducing two point mutations into the wild type epsps gene cloned from maize (Zea mays). This double mutant 2mEPSPS protein has a lower binding affinity for glyphosate, thus allowing sufficient enzyme activity for the plants to develop normally in the presence of this herbicide. Other herbicide tolerant crops have been commercialized using this gene (Wallace et al., 2011).
Isoxaflutole is an herbicide whose target is HPPD, an enzyme found in both plants and animals (Pallett et al., 2001). HPPD plays a catabolic role in the breakdown of tyrosine in all aerobic organisms and in plants there is an anabolic branch where it plays a role in the production of vitamin E and plastoquinones. HPPD is an Fe II-dependent non-heme oxygenase catalyzing the conversion of 4-hydroxyphenylpyruvate (HPP) into homogentisic acid (HGA) (Moran, 2005), a key precursor in both the tocopherol (vitamin E) and plastoquinone/carotenoid pathway. Plant HPPD is the molecular target of several natural compounds and of a range of very effective synthetic herbicides including those in the isoxazoles, triketones, and pyrazolines families that are currently used commercially (Raspail et al., 2011; Schulz et al., 1993; Dayan et al., 2007). The activity of all commercialized HPPD-inhibitors is based on a chelating functionality, which binds to the redoxactive iron center in the enzyme. Inhibition of HPPD results in the depletion of the plant plastoquinone and vitamin E pools, leading to bleaching symptoms (Matringe, et al., 2005). The \textit{hppdPfW336-1Pa} gene introduced into HPPDi cotton was cloned from \textit{Pseudomonas fluorescens} and its tolerance to certain benzoylisoxazoles was increased by a single mutation that resulted in a substitution of glycine to tryptophan at position 336 (Boudec et al., 2001).

Cotton derives most of its economic value from its lint, but its seed plays an important role in oil and feed products as well. Aside from cottonseed oil, human consumption of cotton seed products is limited due to the presence of anti-nutritional factors (\textit{i.e}. gossypol and cyclopropenoic fatty acids) in the seeds. As an important source of nutrition for dairy cattle, cottonseed meal undergoes modest transnational trade (FAO Statistics Division, 2016), including importation to jurisdictions with strict safety Oversight of plant products developed through genetic engineering. Developers of cotton genetically engineered through Agrobacterium-mediated transformation, therefore provide regulatory agencies, including several importation-only jurisdictions, an assessment of the agronomic performance and composition of cotton seed (Ladics et al., 2015; Nakai et al., 2015). These requirements are in spite of numerous studies and over 20 years of experience with the safety of genetically engineered cotton (NASEM, 2016) and rigorous screening processes built in the product development pipeline (Prado et al., 2014).

Comparative assessment, a component in the weight of evidence to demonstrate the safety of new genetically engineered cotton products involves the comparison of both cotton agronomics and composition of the seed with its non-genetically engineered counterpart (OECD 2008, 2009). Bayer CropScience conducted an agronomic and lint quality assessment for HPPDi cotton at 15 field sites across the US, and composition assessment of fuzzy seed samples from eight sites (Fig. 1). Each field trial contained four blocks completely randomized with the following entries: HPPDi cotton treated with trait-specific herbicides glyphosate and isoxaflutole, HPPDi cotton not treated with trait-specific herbicides, Coker 312 (the conventional, untransformed comparator of HPPDi cotton), and three other non-genetically engineered varieties at each site. Agronomic data was collected and lint was tested by high volume instrument (HVI). Fuzzy seed samples were analyzed for proximates, fiber, anti-nutrients, and other components such as compounds in the tyrosine pathway. The goal of these assessments was to compare HPPDi cotton to its conventional comparator and other cotton varieties with respect to commercially important agronomic characteristics, lint properties and compositional components. The objective was to test whether HPPDi cotton is substantially equivalent to its conventional comparator and performs similarly under normal agronomic conditions across a variety of environmental conditions (\textit{i.e}. different production regions).

Figure 1. Map of sites that carried out field production for HPPDi cotton, its conventional comparator, and reference varieties overlaid on 2015 cotton crop data layer. Sites for which samples were analyzed for compositions are bolded and in italics. The agronomic assessment was done at sites 02, 03, 07, 08, 09, 10 and 11 in 2014 and sites 13, 14, 15, 16, 17, 18, 20, and 21 in 2015.
MATERIALS AND METHODS

Seed Quality and Varieties tested. All planting seeds were acid delinted and coated with a commercial mix of fungicides and insecticides typically used in commercial cotton production. Each HPPDi cotton and Coker 312 seed lot was verified to be at least 98% pure for the intended identity, to a 99% confidence interval. The entries included were HPPDi cotton treated with trait-specific herbicides glyphosate and isoxaflutole, HPPDi cotton not treated with trait-specific herbicides, Coker 312 (the non-genetically engineered counterpart for HPPDi cotton), and three of seven non-genetically engineered commercial reference varieties (cultivars) at each site: Acala Maxxa, FM953, FM966, FM989, ST457, ST468, and DP399. Reference varieties were allocated to sites based on optimal adaptation.

Field Trials Locations and Crop Management. Trials were planted at seven field locations in 2014 and eight locations in 2015 according to a standard protocol. Sites were located in North Carolina, Southern Georgia, the Mississippi Delta, several regions across Texas, and the California Central Valley (Fig. 1). Planting dates ranged from June 9 to June 20 in 2014 and May 21 to June 18 in 2015. Each entry was replicated four times in a randomized complete block design, and each field layout was independently randomized. Each plot was a minimum of 27.9 m². Field trials were managed according to local commercial practices. All crop maintenance activities (irrigation, fertilization, tillage, application of herbicides other than isoxaflutole and glyphosate, insecticides, and growth regulators) were applied evenly to all plots in each field trial. Water management at field trial sites included irrigated via underground drip, center pivot, overhead sprinkler, flooding in-furrow, or non-irrigated.

Trait Specific Herbicide Application. Isoxaflutole herbicide used in treated plots was sprayed at a rate of 105 g ai/ha (±10%). Glyphosate used in treated plots was sprayed over the top at a rate of 1121 g ai/ha (±10%). Isoxaflutole treatments were made at planting to the three-leaf stage; glyphosate treatments occurred in the six to nine leaf growth stage. All isoxaflutole and glyphosate applications were made using calibrated spray equipment.

Data Collection and Sampling

Agronomic Parameters. Stand count, plant mapping, yield data, and other agronomic parameters were collected at all trial sites. The stand count was performed on representative sections of each plot (e.g. two 6m rows) when each plot was at the three to eight leaf stage and converted to plants per m².

Plant mapping was performed on five representative plants per plot at crop maturity. Values of each endpoint were averaged among the five plants for analysis. Plant mapping endpoints included height (cotyledonary node to terminal bud), number of nodes, branch descriptions (i.e. fruiting or vegetative) for each node, boll counts (on fruiting and vegetative branches), and notations of boll presence/absence and whether or not the boll was harvestable on the two proximal positions of each fruiting branch. Number of bolls per plant (sum of fruiting and vegetative bolls), first fruiting branch, percent fruit retention, percent harvestable fruiting branch bolls and height to node ratio are derived from plant mapping. Plant mapping also produced data for height, number of nodes, number of vegetative branches, number of potential fruiting sites, and numbers of fruiting branch and vegetative bolls (data not shown).

Two harvest activities were performed at crop maturity. The first harvest was a 25 boll sample collected non-systematically from each plot. The seed cotton was weighed and ginned on tabletop gins to derive seed weight, lint weight, and percent lint. The second was a hand-harvest of all bolls from representative sections of each plot (e.g. two 6m rows) followed by weighing the seed cotton (plot yield). Plot yields were converted and analyzed on a kg/ha basis, and lint yield on a kg/ha basis was calculated from seed cotton weight and percent lint.

Lint Quality. Several significant lint quality measures were conducted using an Ulster HVI 1000 analyzer at Bayer CropScience Breeding Station Fiber Analysis Lab, Leland, MS. HVI lint analysis was performed on individual lint samples harvested from 25 randomly selected bolls within each plot (samples were not pooled by entry). Tests for length uniformity (data not shown), length, strength, elongation, and micronaire were conducted for all samples.

Crop Composition Analysis. Fuzzy seed samples from each plot from eight diverse field sites (Fig. 1) were used for composition analysis. The yield harvest from each site was ginned on tabletop gins to derive approximately 300 g of
fuzzy seed from each plot. The fuzzy seed samples were maintained frozen until analysis. Each fuzzy seed sample was analyzed individually to generate four replicates of each entry per field site. Compositional analyses were conducted using methods considered to be standardized within the analytical community (e.g., methods adopted by the American Oil Chemists’ Society). Analyses included proximates (e.g., carbohydrates, crude fat, crude protein, total dietary fiber and ash), anti-nutrients and other components such as compounds in the tyrosine pathway. Additionally, individual minerals, amino acids, and fatty acid components were analyzed (data not shown).

Statistical Analyses. Statistical analysis was accomplished in SAS version 9.3 (Cary, NC). Each parameter was analyzed with a mixed model analysis of variance. The additive model for the design was

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \delta_{k(j)} + \varepsilon_{ijk},$$

where $Y_{ijk}$ is the individual value measurement, $\mu$ the overall mean, $\alpha_i$ the fixed effect associated with entry, $\beta_j$ the random effect associated with site, $\delta_{k(j)}$ the random effect for the interaction of entry by site, $\delta_{k(j)}$ the random effect associated with block nested within site and $\varepsilon_{ijk}$ the random error. The degrees of freedom were estimated using the method specified by Kenward and Roger (1997).

Based on the mixed model, entry differences were estimated, along with the $p$-values ($t$-test) for the entry differences. Statistical significance was evaluated at $p<0.05$ level. Means and standard deviations were calculated arithmetically.

RESULTS

Agronomic Assessment. Agronomic data was collected throughout the season at each site according to a standard protocol. Early season stand counts indicated that HPPDi cotton and Coker 312 had similar germination and early season survival rates (Fig. 2A). The stand count shown was conducted following isoxaflutole application on the HPPDi cotton treated entry, demonstrating tolerance of HPPDi cotton to isoxaflutole.

![Figure 2](image-url) Figure 2. Stand and yield parameters of Coker 312, HPPDi cotton not treated, HPPDi cotton treated, and seven reference varieties. Column height represents mean and error bars represent standard deviation. Significant differences ($p<0.05$) between Coker 312 and HPPDi cotton treated or HPPDi cotton not treated are indicated with an asterisk (*). A. Stand Count at three to seven leaf stage, B. Bolls per Plant including fruiting branch and vegetative bolls, C. Seed cotton Yield derived from two rows in each plot, and D. Lint Yield calculated from seed cotton yield and lint percent. Lint percent data is not shown.
The results of the plant mapping indicated that Coker 312 and HPPDi cotton produced a similar number of bolls (Fig. 2B). Likewise, first fruiting branch, percent fruit retention, and percent harvestable fruiting branch bolls were not significantly different between Coker 312 and HPPDi cotton (Fig. 3). HPPDi cotton not treated was associated with a slightly higher height to node ratio than Coker 312 (Fig. 3A), however, the difference was numerically small, and the mean HPPDi cotton not treated height to node ratio (4.86 cm/node) was within the range (2.31 to 7.54 cm/node) of reference varieties (Fig. 3A) and therefore this difference could not be considered as biologically relevant. Moreover, no differences between Coker 312 and HPPDi cotton entries were detected for height or number of nodes as individual parameters (data not shown). Additionally, number of vegetative branches, number of potential fruiting sites, number of fruiting branch bolls and number of vegetative bolls were not significantly different between Coker 312 and HPPDi cotton treated or not treated entries (data not shown).

Yield was determined from seed cotton harvest of representative areas of each plot within each field trial through hand-harvest and weighing. Lint yields were calculated on a kg/ha basis from seed cotton weight and percent lint from each plot (percent lint data not shown). Differences were detected for seed cotton yield and lint yield between Coker 312 and HPPDi cotton not treated (Fig. 2). However, the difference was numerically small, and the means of seed cotton and lint yield (2789 and 1070 kg/ha, respectively) were within the range (398 to 5356 and 172 to 2353 kg/ha, respectively) of reference varieties (Fig. 2). No differences were detected in seed cotton yield or lint yield between Coker 312 and HPPDi cotton treated. This indicates that there are no biologically relevant differences in cotton yield or lint yield that can be attributed to the introduced traits in HPPDi cotton.

Figure 3. Plant mapping parameters of Coker 312, HPPDi cotton not treated, HPPDi cotton treated, and seven reference varieties. Column height represents mean and error bars represent standard deviation. Significant differences (*p<0.05) between Coker 312 and HPPDi cotton treated or HPPDi cotton not treated are indicated with an asterisk (*). A. Height to Node Ratio, B. Average First Fruiting Branch (node number above cotyledon), C. Percent Fruit Retention represents the number of bolls divided by total number of buds on first two fruiting branch positions, and D. Percent Harvestable (i.e. open and normal) Fruiting Branch Bolls.
**Lint Quality Assessment.** Standard HVI analyses were carried out on lint from 25 boll samples derived from each plot. Coker 312 and HPPDi cotton treated or not treated did not show differences in length, strength elongation or micronaire (Fig. 4). Additionally, there were no differences between Coker 312 and HPPDi cotton treated or not treated for length uniformity (data not shown).

**Fuzzy Seed Composition.** Levels of proximates including carbohydrates, crude protein, crude fat, and total dietary fiber were quantified (Fig. 5). There were no significant differences in proximate levels between HPPDi cotton and its non-genetically engineered counterpart, Coker 312, except that crude protein was statistically significantly lower in HPPDi cotton, treated and not treated, compared to Coker 312 (Fig. 5B). The difference in crude protein levels was numerically small (within 1% on average) and within the range of reference varieties (15.8 to 28.7% DW). Out of the 18 amino acids analyzed, only cystine and methionine levels were significantly lower in HPPDi cotton treated compared to Coker 312, but means were within the range of reference varieties (data not shown). No other individual amino acid was lower in either treated or not treated HPPDi cotton (data not shown). Therefore, the difference in crude protein levels was not considered to be biologically relevant.

Levels of ash (total mineral content), and vitamins including single and total tocopherols, vitamin A, and vitamin K₁ were determined. There were very few values above the Lower Limit of Quantitation (LOQ) for beta and delta tocopherol, therefore no statistical analysis was conducted on these analytes. No statistically significant differences were observed between Coker 312 and HPPDi cotton treated and not treated, or for gamma tocopherols (data not shown); and ash, total tocopherols, vitamin A, and vitamin K₁ (Fig. 6). Levels of alpha tocopherol were statistically significantly lower in HPPDi cotton, treated and not treated, compared to Coker 312, but were within the range of the reference varieties (data not shown) and therefore this difference is not biologically relevant.

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**Figure 4.** HVI lint properties of Coker 312, HPPDi cotton not treated, HPPDi cotton treated, and seven reference varieties. Column height represents mean and error bars represent standard deviation. No significant differences (p < 0.05) between Coker 312 and HPPDi cotton treated or HPPDi cotton not treated were detected. Lint samples were derived from 25 bolls from each plot. A. Length (UL₅₀), B. Micronaire, C. Strength, and D. Elongation.
Figure 5. Proximate levels of Coker 312, HPPDi cotton not treated, HPPDi cotton treated, and seven reference varieties fuzzy seeds. Column height represents mean and error bars represent standard deviation, expressed as percent dry weight (DW). Significant differences ($p<0.05$) between Coker 312 and HPPDi cotton treated or HPPDi cotton not treated are indicated with an asterisk (*). A. Carbohydrates, B. Crude Protein, C. Crude Fat, and D. Total Dietary Fiber.

Figure 6. Levels of total minerals (ash) and vitamins in Coker 312, HPPDi cotton not treated, HPPDi cotton treated, and seven reference varieties fuzzy seeds. Column height represents mean and error bars represent standard deviation, expressed as percent or mg/kg dry weight (DW). No significant differences ($p<0.05$) between Coker 312 and HPPDi cotton treated or HPPDi cotton not treated were detected. A. Ash, B. Total Tocopherols, C. Vitamin A, and D. Vitamin K$_1$ (phyloquinone).
Cotton seed naturally contain anti-nutrients (toxic substances) that impact the amount of cotton meal that can be incorporated into animal feed. Levels of anti-nutrients including free gossypol (unbound), total gossypol, malvalic acid, and dihydrosterculic acid were compared between Coker 312 and HPPDi cotton (Fig. 7). Sterculic acid levels were also analyzed but not described here as the levels were frequently lower than the limit of quantitation. There was a trend for lower anti-nutrient levels in HPPDi cotton compared to Coker 312 and reference varieties for all anti-nutrients. Differences were statistically significant for free and total gossypol for both HPPDi cotton treated and not treated, and for dihydrosterculic acid for HPPDi cotton treated. On a dry weight basis reference varieties’ fuzzy seed samples ranged from 0.273 to 0.941 mg/kg free gossypol, 0.346 to 1.34 mg/kg total gossypol, 1.26 to 4.07 mg/kg dihydroserculic acid and below the lower limit of quantification to 1.062 mg/kg malvalic acid. The mean HPPDi cotton treated or not treated levels of each of these anti-nutrients fell within these ranges. Anti-nutrient analyses confirmed that HPPDi cotton is commercially acceptable for use as both food and feed.

The statistically significant differences in crude protein, free and total gossypol, dihydrosterculic acid and alpha tocopherol between HPPDi cotton and Coker 312 are not considered biologically meaningful since the levels in HPPDi cotton were within the natural variability as confirmed by the range of the reference varieties and the range of other cotton varieties (ILSI, 2016). HPPDi cotton displays commercially acceptable performance with regards to the nutritional components analyzed within the fuzzy seed produced.

**DISCUSSION**

Agronomic performance, lint quality, and fuzzy seed composition data were generated from plots grown at fifteen field sites in two years representing multiple environmental conditions to support comparisons of HPPDi cotton, its non-genetically engineered counterpart comparator, and non-genetically engineered commercial cotton varieties. HPPDi cotton was compared to its non-genetically engineered counterpart in a mixed model ANOVA for every endpoint. Statistically significant (p<0.05) differences included lower yield (seed cotton and lint) and a higher height to node ratio for the non-treated HPPDi cotton but were not considered to be biologically relevant because these differences could be attributable to manual sampling, natural variability in the field assessment, or weed competition in HPPDi not treated. The effect of weed competition on cotton yield is well-documented (Rowland et al., 1999) and the availability of new herbicide modes would be expected to have a net positive effect on yield. No differences were detected for early season stand counts, bolls per plant, or any lint quality parameter (Figs. 2 and 3), indicating that HPPDi cotton maintains performance and economic value.

Plant mapping endpoints are indicative of varietal productivity including responses to environmental conditions (Jenkins et al., 1990). No differences between Coker 312 and HPPDi cotton with respect to any plant mapping endpoint were detected (Fig. 2B, Fig. 3, data not shown). In contrast, fruiting branch boll retention was greater in insect-resistant cotton expressing the Bt protein than non-Bt cotton because non-Bt cotton abscised bolls under lepidopteran pressure (Hofs et al., 2006). The plant mapping data indicate that Coker 312 and HPPDi cotton were similarly productive and produced similar physiological responses to environmental conditions that occurred throughout the growing seasons in very diverse environments (Fig. 1). Overall, the plant mapping results indicate that HPPDi cotton is not different from its non-genetically engineered counterpart, except for the added benefit of tolerance to isoxaflutole and glyphosate.

Fuzzy seed samples from each plot from eight diverse field sites (Fig. 1) were analyzed for major composition components including proximates, vitamins and minerals, and anti-nutrients. Statistically significant differences were detected for HPPDi cotton (treated or not treated) versus its comparator for crude protein (Fig. 5B). These differences are not considered biologically meaningful since the average levels of protein produced by HPPDi cotton were very similar (within the natural variability) to the average amount in fuzzy seed of reference varieties in this study, and within the range of other cotton varieties (ILSI, 2016). Additionally, individual amino acid levels were not different between HPPDi cotton and its non-genetically engineered counterpart, except for cystine and methionine (data not shown), and there were no differences for other proximates including carbohydrates, ash, or crude fat (Fig. 5). These results confirm that the nutritional composition of HPPDi cotton seed is commercially acceptable.
Anti-nutrients including the terpenoid phytoalexin gossypol and cyclopropenoid fatty acids malvalic acid and dihydrosterculic acid limits cottonseed meal as a protein source for non-ruminant animals. HPPDi cotton contained lower total gossypol, free gossypol and dihydrosterculic acid than its conventional comparator. The statistically significant differences for gossypol are not considered biologically relevant since they are within the range reported for other cotton varieties (ILSI, 2016). Gossypol levels of other glyphosate tolerant cotton lines were unrelated to the transgene in three different genetic backgrounds (Nida et al., 1996). Additionally, this minor reduction in gossypol levels would not impact cottonseed utility for human consumption (Gadelha et al., 2014). Dihydrosterculic acid is a cyclopropane ring product of oleic acid, and malvalic acid is formed from dihydrosterculic acid with sterculic acid as an intermediate. No differences between HPPDi cotton and its non-genetically engineered counterpart for malvalic acid (Fig. 7C), and oleic acid or sterculic acid (data not shown), indicate that the statistically significant reduction in dihydrosterculic acid is likely not attributable to the insertion in HPPDi cotton.

The results of this assessment indicate that HPPDi cotton has commercially acceptable agronomic, lint, and nutritional qualities. These qualities are similar to the conventional comparator, Coker 312, and the commercial comparators. This type of data provides a partial basis for risk assessment, including addressing possible unintended effects of genetic engineering (Devos et al., 2014). This study indicates that HPPDi cotton does not pose unique, unintended risks to cotton production, or to the resultant food, feed and fiber. Likewise, stacked products (multiple events combined by traditional breeding) incorporating HPPDi cotton would not be expected to compromise the contributing events’ safety or efficacy parameters including genetic stability, gene expression, the herbicide tolerance phenotype, or agronomic and compositional components (Kok et al., 2014). The safety profile of this event supports its value as a worthwhile tool, both alone and in combination with other insect resistance and herbicide tolerance traits. When managed appropriately this technology could potentially result in positive change for production, ecological and socio-economic status in rural cotton producing areas (Raven, 2014).

Figure 7. Antinutrient levels of Coker 312, HPPDi cotton not treated, HPPDi cotton treated, and seven reference varieties fuzzy seeds. Column height represents mean and error bars represent standard deviation, expressed as mg/kg dry weight (DW). Significant differences ($p<0.05$) between Coker 312 and HPPDi cotton treated or HPPDi cotton not treated are indicated with an asterisk (*). A. Free (non-protein bound) Gossypol, B. Total Gossypol, C. Malvalic Acid, and D. Dihydrosterculic Acid.
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