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Photosystem II-Inhibitors Play a Limited Role in Sweet Corn Response to 4-Hydroxyphenyl Pyruvate Dioxygenase-Inhibiting Herbicides

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ABSTRACT

Postemergence (POST) application of 4-hydroxyphenyl pyruvate dioxygenase (HPPD) inhibitors in combination with a photosystem II (PSII) inhibitor, such as atrazine [6-chloro-N-ethyl-N9-(1-methylethyl)-1,3,5-triazine-2,4-diamine], is common practice in sweet corn (*Zea mays* L.) production. Given the sensitivity of sweet corn to HPPD-inhibiting herbicides, the objective of this work was to determine the extent to which cytochrome P450 (CYP) genotype and PSII-inhibitors affect crop sensitivity to HPPD-inhibiting herbicides. Greenhouse experiments were used to identify PSII-inhibitors that were least injurious when combined with the HPPD-inhibitors, mesotrione [2-(4-mesyl-2-nitrobenzoyl)-3-hydroxycylohex-2-enone], tembotrione {2-[2-chloro-4-(methylsulfonyl)-3-[(2,2,2-trifluoroethoxy)methyl]benzoyl]-1,3-cyclohexanedione}, and topramezone {[3-(4,5-dihydro-3-isoxazolyl)-2-methyl-4-(methylsulfonyl)phenyl](5-hydroxy-1-methyl-1H-pyrazol-4-yl)methanone}. Subsequently, HPPD-inhibitors were tested individually with PSII-inhibitors atrazine, or bentazon [3-(1-methylethyl)-(1H)-2,1,3-benzothiadiazin-4(3H)-one2,2-dioxide], or alone in field experiments on all three CYP genotypic classes; hybrids homozygous for mutant CYP alleles (cypcyp), hybrids homozygous for functional alleles (CYPCYP), and heterozygous hybrids (CYPcyp). Leaf bleaching within 1 wk of herbicide application increased when a PSII-inhibitor was combined with an HPPD-inhibitor; however, the relatively low level of injury was short-lived. Tank mixing atrazine to mesotrione, tembotrione, or topramezone in sweet corn did not increase risk of yield loss compared to HPPD-inhibitor applied alone. The synergistic effect on weed control between certain PSII- and HPPD-inhibitor combinations reported previously does not hold true regarding sweet corn sensitivity to these herbicides. Among three HPPD-inhibitors tested in sweet corn, topramezone was the safest, regardless of PSII combination. Mutant CYP alleles, namely CYPcyp and cypcyp hybrids, are the main cause of sweet corn sensitivity to mesotrione, tembotrione, and other CYP-metabolized herbicides; therefore, breeding efforts to eliminate mutant CYP alleles should remain a high priority.

Postemergence applications of HPPD-inhibiting herbicides are common in sweet corn production for broadleaf weed control. Currently three HPPD-inhibiting herbicides are registered for use on sweet corn in the United States; mesotrione, tembotrione, and topramezone. A survey of growers' fields from 2005 to 2007 identified mesotrione as the second most widely used herbicide in sweet corn (Williams et al., 2010). With the commercialization of topramezone and tembotrione in 2006 and 2008, respectively, collectively the HPPD-inhibiting herbicides play an even greater role today in managing weeds of sweet corn (authors, personal observation, 2010). Moreover, HPPD-inhibitors represented the last major class of chemistry without herbicide resistance, until recently when HPPD-resistance was identified in multiple waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer] (Hausman et al., 2011; McMullan and Green, 2011) and palmer amaranth (*A. palmeri* S. Wats.) populations (McCabe, 2013). In susceptible plant biotypes, competitive inhibition of the HPPD enzyme by the herbicide leads to oxidative degradation of chlorophyll, compromising chloroplast synthesis and function.

Applications of HPPD-inhibitors are often tank mixed with a PSII-inhibitor in corn production systems. Numerous authors have found weed control efficacy of HPPD-inhibitors is increased with relatively low rates of a PSII-inhibitor, such as atrazine, bromoxynil (3,5-dibromo-4-hydroxybenzonitrile), or metribuzin [4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one] (Abendroth et al., 2006; Armel et al., 2005; Creech et al., 2004; Woodyard et al., 2009a, 2009b). For instance, addition of atrazine to mesotrione showed a synergistic effect on control of numerous weed species, whereby plant response was greater than expected based on individual product performance (Abendroth et al., 2006; Bollman et al., 2006; Hugie et al., 2008; Walsh et al., 2012; Woodyard et al.,

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Abbreviations: CYP gene, cytochrome P450 gene; DAT, days after treatment; GDD, growing degree days; HPPD-inhibitor, 4-hydroxyphenyl pyruvate dioxygenase inhibitor; LAI, leaf area index; PAR, photosynthetically active radiation; POST application, postemergence application; PSII-inhibitor, photosystem II inhibitor.

2009a, 2009b). Synergism from the atrazine plus mesotrione combination was explained by a reduction in photosynthetic rate compared to mesotrione alone, due to a complementary mode of action of mesotrione and atrazine in susceptible plant species (Creech et al., 2004; Armel et al., 2005; Hess, 2000; Fuerst and Norman, 1991). Both HPPD- and PSII-inhibitor mechanisms in plants are light dependent and result in foliar tissue membrane damage to susceptible plants due to the degradation of polyunsaturated fatty acids from lipid peroxidation (Hess, 2000). However, their sites of action are different. The PSII-inhibitor atrazine interferes with electron transfer on the D1 protein of photosystem II by binding to the Q_B site (Hess, 2000; Fuerst and Norman, 1991). In contrast, an HPPD-inhibitor results in depletion of the plastoquinone pool; thereby decreasing electron transport during photosynthesis and ultimately inhibiting carotenoid biosynthesis (Schultz et al., 1985; Lee et al., 1997). However, both result in inhibition of electron transfer between the photosystems, resulting in oxidative stress and reactive oxygen radicals that damage cellular constituents.

Selective herbicide metabolism accounts for field corn tolerance to POST applications of mesotrione (Mitchell et al., 2001), tembotrione (Schulte and Köcher, 2009), and topramezone (Grossmann and Ehrhardt, 2007). Nonetheless, sweet corn sensitivity to HPPD-inhibitors is well documented (Bollman et al., 2008; O'Sullivan et al., 2002; Williams et al., 2005). Sweet corn sensitivity to mesotrione, tembotrione, and possibly topramezone is conditioned by a single recessive gene (Williams et al., 2005; Williams and Pataky, 2008, 2010). This is apparently the same gene that conditions sensitivity to several other cytochrome P450 (CYP) metabolized herbicides from different chemical families (Pataky et al., 2006; Nordby et al., 2008). The gene in herbicide-sensitive sweet corn inbred Cr1 was mapped to the region on the short arm of chromosome 5 as a previously sequenced CYP gene (Nordby et al., 2008), referred to as the *nsf1* or *ben1* gene, which is one of two genes also responsible for bentazon sensitivity (Kang, 1993; Williams et al., 2006; Dam et al., 2010). In trials throughout North America, hybrids homozygous for mutant CYP alleles (cypcyp) were injured most frequently by P450-metabolized herbicides, hybrids homozygous for functional alleles (CYPCYP) were injured least, and heterozygous hybrids (CYPcyp) often had an intermediate response (Pataky et al., 2008).

Atrazine applied POST plays a major role in reducing risk of weed control failure in sweet corn production throughout North America (Williams et al., 2011a) and is used on more sweet corn acres than any other single herbicide (Williams et al., 2010). Does adding a PSII-inhibitor to an HPPD-inhibitor application increase risk of injury or yield loss in sweet corn? Previous investigation of the genetics of sweet corn tolerance focused on HPPD-inhibitor applied alone, not in combination with PSII-inhibitors (Williams et al., 2005; Williams and Pataky, 2008, 2010; Meyer et al., 2010). Bollman et al. (2008) evaluated weed control in sweet corn using HPPD-inhibitor with and without atrazine in hybrids of unknown CYP genotype. While some research has been conducted on combinations of HPPD-inhibitors and atrazine in sweet corn, the focus has been on weed control and yield stability (Williams et al., 2011b). Therefore, the objective of this work was to determine the extent to which CYP genotypes in sweet corn and PSII-inhibiting herbicide combination influences sensitivity to HPPD-inhibiting herbicides.

MATERIALS AND METHODS

Greenhouse Experiment

The HPPD- and PSII-inhibitor combinations were tested on CYP genotypes at the University of Illinois Plant Care Facility in 2010. The CYPCYP, CYPcyp, and cypcyp genotypes of near-isogenic sweet corn hybrid lines were created from crosses of near-isogenic inbred lines selected in the S_6 generations for functional or mutant CYP alleles. The experimental design was a split plot with three blocks. Ten kernels of three genotypes were planted into three separate rows in a 30 by 30 by 7 cm flat filled with potting mix. The main plot factor was herbicide treatments and subplot factor was CYP genotypes. Flats were arranged in a randomized complete-block design. Herbicide treatments (2X use rates) were mesotrione (Callisto, Syngenta, Greensboro, NC) at 210 g a.i. ha^{-1}, tembotrione (Laudis, Bayer CropScience, Research Triangle Park, NC) at 184 g a.i. ha^{-1}, and topramezone (Impact, AMVAC Chemical Corporation, Los Angeles, CA) at 49 g a.i. ha^{-1} applied alone or in combination with one of four PSII-inhibiting herbicides; atrazine at 2018 g a.i. ha^{-1}, bentazon (Basagran, Winfield Solutions, St. Paul, MN) at 2242 g a.i. ha^{-1}, bromoxynil (Buctril, Bayer CropScience, Research Triangle Park, NC) at 210 g a.i. ha^{-1}, and metribuzin (Metribuzin 75, Loveland Products, Inc., Greeley, CO) at 140 g a.i. ha^{-1}. The 2X herbicide rates were used to reflect application overlap.

Herbicides were applied when sweet corn had one to two fully emerged collars using a spray chamber in which the nozzle traveled over the stationary flats delivering the spray solution at 207 kPa. Untreated controls were included. Adjuvants for all treatments included 1% (v/v) crop oil concentrate (COC) and 2% (v/v) urea ammonium nitrate (UAN) with 28% N. Natural sunlight was supplemented with metal halide lamps for an intensity of 1000 μmol m^{-1} s^{-1} for 14 h. Greenhouse temperature was maintained at 24 ± 4°C. The experiment was conducted twice.

Plant injury was evaluated 7 days after treatment (DAT). Since HPPD-inhibitors used in this study interrupt carotenoid biosynthesis, leaf bleaching is a common, initial response among susceptible plants. A chlorophyll meter (SPAD 502 plus chlorophyll meter, Konica Minolta) was used to measure leaf greenness. The average of five SPAD readings was collected per row along the mid-length of the oldest emerging leaf. Percent leaf bleaching was calculated as: [(mean SPAD reading of untreated control minus SPAD reading of herbicide treated) divided by mean SPAD reading of untreated control] × 100.

Field Experiments

Results of greenhouse experiments, described later, were used to narrow the list of herbicide treatments for inclusion in subsequent field experiments. Each field experiment focused on an HPPD-inhibitor (mesotrione, tembotrione, or topramezone) used alone or in combination with certain PSII-inhibitors. Tembotrione and topramezone experiments were performed in 2011 and mesotrione experiments were performed in 2012.

Each HPPD-inhibitor experiment was conducted at two locations: the University of Illinois Crop Sciences Research and Education Center, Urbana, IL, and the research farm managed by the United States Department of Agriculture- Agricultural Research Service near Paterson, WA. These locations represent the two regions (Midwest and Pacific Northwest) where U.S. processing sweet corn is grown. The soil type at Urbana was Flanagan

silt loam (fine, smectitic, mesic Aquic Argiudoll). The soil type at Patterson was Quincy sand (mixed, mesic Xeric Torripsamment). Production practices common to each region, including crop seeding rate, fertilization, irrigation, and pest control, were used.

The experimental design was a split plot with four blocks. Levels of the main plot factor were the three CYP genotypes (cypcyp, CYPcyp, and CYPCYP), with each genotype represented by two hybrids. Hybrids were identified for CYP genotypes from previous experiments (Pataky et al., 2008; 2009). They included Merit (Seminis, Inc.) and 177A (Illinois Foundation Seed, Inc.) as cypcyp hybrids, Super Sweet Jubilee (Syngenta Seeds, Inc.) and 277A (Illinois Foundation Seed, Inc.) as CYPcyp hybrids, and Ambrosia (Crookham Seed Co.) and Marvel (Crookham Seed Co.) as CYPCYP hybrids. Levels of the subplot factor were four herbicide treatments: HPPD-inhibitor alone, HPPD-inhibitor plus atrazine, HPPD-inhibitor plus bentazon, and an untreated control. Herbicide use rates and adjuvants were identical to the greenhouse experiment.

Sweet corn was seeded in 76-cm spaced rows targeting a population of 56,800 and 77,000 plants ha^{-1} at Illinois and Washington, respectively. Main plots were four rows wide by 44 m long. Subplots were four rows wide by 9 m long. Within each main plot, a 2 m wide alley was maintained between subplots. Experiments were kept weed-free. Early season weeds were controlled with a pre-emergence application (1X use rate) of 1800 g a.i. ha^{-1} S-metolachlor plus 2200 g a.i. ha^{-1} atrazine (Bicep II Magnum, Syngenta, Greensboro, NC). Herbicide treatments were applied when sweet corn had three to five visible collars using a hand-held compressed-air backpack sprayer delivering 187 L ha^{-1} of spray solution at 276 kPa. Both greenhouse and field herbicide applications were performed within the recommended application timing.

Response variables were taken from the middle two rows of each four-row plot. Leaf bleaching at 7 and 14 DAT was evaluated using a chlorophyll meter as described earlier. Crop height 7 DAT, 14 DAT, and at silking was measured on three to five plants per plot. Height was measured from the surface to the uppermost leaf. Leaf area index (LAI) and photosynthetically active radiation (PAR) above and below the crop canopy were measured post-silking using a linear ceptometer (LP-80 AccuPAR, Decagon Devices, Pullman, WA). Light interception was calculated as: [(mean above canopy PAR minus mean below canopy PAR) divided by mean above canopy PAR] × 100. Mid-silk dates were measured in Illinois. Upon initial tassel emergence, plants with emerged silks were counted daily until at least 50% of plants per plot had silked; hereafter called the mid-silk date. Thermal time to mid-silk was determined as cumulative growing degree days (GDD) from crop emergence to mid-silk date, whereby GDDs were determined using a base temperature of 10°C and daily temperature data from a weather station with 1 km of each field (Illinois State Water Survey, Champaign, IL, and Washington Agricultural Weather Network, Prosser, WA). Relative plant height and crop LAI at silking was calculated as: (response variable of herbicide treatment divided by mean response of untreated control) × 100. All measurements taken from multiple plants per plot were averaged by each plot for statistical analyses.

Sweet corn ears were harvested approximately 21 and 26 d after mid-silk in Illinois and Washington, respectively. All ears >4.4 cm in diameter were hand harvested from the center two rows, 6 m in length, of each subplot. Ear number and mass were recorded. For each hybrid, relative yield was calculated as: (ear response variable of herbicide treatment divided by mean ear response variable of untreated control) × 100.

Statistical Analysis

Response variables of greenhouse and field experiments were analyzed individually using PROC MIXED in SAS version 9.2 (SAS Institute, 2007). Hybrids used in field experiments were not considered a main effect because their CYP genotypes were already evaluated from a previous study and because this experiment focused on overall performance of CYP genotypes, rather than individual hybrid performance. Therefore, hybrid to hybrid variation was analyzed as a part of the main plot error. Runs and blocks within run of the greenhouse experiment were considered random factors. Locations and blocks within location of field experiments were considered random factors. Genotype and herbicide treatments were considered fixed factors in both greenhouse and field experiments. Data complied with ANOVA assumptions of homogeneity of variance based on the modified Levene's test (Neter et al., 1996) and normality based on diagnostic test of residuals. Since the interaction of genotype and herbicide treatment with runs was not significant, greenhouse data was pooled over runs. Since the interaction of genotype and herbicide treatment with locations was not significant, field data were pooled over locations.

The experimental design used for the data analyses was:

$$Y_{ijkl} = \mu + \alpha_i + \beta_{(i)j} + \tau_k + (\alpha\tau)_{ik} + \varepsilon_{(i)jk} + \delta_l + (\alpha\delta)_{jl} + (\tau\delta)_{kl} + (\alpha\tau\delta)_{ijk} + \varepsilon_{(i)jkl}$$

where α_i, effect of run or location in greenhouse and field trials, respectively; $\beta_{(i)j}$, block effects nested within run or location; τ_k, main plot effects; $(\alpha\tau)_{ik}$, interaction between run/location and main plot effects; δ_l, subplot effects; $(\alpha\delta)_{jl}$, interaction between run/location and subplot effects; $(\tau\delta)_{kl}$, interaction between main plot and subplot effects; $(\alpha\tau\delta)_{ijk}$, interaction between run/location, main plot and subplot effects; $\varepsilon_{(i)jk}$ and $\varepsilon_{(i)jkl}$ are error terms.

Subplot means within the same main plot were separated by Fisher's least significant difference (LSD) at $\alpha = 0.01$ in SAS.

RESULTS

Greenhouse Experiments

Leaf bleaching 7 DAT revealed an interaction between HPPD-inhibiting herbicide and CYP genotypic class (<0.001). Topramezone applied alone was safe across all CYP genotypes (Fig. 1). In contrast, mesotrione and tembotrione injured sweet corn hybrids, with severity varying by CYP genotype. For example, the cypcyp near-isogenic hybrid was injured 2.8 and 6.4 times more from mesotrione and tembotrione, respectively, than the CYPCYP near-isogenic hybrid.

Plant leaf bleaching to HPPD-inhibiting herbicide was not reduced by the addition of a PSII-inhibitor, and in most cases, increased crop injury. For example, addition of metribuzin to all three HPPD-inhibitors increased leaf bleaching twofold or more, relative to the HPPD-inhibitor alone (Fig. 2).

These greenhouse results were used to narrow the list of candidate PSII-inhibiting herbicides considered for inclusion in subsequent field trials. Metribuzin was too injurious on sweet

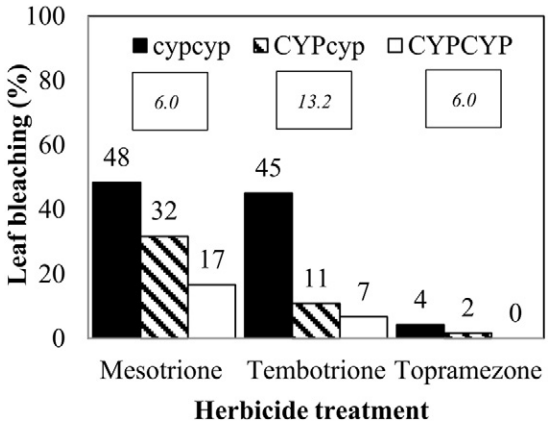

Fig. 1. Percent leaf bleaching of sweet corn cytochrome P450 (CYP) Genotypes 7 d after treatment with 4-hydroxyphenyl pyruvate dioxygenase (HPPD) inhibiting herbicides applied alone in greenhouse experiments. Percent leaf bleaching of the untreated control had the mean of 0% and was not included in the figure. Fisher's least significant difference, reported in italic, was calculated for CYP genotype comparison at the same herbicide treatment at $\alpha = 0.01$.

corn when combined with mesotrione, tembotrione, or topramezone. Although bromoxynil was not excessively injurious in greenhouse trials, it is not registered for use on sweet corn in North America. Given the limitations on field space and seed availability, herbicide treatments for use in sweet corn were narrowed to three: (i) HPPD-inhibitor alone, (ii) HPPD-inhibitor plus atrazine, and (iii) HPPD-inhibitor plus bentazon.

Field Experiments

Leaf bleaching 7 DAT revealed an interaction between CYP genotypic class and PSII-inhibitor combination for mesotrione and tembotrione trials (<0.001) but not for topramezone trials (0.375). Topramezone alone injured sweet corn the least across the CYP genotypes. Combinations of topramezone with atrazine or bentazon resulted in low levels of leaf bleaching (≤17%) (Fig. 3). Tembotrione also was safe, except for cypcyp hybrids, where all herbicide treatments resulted in >50% leaf bleaching. Injury was greatest in mesotrione trials, where all genotypic classes were bleached (up to 76%) by one or more herbicide treatments. Injury to CYPCYP hybrids was notable at 20% with mesotrione plus bentazon application.

An interaction between CYP genotypic class and PSII-inhibitor combination for mesotrione and tembotrione was observed for early season crop height (≤0.004). At 7DAT, crop stunting (up to 45%) was observed in cypcyp hybrids treated with or without PSII-inhibitors in mesotrione and tembotrione trials (data not shown). This effect also was observed at 14DAT (data not shown). However, by mid-silk, cypcyp hybrids in mesotrione alone treatment were comparable to the untreated control (Fig. 4). In contrast, cypcyp hybrids were ~40% shorter than the untreated control when they had received tembotrione alone or tembotrione plus atrazine at mid-silk. These hybrids were stunted 19% by tembotrione plus bentazon. Sweet corn height was not reduced by any treatments in topramezone trials.

The cypcyp hybrids treated with mesotrione and tembotrione often had smaller plant canopies at mid-silk, up to 63% LAI reductions, compared to the untreated control (Fig. 5). Such leaf area reductions impeded the crop's ability to intercept PAR (data not shown).

Most treatments did not delay silk emergence. The exception was mesotrione plus atrazine or bentazon in cypcyp hybrids. In these cases, silk emergence was delayed 86 GDD on average, or about 6 d for central Illinois (data not shown).

Yield response was dependent on HPPD-inhibitor, herbicide combination, and genotypic class of sweet corn. For example, relative ear mass yields in all topramezone treatments, across genotypic classes, were similar to the untreated control (Fig. 6). Similar to growth response variables, negative effects on ear mass from tembotrione treatments were isolated to cypcyp hybrids. Yields were reduced as much as 49% in tembotrione alone and tembotrione plus atrazine treatments. Yields were reduced only 24% by the tembotrione plus bentazon treatment for cypcyp hybrids, which were comparable to untreated control. The cypcyp hybrids demonstrated yield losses across all mesotrione combinations. Ear number data showed a similar result (data not shown).

DISCUSSION

A PSII-inhibitor, such as atrazine, is often added to POST applications of an HPPD-inhibitor to expand weed control spectrum. However, certain sweet corn hybrids are injured by applications of HPPD-inhibitors applied alone (Williams and Pataky, 2010; Pataky et al., 2008, 2009; Meyer et al., 2010). Studies have

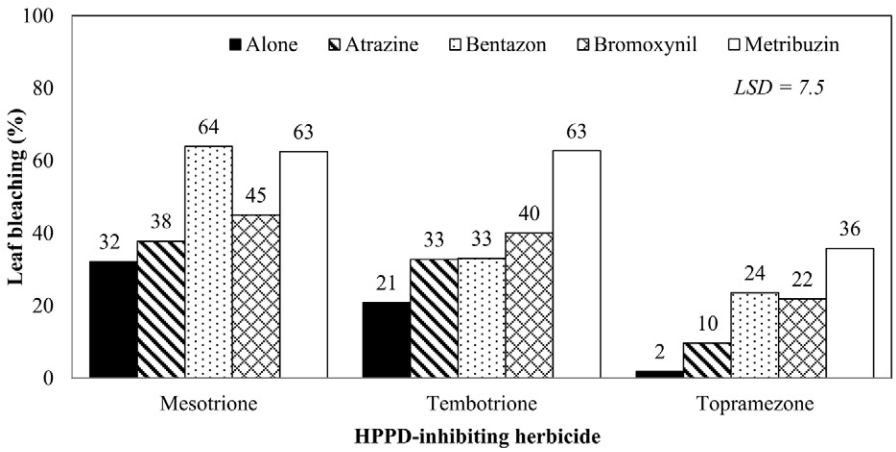

Fig. 2. Percent leaf bleaching 7 d after treatment with photosystem II (PSII) and HPPD-inhibitor combinations across all CYP genotypes in greenhouse experiments. Percent leaf bleaching of the untreated control had the mean of 0% and was not included in the figure. Fisher's least significant difference, reported in italic, was calculated for herbicide treatment comparison at $\alpha = 0.01$.

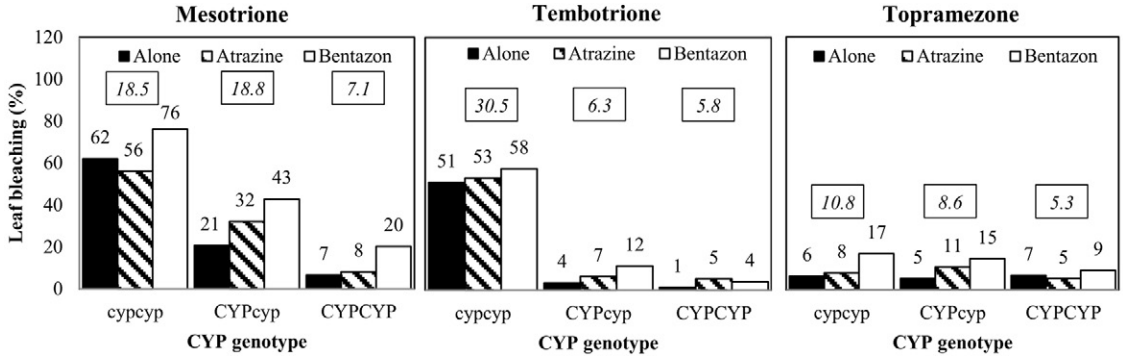

Fig. 3. Percent leaf bleaching of sweet corn CYP genotypes 7 d after treatment with PSII- and HPPD-inhibitor combinations in field experiments. Percent leaf bleaching of the untreated control had the mean of 0% and was not included in the figure. Fisher's least significant difference, reported in italic, was calculated for herbicide treatment subplot comparison at the same CYP genotype main plot level at $\alpha = 0.01$.

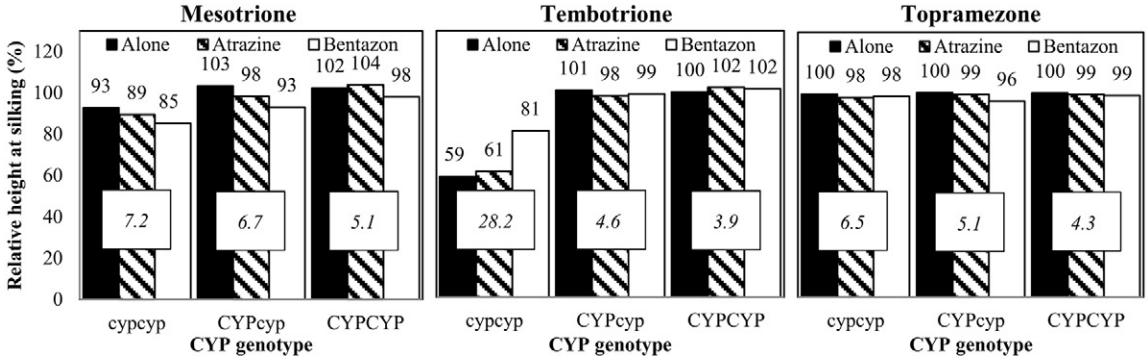

Fig. 4. Relative height at silking of sweet corn CYP genotypes as a result of PSII- and HPPD-inhibitor combinations in field experiments. Relative height at silking of the untreated control had the mean of 100% and was not included in the figure. Fisher's least significant difference, reported in italic, was calculated for herbicide treatment subplot comparison at the same CYP genotype main plot level at $\alpha = 0.01$.

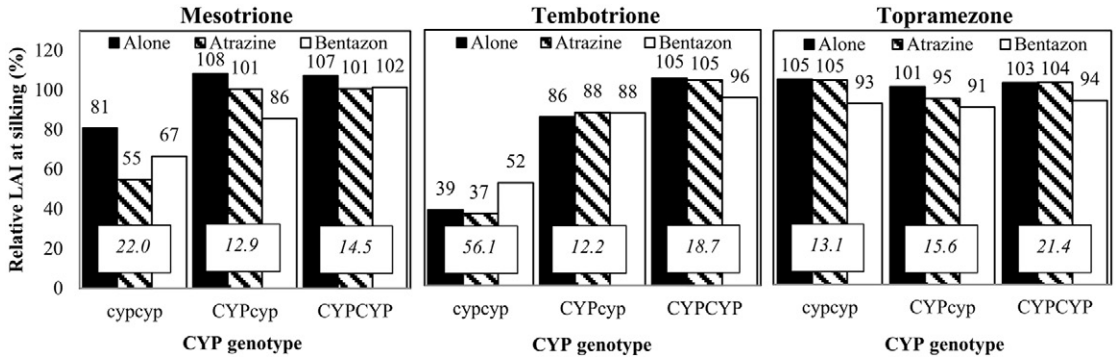

Fig. 5. Relative crop leaf area index (LAI) at silking of sweet corn CYP genotypes as a result of PSII- and HPPD-inhibitor combinations in field experiments. Relative crop LAI of the untreated control had the mean of 100% and was not included in the figure. Fisher's least significant difference, reported in italic, was calculated for herbicide treatment subplot comparison at the same CYP genotype main plot level at $\alpha = 0.01$.

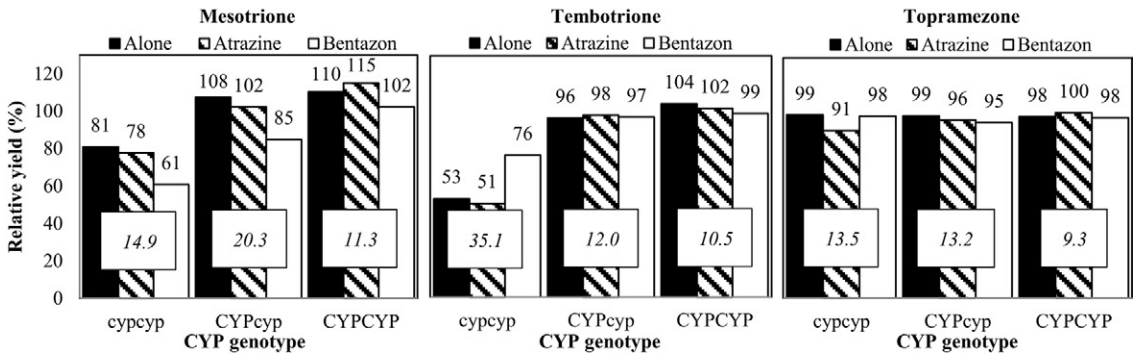

Fig. 6. Relative ear mass yield of sweet corn CYP genotypes as a result of PSII- and HPPD-inhibitor combinations in field experiments. Relative ear mass yield of the untreated control had the mean of 100% and was not included in the figure. Fisher's least significant difference, reported in italic, was calculated for herbicide treatment subplot comparison at the same CYP genotype main plot level at $\alpha = 0.01$.

determined that a single gene in sweet corn is responsible for sensitivity to multiple herbicides including HPPD-inhibitors and other CYP-metabolized herbicides (Pataky et al., 2006; Nordby et al., 2008; Williams and Pataky, 2008). Moreover, degree of sensitivity to these herbicides is driven by the number of mutant CYP alleles in the hybrid and environmental conditions influencing the plant growth (Pataky et al., 2008; Williams and Pataky, 2010). Therefore, this work aimed to quantify how plant growth and yield of different sweet corn CYP genotypes respond to HPPD-inhibitors when combined with different PSII-inhibitors.

Crop responses among CYP genotypes to HPPD-inhibitors, applied alone, are consistent with previous research. For instance, sweet corn tolerance to topramezone was confirmed for all genotypic classes in greenhouse and field experiments. Previous research showed application of topramezone at 36 g a.i. ha^{-1} resulted in £5% injury on 746 sweet corn hybrids tested, including cypcyp hybrids (Williams and Pataky, 2010). Excellent crop safety also was observed for tembotrione applied alone, with the exception of cypcyp hybrids. Of 68 sweet corn hybrids with known CYP genotypes, Williams and Pataky (2008) showed that only cypcyp hybrids were injured by tembotrione (185 g a.i. ha^{-1}). These hybrids (e.g., Merit) were the only hybrids injured by tembotrione in additional trials (Bollman et al., 2008). Of the three HPPD-inhibitors tested in this research, mesotrione often injured cypcyp hybrids the most and CYPCYP hybrids the least; with CYPcyp hybrids having an intermediate crop response to mesotrione (Williams et al., 2008; Meyer et al., 2010). Although sweet corn shares a common genetic basis for response to mesotrione and tembotrione, mesotrione lacks the crop safener isoxadifen-ethyl formulated with tembotrione, which reduces crop injury to CYPcyp and CYPCYP hybrids (Williams and Pataky, 2008).

Does adding a PSII-inhibitor to an HPPD-inhibitor application increase risk of injury or yield loss to sweet corn? In the greenhouse experiment, leaf bleaching within 1 wk of herbicide application increased when a PSII-inhibitor was combined with an HPPD-inhibitor. However, the relatively low level of injury from addition of a PSII-inhibitor was short-lived in field experiments, as evidenced by plant growth, canopy development, and yield measured later in the season.

Yield response varied with HPPD-inhibitor and herbicide treatment. Yield response to HPPD-inhibitor plus atrazine was similar to yield response to HPPD-inhibitor applied alone. However, bentazon reduced yield loss from tembotrione in cypcyp hybrids. Bentazon dampened adverse plant response when mixed with saflufenacil (Frihauf et al., 2010), imazethapyr (Bauer et al., 1995), acifluorfen (Sorensen et al., 1987), and triotosulfuron (Lycan and Hart, 1999) by reducing foliar herbicide absorption in various weed and crop species. Differential absorption rates of tembotrione were not supported in the present work, because leaf bleaching 7 DAT was similar for tembotrione and tembotrione plus bentazon on cypcyp hybrids. Since isoxadifen-ethyl appears to play an important role in CYP metabolism of tembotrione (Williams and Pataky, 2008), perhaps the safener also enhanced metabolism of bentazon. Afterall, *nsf1* (also called *ben1*) is one of two genes responsible for bentazon sensitivity (Bradshaw et al., 1994). The other gene involved in bentazon sensitivity is *ben2*; hybrids used in this work had unknown genotypes for *ben2*. While *Nsf1/Ben1* is responsible for detoxifying multiple P450-metabolized herbicides, *Ben2* only metabolizes bentazon, there is no direct evidence it encodes a CYP, and it is not induced by safeners (Barrett et al., 1997). The present work shows that adding bentazon to mesotrione, which lacks isoxadifen-ethyl, did not reduce yield loss for hybrids with mutant CYP alleles, relative to mesotrione applied alone.

Under current sweet corn management practice, adding atrazine to POST applications of mesotrione, tembotrione, or topramezone does not increase risk of yield loss from these HPPD-inhibitors. The synergistic effect on weed control between certain PSII- and HPPD-inhibitor combinations reported previously (Abendroth et al., 2006; Bollman et al., 2006; Hugie et al., 2008; Woodyard et al., 2009a, 2009b; Walsh et al., 2012) does not hold true regarding sweet corn sensitivity to atrazine plus mesotrione, tembotrione, or topramezone. Although both HPPD- and PSII-inhibitors destabilize tissue membranes in susceptible plants (Hess, 2000), detoxifying mechanisms in corn are different. Glutathione S-transferases (GSTs) catalyze the formation of an atrazine-glutathione conjugate (Shimabukuro et al., 1971; Timmerman, 1989) and plants with high levels of GST activity are more tolerant than plants with low levels (Timmerman, 1989). Corn GST isozymes that differ in subunit composition are involved in detoxification of atrazine through several metabolic pathways (Cherifi et al., 2001). Yields of cypcyp hybrids in the atrazine plus mesotrione or tembotrione treatments were similar to the HPPD-inhibitor applied alone, suggesting atrazine metabolism was not impacted by lack of CYP activity. Yield response from atrazine plus mesotrione or tembotrione is due primarily to CYP alleles regulating metabolism of mesotrione and tembotrione in sweet corn.

Although environmental factors and crop safeners can influence herbicide sensitivity in sweet corn, mutant CYP alleles remain the main cause for sweet corn sensitivity to certain HPPD-inhibitors; specifically, hybrids homozygous for mutant CYP alleles. While mutant CYP alleles appear to cause little problem for applications of topramezone, their presence in commercial hybrids is problematic for mesotrione and tembotrione applications. Whether mesotrione or tembotrione are applied alone or in combination with PSII-inhibitors, hybrids homozygous for mutant CYP alleles suffer unacceptable yield loss. Even heterozygous hybrids that we tested (CYPcyp) are compromised by a combination such as mesotrione plus bentazon.

CONCLUSIONS

This study provides a more complete understanding of how combinations of PSII- and HPPD-inhibitors affect plant growth and yield of different sweet corn CYP genotypes. Because cypcyp and CYPcyp hybrids are still commercially available, breeding efforts to eliminate mutant CYP alleles in sweet corn remain a high priority. In the meantime, the choice of PSII- and HPPD-inhibitor combinations should be considered carefully. Postemergence application of mesotrione should be considered only for CYPcyp and CYPCYP genotypes, and of the PSII-inhibitors tested, only with atrazine. Tembotrione, with or without atrazine or bentazon, is unlikely to negatively influence yield of sweet corn hybrids with at least one functional CYP allele. Among the three HPPD-inhibitors tested on sweet corn, topramezone was the safest, regardless of combination with atrazine or bentazon, for all CYP genotypes.

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REFERENCES

Abendroth, J., A. Martin, and F. Roeth. 2006. Plant response to combinations of mesotrione and photosystem II inhibitors. Weed Technol. 20:267–274. doi:10.1614/WT-05-020R.1

Armel, G.R., G.J. Hall, H.P. Wilson, and N. Cullen. 2005. Mesotrione plus atrazine mixtures for control of canada thistle (*Cirsium arvense*). Weed Sci. 53:202–211. doi:10.1614/WS-04-039R

Barrett, M., N. Polge, R. Baerg, L. Bradshaw, and C. Poneleit. 1997. Role of cytochrome P-450s in herbicide metabolism and selectivity and multiple herbicide metabolizing cytochrome P-450 activities in maize. In: K.K. Hatzios, editor, Regulation of enzymatic systems detoxifying xenobiotics in plants. Kluwer Academic Publ., Dordrecht, the Netherlands. p. 35–50.

Bauer, T.A., K.A. Renner, and D. Penner. 1995. Olathe pinto bean (*Phaseolus vulgaris*) response to postemergence imazethapyr and bentazon. Weed Sci. 43:276–282.

Bollman, J.D., C.M. Boerboom, R.L. Becker, and V.A. Fritz. 2008. Efficacy and tolerance to HPPD-inhibiting herbicides in sweet corn. Weed Technol. 22:666–674. doi:10.1614/WT-08-036.1

Bollman, S.L., J.J. Kells, and D. Penner. 2006. Weed response to mesotrione and atrazine applied alone and in combination preemergence. Weed Technol. 20:903–907. doi:10.1614/WT-04-304.1

Bradshaw, L.D., M. Barrett, and C.G. Poneleit. 1994. Inheritance of bentazon susceptibility in a corn (*Zea mays*) line. Weed Sci. 42:641–647.

Cherifi, M., M. Raveton, A. Picciocchi, P. Ravanel, and M. Tissut. 2001. Atrazine metabolism in corn seedlings. Plant Physiol. Biochem. 39:665–672. doi:10.1016/S0981-9428(01)01281-5

Creech, J.E., T.A. Monaco, and J.O. Evans. 2004. Photosynthetic and growth responses of *Zea mays* L. and four weed species following post-emergence treatments with mesotrione and atrazine. Pest Manag. Sci. 60:1079–1084. doi:10.1002/ps.924

Dam, T., A.D. Guida, Jr., C.B. Hazel, B. Li, and M.E. Williams. 2010. Methods of identifying a plant with an *nsf1* locus. U.S. Patent no. 7838,263 B2. Date issued: 23 Nov. 2010.

Frihauf, J.C., P.W. Stahlman, and K. Al-Khatib. 2010. Saflufenacil absorption and translocation in winter wheat (*Triticum aestivum* L.). Pestic. Biochem. Physiol. 98:243–247. doi:10.1016/j.pestbp.2010.06.014

Fuerst, E., and M. Norman. 1991. Interactions of herbicides with photosynthetic electron-transport. Weed Sci. 39:458–464.

Grossmann, K., and T. Ehrhardt. 2007. On the mechanism of action and selectivity of the corn herbicide topramezone: A new inhibitor of 4-hydroxyphenylpyruvate dioxygenase. Pest Manag. Sci. 63:429–439. doi:10.1002/ps.1341

Hausman, N.E., S. Singh, P.J. Tranel, D.E. Riechers, S.S. Kaundun, N.D. Polge et al. 2011. Resistance to HPPD-inhibiting herbicides in a population of waterhemp (*Amaranthus tuberculatus*) from Illinois, United States. Pest Manag. Sci. 67:258–261. doi:10.1002/ps.2100

Hess, F.D. 2000. Light-dependent herbicides: An overview. Weed Sci. 48:160–170. doi:10.1614/0043-1745(2000)048[0160:LDHAO]2.0.CO;2

Hugie, J.A., G.A. Bollero, P.J. Tranel, and D.E. Riechers. 2008. Defining the rate requirements for synergism between mesotrione and atrazine in redroot pigweed (*Amaranthus retroflexus*). Weed Sci. 56:265–270. doi:10.1614/WS-07-128.1

Kang, M.S. 1993. Inheritance of susceptibility to nicosulfuron herbicide in maize. J. Hered. 84:216–217.

Lee, D., M. Prisbylla, T. Cromartie, D. Dagarin, S. Howard, W. Provan et al. 1997. The discovery and structural requirements of inhibitors of p-hydroxyphenylpyruvate dioxygenase. Weed Sci. 45:601–609.

Lycan, D.W., and S.E. Hart. 1999. Physiological response of soybean (*Glycine max*) and two weed species to thifensulfuron and bentazon combinations. Weed Sci. 47:143–148.

McCabe. 2013. Palmer amaranth herbicide resistance confirmed in Nebraska. Prairie Farmer. Farm Progress Co., Inc. http://farmprogress.com/story-palmer-amaranth-herbicide-resistance-confirmed-nebraska-9-103317 (accessed January 2014).

McMullan, P.M., and J.M. Green. 2011. Identification of a tall waterhemp (*Amaranthus tuberculatus*) biotype resistant to HPPD-inhibiting herbicides, atrazine, and thifensulfuron in iowa. Weed Technol. 25:514–518. doi:10.1614/WT-D-10-00150.1

Meyer, M.D., J.K. Pataky, and M.M. Williams, II. 2010. Genetic factors influencing adverse effects of mesotrione and nicosulfuron on sweet corn yield. Agron. J. 102:1138–1144. doi:10.2134/agronj2010.0093

Mitchell, G., D. Bartlett, T. Fraser, T. Hawkes, D. Holt, J. Townson, and R. Wichert. 2001. Mesotrione: A new selective herbicide for use in maize. Pest Manag. Sci. 57:120–128. doi:10.1002/1526-4998(200102)57:23.0.CO;2-E

Neter, J., M.H. Kutner, C.J. Nachtsheim, and W. Wasserman. 1996. Applied linear statistical models. 4th ed. Irwin, Chicago, IL.

Nordby, J.N., M.M. Williams, II, J.K. Pataky, D.E. Riechers, and J.D. Lutz. 2008. A common genetic basis in sweet corn inbred Cr1 for cross sensitivity to multiple cytochrome P450-metabolized herbicides. Weed Sci. 56:376–382. doi:10.1614/WS-07-145.1

O'Sullivan, J., J. Zandstra, and P. Sikkema. 2002. Sweet corn (*Zea mays*) cultivar sensitivity to mesotrione. Weed Technol. 16:421–425. doi:10.1614/0890-037X(2002)016[0421:SCZMCS]2.0.CO;2

Pataky, J.K., M.D. Meyer, J.D. Bollman, C.M. Boerboorn, and M.M. Williams, II. 2008. Genetic basis for varied levels of injury to sweet corn hybrids from three cytochrome P450-metabolized herbicides. J. Am. Soc. Hortic. Sci. 133:438–447.

Pataky, J.K., J.N. Nordby, M.M. Williams, II, and D.E. Riechers. 2006. Inheritance of cross-sensitivity in sweet corn to herbicides applied postemergence. J. Am. Soc. Hortic. Sci. 131:744–751.

Pataky, J.K., M.M. Williams, II, D.E. Riechers, and M.D. Meyer. 2009. A common genetic basis for cross-sensitivity to mesotrione and nicosulfuron in sweet corn hybrid cultivars and inbreds grown throughout north america. J. Am. Soc. Hortic. Sci. 134:252–260.

SAS Institute. 2007. Version 9.2. SAS Inst., Cary, NC.

Schulte, W., and H. Köcher. 2009. Tembotrione and combination partner isoxadifen-ethyl–mode of herbicidal action. Bayer CropSci. J. 62:35–52.

Schultz, G., J. Soll, E. Fiedler, and D. Schulzesiebert. 1985. Synthesis of prenylquinones in chloroplasts. Physiol. Plant. 64:123–129. doi:10.1111/j.1399-3054.1985.tb01222.x

Shimabukuro, R.H., D. Frear, H. Swanson, and W. Walsh. 1971. Glutathione conjugation- enzymatic basis for atrazine resistance in corn. Plant Physiol. 47:10–14. doi:10.1104/pp.47.1.10

Sorensen, V.M., W.F. Meggitt, and D. Penner. 1987. The interaction of acifluorfen and bentazon in herbicidal combinations. Weed Sci. 35:449–456.

Timmerman, K. 1989. Molecular characterization of corn glutathione S-transferase isozymes involved in herbicide detoxication. Physiol. Plant. 77:465–471. doi:10.1111/j.1399-3054.1989.tb05668.x

Walsh, M.J., K. Stratford, K. Stone, and S.B. Powles. 2012. Synergistic effects of atrazine and mesotrione on susceptible and resistant wild radish (*Raphanus raphanistrum*) populations and the potential for overcoming resistance to triazine herbicides. Weed Technol. 26:341–347. doi:10.1614/WT-D-11-00132.1

Williams, M., S. Sowinski, T. Dam, and B.L. Li. 2006. Map-based cloning of the *nsf1* gene of maize. Program and Abstracts of the 48th Maize Genetics Conference, Pacific Grove, CA. March 2006. p. 49.

Williams, M.M., II, C.M. Boerboom, and T.L. Rabaey. 2010. Significance of atrazine in sweet corn weed management systems. Weed Technol. 24:139–142. doi:10.1614/WT-D-09-00074.1

Williams, M.M., II, R.A. Boydston, R.E. Peachey, and D. Robinson. 2011a. Performance consistency of reduced atrazine use in sweet corn. Field Crops Res. 121:96–104. doi:10.1016/j.fcr.2010.11.020

Williams, M.M., II, R.A. Boydston, R.E. Peachey, and D. Robinson. 2011b. Significance of atrazine as a tank-mix partner with tembotrione. Weed Technol. 25:299–302. doi:10.1614/WT-D-10-00140.1

Williams, M.M., II, J. Pataky, J. Nordby, D. Riechers, C. Sprague, and J. Masiunas. 2005. Cross-sensitivity in sweet corn to nicosulfuron and mesotrione applied postemergence. HortScience 40:1801–1805.

Williams, M.M., II, and J.K. Pataky. 2008. Genetic basis of sensitivity in sweet corn to tembotrione. Weed Sci. 56:364–370. doi:10.1614/WS-07-149.1

Williams, M.M., II, and J.K. Pataky. 2010. Factors affecting differential sensitivity of sweet corn to HPPD-inhibiting herbicides. Weed Sci. 58:289–294. doi:10.1614/WS-D-09-00058.1

Williams, M.M., II, L.M. Wax, J.K. Pataky, and M.D. Meyer. 2008. Further evidence of a genetic basis for varied levels of injury to sweet corn hybrids from cytochrome P450-metabolized herbicides applied postemergence. HortScience 43:2093–2097.

Woodyard, A.J., G.A. Bollero, and D.E. Riechers. 2009a. Broadleaf weed management in corn utilizing synergistic postemergence herbicide combinations. Weed Technol. 23:513–518. doi:10.1614/WT-08-188.1

Woodyard, A.J., J.A. Hugie, and D.E. Riechers. 2009b. Interactions of mesotrione and atrazine in two weed species with different mechanisms for atrazine resistance. Weed Sci. 57:369–378. doi:10.1614/WS-08-175.1