Genetic Factors Influencing Adverse Effects of Mesotrione and Nicosulfuron on Sweet Corn Yield

Michael D. Meyer, Jerald K. Pataky, and Martin M. Williams II*

ABSTRACT

Sensitivity to certain P450-metabolized herbicides in corn (*Zea mays* L.) is largely conditioned by a single cytochrome P450 (CYP) gene. Little to no research has been done to evaluate the effect of CYP genotype on sweet corn yield. Yield of 23 sweet corn hybrids of known CYP genotype was evaluated in 2007, 2008, and 2009 following postemergence applications of mesotrione, an hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor, or nicosulfuron, an acetolactate synthase (ALS) inhibitor, at two growth stages. Mesotrione and nicosulfuron were evaluated in separate experiments. Treatments included herbicide application during the V3 to V5 or V5 to V7 growth stages and a nontreated control. Crop injury, measured 7 d after treatment (DAT), ranged from 0 to 87% for mesotrione and 0 to 54% for nicosulfuron among CYP genotypes. Injury from both mesotrione and nicosulfuron was most severe following application during V3 to V5 growth stages on hybrids with mutant (i.e., nonfunctional) cyp alleles. Only hybrids homozygous for mutant cyp alleles (i.e., *cypcyp*) suffered yield losses from mesotrione, ranging from 9 to 40%. These hybrids were not evaluated for nicosulfuron because applications of ALS-inhibiting herbicides kill *cypcyp* hybrids. Nicosulfuron reduced the yield of *CYPcyp* hybrids only; whereas mesotrione did not. Yield losses from nicosulfuron ranged from 9 to 35% among *CYPcyp* hybrids and were associated with moderate to severe loss of kernel rows (i.e., ear pinching). Yield of *CYPCYP* hybrids was not affected by mesotrione or nicosulfuron.

Sensitivity of certain sweet corn hybrids to postemergence applications of cytochrome P450-metabolized herbicides, including mesotrione, a HPPD inhibitor, and nicosulfuron, an ALS inhibitor, has been well documented in the past 20 yr (Diebold et al., 2003, 2004; Grey et al., 2000; Monks et al., 1992; Morton and Harvey, 1992; O'Sullivan and Bouw, 1998; O'Sullivan et al., 2001, 2002; Robinson et al., 1993; Stall and Bewick, 1992). However, not all sweet corn hybrids are sensitive to P450-metabolized herbicides. Variation among hybrid responses to sulfonylurea herbicides, such as nicosulfuron, can be attributed to differential rates of herbicide metabolism. Tolerant plants detoxify herbicides more rapidly than sensitive plants (Green and Ulrich, 1993). Cytochrome P450 enzymes are an important class of enzymes that are responsible for the primary detoxification of some corn herbicides (Barrett, 1995; Kreuz et al., 1996). Although corn has many CYP genes, the number of P450 enzymes involved in metabolic inactivation of herbicides, their expression levels, and their levels of herbicide metabolism are not clearly understood (Barrett, 1995, 2000; Frey et al., 1995, 1997; Persans et al., 2001). The possibility of a "super P450" that metabolizes multiple corn herbicides was proposed over 15 yr ago (Barrett et al., 1994).

Univ. of Illinois, 1102 S. Goodwin Ave., Urbana, IL 61801. Received 4 Mar. 2010. *Corresponding author (mmwillms@illinois.edu).

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Recent studies have shown that sensitivity of corn to multiple P450-metabolized herbicides is regulated primarily by a single CYP gene or a group of closely-linked CYP genes on the short arm of chromosome 5. A recessive gene from the corn inbred W703a conditioned a sensitive response to nicosulfuron and was designated as *nsf1* (Kang, 1993), while a recessive gene from the corn inbred GA209 conditioned a sensitive response to bentazon and was designated as *ben* (Fleming et al., 1988). The bentazon-sensitive inbred GA209 also displayed greater sensitivity to dicamba, imazethapyr, nicosulfuron, and primisulfuron than a bentazon-tolerant inbred B73 (Barrett et al., 1997). Using a map-based cloning approach, the *nsf1* gene was located on the short arm of chromosome 5 and the dominant, functional allele, *Nsf1*, was sequenced from a nicosulfuron-tolerant inbred, B73 (Williams et al., 2006). The *Nsf1* gene was one of four closely-linked genes with significant sequence similarity to CYP genes. This gene also was very similar in sequence and function to a CYP gene in rice (*Oryza sativa* L.) that conditions tolerant responses to multiple herbicides (Pan et al., 2006). Nicosulfuron- and bentazon-sensitive inbreds W703a and GA209 contained a 392 bp insertion in the *Nsf1* gene sequence relative to B73 (Williams et al., 2006). Thus, it appears that the *nsf1* and *ben* alleles identified from W703a and GA209, respectively, are the same 392 bp insertion mutation of a CYP allele, very likely inhibiting the function of the "super P450" Barrett et al. (1994) proposed.

The sweet corn inbred Cr1 is sensitive to nicosulfuron, mesotrione, and at least seven other P450-metabolized herbicides with five different modes of action (Nordby et al., 2008; Pataky et al., 2006; Williams et al., 2005; Williams and Pataky, 2008). A locus or group of closely linked loci that condition cross-sensitivity to these P450-metabolized herbicides were detected in a segregating population of Cr1 × Cr2 (a herbicide tolerant inbred) on the

Abbreviations: CYP genes, cytochrome P450 genes; DAT, days after treatment.

short arm of chromosome 5 in tight linkage disequilibrium with the CYP locus previously identified as the *nsf1* and *ben* genes (Nordby et al., 2008). Based on segregation of progeny from F_2 generations and from testcrosses with Cr1 and Cr2, 45 sweet corn hybrids and 29 sweet corn inbreds were identified as possessing a gene that is the same as or very closely linked to the gene in Cr1 that conditions sensitivity to multiple postemergence herbicides (Pataky et al., 2009). This group of hybrids and inbreds consists of lines developed by 12 independent, commercial breeding programs and includes sugary, sugary enhancer, and shrunken-2 endosperm types that are grown for processing and fresh consumption in worldwide markets. Thus, a common genetic basis for herbicide sensitivity occurs throughout the sweet corn industry.

Although this mutant (i.e., nonfunctional) CYP allele appeared to be recessive based on initial inheritance studies of nicosulfuron and bentazon sensitivity (Fleming et al., 1988; Kang, 1993), the rate of herbicide metabolism of *CYPcyp* hybrids (i.e., hybrids heterozygous for a functional and mutant CYP allele) appears to be intermediate to that of *cypcyp* and *CYPCYP* hybrids (i.e., hybrids homozygous for mutant or functional CYP alleles, respectively). The intermediate rate of metabolism of *CYPcyp* hybrids results in varied phenotypic responses under different conditions (Pataky et al., 2008; Williams and Pataky, 2010). For example, when low rates of HPPD-inhibiting herbicides (e.g., mesotrione, tembotrione, and topramezone) were applied, *CYPcyp* hybrids generally were not injured and had a phenotype similar to *CYPCYP* hybrids as compared to *cypcyp* hybrids, which were injured. Thus, the CYP allele appeared to have dominant gene action. Conversely, at higher application rates, phenotypes of *CYPcyp* hybrids were intermediate to those of uninjured *CYPCYP* and severely injured *cypcyp* hybrids, which would be interpreted as partially-dominant or codominant gene action (Williams and Pataky, 2010). Similarly, injury following postemergence applications of mesotrione, nicosulfuron, or foramsulfuron in 12 herbicide trials in six states was 1.5 to 2.3 times greater on *CYPcyp* hybrids than on *CYPCYP* hybrids, but substantially less than on *cypcyp* hybrids, which were severely injured or killed in many trials (Pataky et al., 2008). However, responses of *CYPcyp* hybrids varied among trials presumably in association with dry or humid environments.

Although applications of certain P450-metabolized herbicides can reduce yields of sweet corn, effects on yield vary in a manner similar to variable phenotypic responses based on visual injury. Mesotrione applied postemergence at 100 g ha^{-1} reduced the yield of 'Del Monte 20-38' by 7% (O'Sullivan et al., 2002). Increasing the application rate to 200 g ha^{-1} resulted in a 28% yield reduction. Despite the presence of injury 7 and 14 DAT, yield of eight sweet corn hybrids was unaffected by postemergence applications of nicosulfuron in one study (Morton and Harvey, 1992), whereas in a similar study, nicosulfuron reduced the yield of 'Silver Xtra Sweet' by 74 to 100% (Robinson et al., 1993). Nicosulfuron plus rimsulfuron applied postemergence reduced the yield of six sweet corn hybrids including 'Del Monte 20-38', 'GH 1698', 'Cabara', 'Supersweet Jubilee', 'GH 1685', and 'Even Sweeter' by 30 to 100% (O'Sullivan and Bouw, 1998). Yield reductions were more common when the application rate was increased from 25 to 50 g ha^{-1}. Foramsulfuron, an ALS-inhibiting herbicide, applied postemergence killed plants or reduced yield by more than 92% to 'Del Monte 20-38'; whereas yield of the hybrids 'Calico Belle' and 'GH 2684' was reduced only 34 to 54% (Diebold et al., 2003). Bentazon, a photosystem II-inhibiting herbicide, applied postemergence reduced the yield of 'Del Monte 20-38' by 94% (Diebold et al., 2004).

In field corn, postemergence applications of foramsulfuron resulted in yield losses of 6 and 15% in two trials (Bunting et al., 2004). Applications made at later growth stages (i.e., V8–V12) resulted in greater levels of injury and yield reduction than those made at earlier growth stages (i.e., V2–V6) (Bunting et al., 2004). The ALS-inhibiting herbicides applied at early growth stages appear to be metabolized before the initiation of ear development, whereas symptoms of ear malformation and loss of kernel rows (ear pinching) result from applications of ALS-inhibiting herbicides at later growth stages. At the V12 growth stage, applications of foramsulfuron caused pinching in 25 to 40% of ears harvested from two different hybrids (Bunting et al., 2004).

Although differential yield responses of sweet corn hybrids to postemergence herbicides occur, little or no research relates sweet corn yield to hybrid genotype for the CYP alleles affecting herbicide metabolism. The objective of this research was to determine if yield is affected by the genetic condition of sweet corn hybrids at the CYP locus controlling P450 metabolism of herbicides when mesotrione and nicosulfuron are applied during the V3 to V5 and V5 to V7 growth stages.

MATERIALS AND METHODS

Twenty-three hybrids developed by seven seed companies and the University of Illinois were evaluated in 2007, 2008, and 2009 at the University of Illinois Vegetable Crops Farm, Champaign, IL (Table 1). Mesotrione and nicosulfuron were evaluated in separate experiments. Each trial included 16 hybrids. Eleven hybrids were common to both trials. The genotype of each hybrid at the CYP locus affecting herbicide metabolism was identified in a previous experiment based on segregation of F_2 and testcross progeny (Pataky et al., 2008). Mesotrione trials included five hybrids homozygous for functional CYP alleles, five hybrids homozygous for mutant cyp alleles, and six hybrids heterozygous for functional and mutant CYP alleles. Nicosulfuron trials included eight hybrids homozygous for functional CYP alleles and eight heterozygous hybrids. Hybrids homozygous for mutant alleles were not included in nicosulfuron trials, because ALS-inhibiting herbicides kill these hybrids. Each trial included 13 or 14 commercially-available hybrids and a set of near isogenic hybrids differing primarily for alleles at the CYP locus on chromosome 5S (Table 1). Near isogenic hybrids (46 × 52 or 95 × 69) were produced from crosses of near isogenic inbred lines selected in the F_6 generation for tolerant and sensitive reactions to mesotrione.

The experimental design was a split-block arrangement of a randomized complete block, with four replicates. Each CYP genotype was replicated within a block. Herbicide treatments were applied to main plots. Hybrids were grown in subplots. An experimental unit was four 3.5 m-long rows, spaced 76 cm apart with approximately 16 plants, representing a commercial field population of 67,700 plants ha^{-1} (≈27,400 plants $acre^{-1}$). The soil type was a Flanagan silt loam (fine, smectitic, mesic Aquic Argiudoll). Fields were fertilized with 202 kg N ha^{-1}. Preplant tillage included two passes each of a field cultivator and spring-tine harrow. Early-season weeds were controlled with a preemergence application of 2.2 kg atrazine ha^{-1} plus 1.8 kg *S*-metolachlor ha^{-1}. Hand weeding was done as necessary.

Table 1. Sweet corn hybrids differing in number of functional CYP alleles grown to evaluate yield responses to postemergence applications of mesotrione or nicosulfuron in field experiments in 2007, 2008, and 2009.

Injury phenotype, CYP genotype†, and hybrid cultivar	Seed source‡	Experiment
Tolerant (*CYPCYP*)		
46 × 52 TT	UI	nicosulfuron
95 × 69 TT	UI	mesotrione
Ambrosia	Cr	both
GH 6462	Rog	both
GSS 1477	Rog	both
HM 2390	HM	both
Luscious TSW	MM	nicosulfuron
Obsession	Sem	nicosulfuron
Rustler	HM	nicosulfuron
Intermediate (*CYPcyp*)		
46 × 52 TS	UI	nicosulfuron
95 × 69 TS	UI	mesotrione
277A	IFSI	both
Argent	Cr	both
Coho	HM	both
Double Gem	MM	nicosulfuron
GH 2669	Rog	both
Max	HM	nicosulfuron
Supersweet Jubilee Plus	Rog	both
Sensitive (*cypcyp*)		
95 × 69 SS	UI	mesotrione
177A	IFSI	mesotrione
Del Monte 20–38	DMC	mesotrione
EX 08705770	Sem	mesotrione
Merit	Sem	mesotrione

† Hybrid genotype at a single CYP locus identified in a previous experiment based on segregation of F_2 and testcross progeny (Pataky et al., 2008).

‡ Cr = Crookham Seed Co.; DMC = Del Monte Corp.; HM = Harris Moran Seed Co.; IFSI = Illinois Foundation Seed, Inc.; MM = Mesa Maize; Rog = Syngenta Seeds, Inc., Rogers Brands; Sem = Seminis, Inc.; UI = Dr. Jerald Pataky, University of Illinois.

Three treatments included a nontreated control and herbicide applications between the growth stages of V3 and V5 or V5 and V7. Mesotrione was applied at 210 g ha^{-1} a.i. plus 1% v/v petroleum oil concentrate. Nicosulfuron was applied at 70 g ha^{-1} a.i. plus 1% v/v petroleum oil concentrate and 2.2 kg ha^{-1} ammonium sulfate. Herbicides were applied at 190 L ha^{-1} spray volume between 29 May and 26 June (Table 2). Plants were assessed visually 7 DAT for the percentage leaf area chlorotic or necrotic and for stunting.

The date that silks emerged 1 to 2 cm from 50% of plants in an experimental unit (i.e., mid-silk stage) was recorded for each hybrid. Primary ears of 10 consecutive plants in the middle two rows of each experimental unit were harvested and weighed 21 d after the mid-silk stage. For each hybrid, yield loss was calculated as: [(Yield of nontreated control – Yield of herbicide treated)/Yield of nontreated control] × 100. After weighing, ears harvested from nicosulfuron trials were husked to assess loss of kernel rows (i.e., pinched ears).

Statistical Analysis

Yield, crop injury, and ear pinching were analyzed separately by ANOVA using the Proc Mixed procedure of SAS, version 9.2 (SAS Institute, 2007). Years and blocks were considered random variables. Genotypes and herbicide treatments were considered fixed variables. Effects were declared significant at $P \leq 0.05$. Residuals were tested for normality using the Proc Univariate procedure of SAS. Residuals were plotted against predicted values using the Proc Gplot procedure of SAS to assess homogeneity of error variance. Assumptions of ANOVA were met. Slice statements were used to partition the genotype × herbicide treatment interaction by genotype. Comparisons of herbicide treatments within genotypic classes were done with single degree of freedom contrast statements. Hybrid means were separated by Fisher's least significant difference (LSD) using the pdmix800 SAS macro (Saxton, 1998).

RESULTS

Years were a significant source of variation in the statistical analysis of yield, crop injury, and ear pinching. Therefore, analyses were done separately for each year.

Mesotrione Trials

In 2007 and 2009, injury from mesotrione applications during the V3 to V5 or V5 to V7 growth stages differed among hybrids with different CYP genotypes. Crop injury 7 DAT was greater on hybrids with fewer functional CYP alleles (Fig. 1). Injury also was greater following application of mesotrione during the V3 to V5 growth stages than during the V5 toV7 growth stages (Fig. 1). Injury 7 DAT with mesotrione at the V3 to V5 stage varied among years, ranging from 4 to 18% on *CYPCYP* hybrids, 16 to 44% on *CYPcyp* hybrids, and 39 to 87% on *cypcyp* hybrids. Injury 7 DAT with mesotrione at the V5 to V7 stage also varied among years and ranged from 0 to 1%, 1 to 8%, and 21 to 33% on *CYPCYP* hybrids, *CYPcyp* hybrids, and *cypcyp* hybrids, respectively. Injury from mesotrione was appreciably lower on all genotypes in 2008 than in 2007 or 2009. Severity of injury was nearly equivalent in 2007 and 2009. No injury occurred in the nontreated control plots.

The effect of mesotrione on sweet corn yield differed among hybrids with different CYP genotypes. The CYP genotype by herbicide treatment interaction term also was significant in the ANOVA of yield in 2007 and 2009. Slice statements partitioning

Table 2. Environmental conditions during and total precipitation 7 d before application of mesotrione or nicosulfuron to sweet corn hybrids at the V3-V5 or V5-V7 growth stages in 2007, 2008, and 2009.

Conditions	2007		2008		2009	
	V3-V5†	V5-V7	V3-V5	V5-V7	V3-V5	V5-V7
Application date	May 29	June 6	June 18	June 24	June 17	June 26
Temperature at application, °C	33	24	26	28	27	30
Relative humidity at application, %	35	35	27	39	65	57
Total precipitation prior, cm‡	1.3	0.8	0.2	0.3	7.1	4.5

† Number of visible leaf collars when herbicides were applied.

‡ Total rainfall in the 7 d before application.

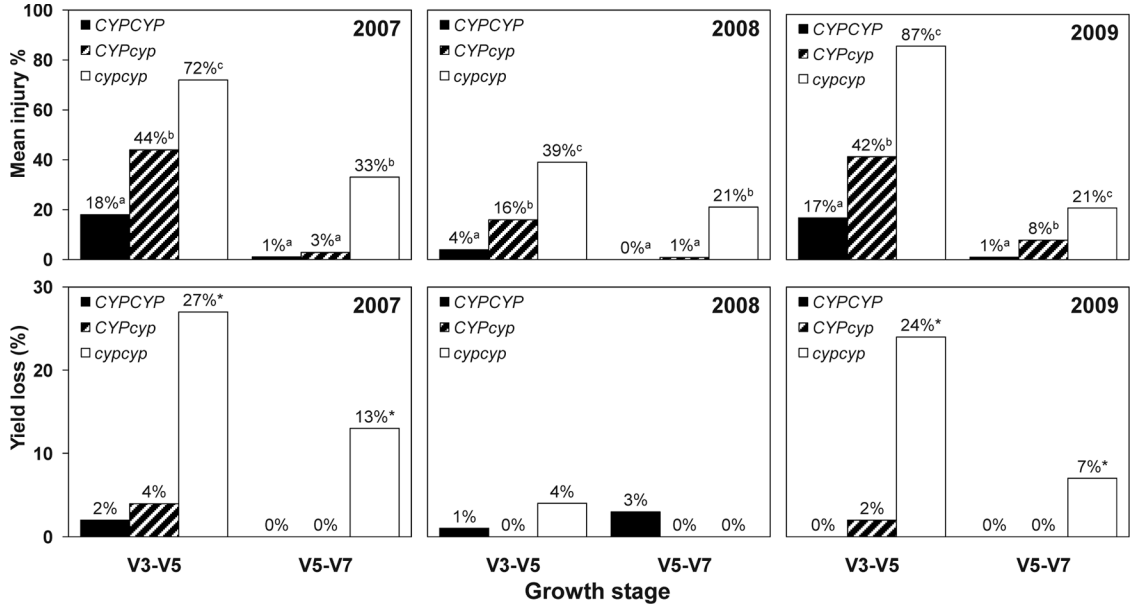

Fig. 1. Injury 7 d after treatment (DAT) and yield loss in sweet corn hybrids differing in number of functional *CYP* alleles following mesotrione application during the V3-V5 or V5-V7 growth stages. Within a given year and growth stage, injury values followed by different letters differ significantly at $P \leq 0.05$ based on single degree of freedom contrasts. Standard errors for injury were 1.83, 1.00, and 1.84 in 2007, 2008, and 2009, respectively. Mean yield loss followed by an asterisk differs significantly from the nontreated control at $P \leq 0.05$ based on single degree of freedom contrasts. Standard errors for yield loss were 1.00, 0.78, and 0.75 in 2007, 2008, and 2009, respectively.

the interaction by CYP genotype indicated the effect of mesotrione treatments were significant only for *cypcyp* hybrids. Yield of *CYPCYP* and *CYPcyp* hybrids was unaffected by mesotrione (Fig. 1). Yield of the group of *cypcyp* hybrids was reduced 27 and 24% by mesotrione applications during the V3 to V5 growth stages in 2007 and 2009, respectively; whereas yield of this group of hybrids was reduced 13 and 7% by mesotrione applications during the V5 to V7 growth stages in 2007 and 2009, respectively (Fig. 1). Mesotrione treatments did not affect yield in 2008.

Among the five *cypcyp* hybrids, yield reductions resulting from mesotrione applications during the V3 to V5 growth stages ranged from 12 to 36% in 2007 and from 14 to 40% in 2009 (Table 3). Applications of mesotrione during the V5 to V7 growth stages reduced yields of individual *cypcyp* hybrids from 9 to 22% in 2007 and from 10 to 12% in 2009 (Table 3). Yields were not reduced on *cypcyp* hybrids in 2008 (Table 3). Among these five hybrids, yield of '177 A' was affected most and yield of 'EX 08705770' was affected least by mesotrione.

Nicosulfuron Trials

In 2007 and 2009, injury from nicosulfuron applications during the V3 to V5 or V5 to V7 growth stages differed between *CYPCYP* and *CYPcyp* hybrids. Crop injury 7 DAT was greatest on *CYPcyp* hybrids following application during the V3 to V5 growth stages (Fig. 2). Injury 7 DAT with nicosulfuron during the V3 to V5 growth stages was 5 and 21% on *CYPCYP* hybrids and 28 and 54% on *CYPcyp* hybrids in 2007 and 2009, respectively (Fig. 2). Injury 7 DAT with nicosulfuron during the V5 to V7 growth stages was 4 and 7% on *CYPCYP* hybrids and 16% on *CYPcyp* hybrids in 2007 and 2009, respectively (Fig. 2). No visible injury resulted from nicosulfuron applications in 2008.

The effect of nicosulfuron on sweet corn yield differed among hybrids with different CYP genotypes, as indicated by a significant CYP genotype × herbicide treatment interaction in the ANOVA of yield in 2007 and 2009. Applications of nicosulfuron did not affect the yield of *CYPCYP* hybrids (Fig. 2). Yield of *CYPcyp* hybrids was reduced 8 to 22% by nicosulfuron applications during the V3 to V5 or V5 to V7 growth stages (Fig. 2).

Table 3. Relative yield (ear weight) of sweet corn hybrids homozygous for mutant (nonfunctional) cyp alleles, expressed as a percentage yield of the nontreated control, following applications of mesotrione during the V3-V5 or V5-V7 growth stages in 2007, 2008, and 2009. Standard errors of yield as a percentage of the control were 2.85, 5.83, and 3.35 for 2007, 2008, 2009, respectively.

Hybrid	2007		2008		2009		Mean
	V3-V5†	V5-V7	V3-V5	V5-V7	V3-V5	V5-V7	
	yield as % of control						
177A	66*‡	81*	85	93	60*	90*	79
95 × 69 SS	64*	90*	116*	102	75*	90*	90
Del Monte 20–38	76*	78*	92	101	77*	88*	85
EX 08705770	88*	94	103	103	86*	103	97
Merit	69*	91*	95	101	82*	90*	88
LSD	8.28		15.97		7.78		

† Growth stage determined by the number of visible leaf collars.

‡ Within a given year and hybrid combination, values with an asterisk differed significantly from the nontreated control at $P \leq 0.05$ based on Fisher's least significant difference test.

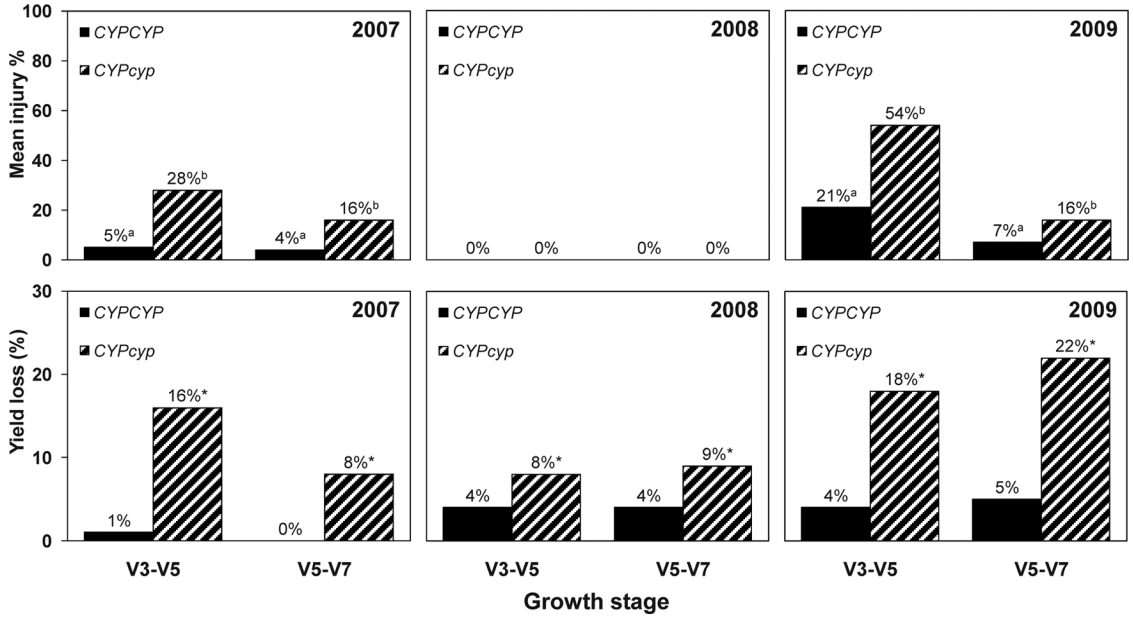

Fig. 2. Injury 7 d after treatment (DAT) and yield loss in sweet corn hybrids differing in number of functional *CYP* alleles following nicosulfuron application during the V3-V5 or V5-V7 growth stages. Within a given year and growth stage, injury values followed by different letters differ significantly at $P \leq 0.05$ based on single degree of freedom contrasts. Standard errors for injury were 0.82 and 1.56 in 2007 and 2009, respectively. Mean yield loss followed by an asterisk differs significantly from the nontreated control at $P \leq 0.05$ based on single degree of freedom contrasts. Standard errors for yield loss were 0.78, 0.63, and 0.95 in 2007, 2008, and 2009, respectively.

Among eight *CYPcyp* hybrids, yield reductions due to nicosulfuron applications during the V3 to V5 growth stages ranged from 9 to 57% in 2007, 11 to 28% in 2008, and 8 to 57% in 2009 (Table 4). When nicosulfuron was applied during the V5 to V7 growth stage, yield reductions to individual *CYPcyp* hybrids ranged from 9 to 18% in 2007, 12 to 18% in 2008, and 15 to 50% in 2009 (Table 4). Although the effect of nicosulfuron on yield of these hybrids varied among hybrids, years, and time of application, 'Double Gem' was consistently affected more severely than the other seven hybrids.

Nicosulfuron treatments caused a reduction in kernel rows of harvested ears. The percentage of affected ears differed among hybrids with different CYP genotypes in 2007 and 2009. Ear pinching was more severe on *CYPcyp* hybrids, ranging from 9 to 12% and 13 to 66% at the V3 to V5 and V5 to V7 growth stages, respectively (data not shown). On *CYPCYP* hybrids, <10% of ears lost kernel rows following applications of nicosulfuron except for the V5 to V7 applications in 2008 and 2009, which resulted in 14 and 25% affected ears, respectively (data not shown).

DISCUSSION

A single CYP gene or group of closely linked CYP genes on the short arm of chromosome 5 affect the response of sweet corn hybrids to several postemergence corn herbicides with multiple modes of action, including nicosulfuron and mesotrione (Pataky et al., 2009). Levels of injury differ among sweet corn hybrids with different CYP genotypes probably as a result of differential rates of herbicide metabolism (Pataky et al., 2008). Hybrids homozygous for functional CYP alleles (i.e., *CYPCYP*) are not substantially or consistently injured by P450-metabolized herbicides. Hybrids homozygous for mutant alleles (i.e., *cypcyp*) are frequently killed or severely injured by P450-metabolized herbicides. Injury to hybrids heterozygous for a functional and a mutant allele (i.e., *CYPcyp*) is variable

Table 4. Relative yield (ear weight) of sweet corn hybrids heterozygous for functional and mutant (nonfunctional) CYP alleles, expressed as a percentage yield of the nontreated control, following applications of nicosulfuron during V3-V5 or V5-V7 growth stages in 2007, 2008, and 2009. Standard errors of yield as a percentage of the control were 3.31, 3.78, and 2.94 for 2007, 2008, 2009, respectively.

Hybrid	2007		2008		2009		Mean
	V3-V5†	V5-V7	V3-V5	V5-V7	V3-V5	V5-V7	
	yield as % of control						
277A	88*‡	86*	94	98	92*	94	92
46 × 52 TS	94	94	91	89*	90*	83*	90
Argent	84*	99	95	95	89*	92*	92
Coho	90*	93	93	82*	92*	84*	89
Double Gem	43*	82*	72*	88*	43*	50*	63
GH 2669	91*	98	103	93	92*	85*	94
Max	87*	91*	99	102	87*	79*	91
Supersweet Jubilee Plus	90*	89*	88*	87*	82*	65*	84
LSD	8.37		9.48		7.04		

† Growth stage determined by the number of visible leaf collars.

‡ Within a given year and hybrid combination, values with an asterisk differed significantly from the nontreated control at $P \leq 0.05$ based on Fisher's least significant difference test.

but typically more similar to injury on *CYPCYP* hybrids than *cypcyp* hybrids. Environment and other factors also affect the degree to which sweet corn is injured by these herbicides. When trials were conducted in arid regions, such as Idaho and Colorado, *CYPcyp* and *cypcyp* hybrids were uninjured by P450-metabolized herbicides whereas these hybrids were injured substantially in humid environments (Pataky et al., 2008).

Results of this experiment demonstrate that the genetic condition of hybrids at the CYP locus on chromosome 5S also affects the yield of sweet corn following application of mesotrione or nicosulfuron at the V3 to V5 or the V5 to V7 growth stages, although yield is not affected entirely the same as injury responses. Injury to hybrids with different CYP genotypes was not always associated with significant yield losses. *CYPCYP* hybrids were generally uninjured by mesotrione or nicosulfuron and yield of these hybrids was not affected by either herbicide. Following mesotrione application, *cypcyp* hybrids were severely injured and yield was reduced except in 2008 when weather was dry before application. Hybrids homozygous for mutant cyp alleles were not included in the nicosulfuron studies because they are killed by ALS-inhibiting herbicides. Injury from mesotrione was more severe on *CYPcyp* hybrids than on *CYPCYP* hybrids; however, despite levels of injury as high as 44% following applications of mesotrione, yield of *CYPcyp* hybrids was not reduced by this herbicide. Conversely, yields of *CYPcyp* hybrids were reduced by nicosulfuron;. Thus, CYP genotype affects whether or not a hybrid is likely to be injured by a P450-metabolized herbicide, but adverse effects on yield from herbicide injury also depend on the type and severity of injury caused by herbicides with different modes of action.

Several sweet corn hybrids evaluated in previous studies, before knowledge of the genetic basis of herbicide cross-sensitivity, have subsequently been identified as homozygous or heterozygous for CYP alleles on the short arm of chromosome 5 (Pataky et al., 2009). As in this study, yields of *cypcyp* hybrids were reduced by mesotrione or ALS-inhibiting herbicides. Effects of ALS-inhibiting herbicides on yield of *CYPcyp* hybrids were variable. Yields of *CYPCYP* hybrids usually were not affected by ALS-inhibiting herbicides. For instance, yields of 'Merit' and 'Del Monte 20-38' (now known to be *cypcyp* hybrids) were reduced in each of 30 evaluations of ALS-inhibiting herbicides in 16 previous trials (Diebold et al., 2003; Monks et al., 1992; O'Sullivan and Bouw, 1998; O'Sullivan and Sikkema 2001; O'Sullivan et al., 1998). Yields of both hybrids were reduced 92 to 100% because application of ALS-inhibiting herbicides usually killed these hybrids. Yield of 'Del Monte 20-38' also was reduced 7 and 28% in two previous evaluations of mesotrione (O'Sullivan et al., 2002). Yields of 'Delectable', 'Eliminator', 'Even Sweeter', 'How Sweet It Is', 'Jubilee', and 'Supersweet Jubilee' (identified recently as *CYPcyp* hybrids) were reduced in 8 of 52 evaluations of ALS-inhibiting herbicides in 18 previous trials. Among these hybrids, yield losses ranged from 14 to 48% (Grey et al., 2000; O'Sullivan and Bouw, 1998; O'Sullivan et al., 1995; Robinson et al., 1993). Yield of 'Supersweet Jubilee' was unaffected by mesotrione in one trial (O'Sullivan et al., 2002). Yields of 'Challenger', 'Incredible', and 'Miracle' (identified recently as *CYPCYP* hybrids) were not affected in 22 of 24 evaluations of ALS-inhibiting herbicides in nine previous trials (Diebold et al., 2003; Monks et al., 1992; O'Sullivan and Bouw, 1998; O'Sullivan and Sikkema, 2001; O'Sullivan et al., 1995, 1998). Yield of 'Challenger' was reduced 15 to 23% by applications of nicosulfuron plus rimsulfuron in one trial (O'Sullivan et al., 1995). Yield losses on *CYPCYP* hybrids following application of mesotrione have not yet been reported.

The potential for crop injury and yield loss following applications of P450-metabolized herbicides is affected substantially by CYP genotype. Environmental conditions and other inherent genetic differences among hybrids also affect the potential for injury and yield loss. In this experiment, yield losses from mesotrione were substantial for *cypcyp* hybrids, but varied among years. In 2008 when mesotrione caused less crop injury and yields were not reduced among *cypcyp* hybrids, abnormally dry weather conditions preceded herbicide applications (Table 2), and plants exhibited symptoms of drought stress. In a previous study with a total of 18 trials (sites-years), the lowest levels of mesotrione injury to sweet corn occurred in arid climates or when abnormally dry conditions preceded herbicide application (Pataky et al., 2008; Williams et al., 2008), perhaps the result of poor cuticular penetration and/or translocation of the herbicide.

In addition to CYP genotype and environmental conditions, applications of P450-metabolized insecticides or other herbicides could affect the potential for injury and yield loss following applications of P450-metabolized herbicides. Organophosphate insecticides are known to interfere with P450-metabolism in corn (Baerg et al., 1996). Terbufos, an organophosphate insecticide, reduces metabolism of nicosulfuron in corn while increasing its uptake (Diehl et al., 1995). Reduced rates of nicosulfuron metabolism due to organophosphate application in *CYPcyp* and *cypcyp* hybrids could result in increased injury and yield loss. Injury from nicosulfuron on 'Jubilee', a *CYPcyp* hybrid, was greater when plants also were treated with terbufos (Morton et al., 1991, 1993). Nicosulfuron reduced the yield of 'Jubilee' plants treated with terburos, while yield of plants not treated with terbufos was unaffected by nicosulfuron (Morton et al., 1993). Similarly, applications of herbicides with different detoxification pathways could enhance injury from P450-metabolized herbicides on hybrids with mutant cyp allele(s). Mesotrione and atrazine, a photosystem II inhibitor, are known to have synergistic activity (Abendroth et al., 2006). Recently, postemergence applications of topramezone (another HPPD-inhibiting herbicide) and atrazine killed *cypcyp* sweet corn inbreds and hybrids in Idaho nurseries (J.K. Pataky, personal observation, 2009). Additional research is necessary to determine the extent to which P450-metabolized insecticides and other herbicides interact with P450-metabolized herbicides to affect crop injury and yield of *CYPcyp* and *cypcyp* hybrids.

Loss of kernel rows caused by nicosulfuron occurred on both *CYPCYP* and *CYPcyp* hybrids. Previously, loss of kernel rows following application of foramsulfuron, an ALS-inhibiting herbicide, was reported on dent corn hybrids differing in foramsulfuron tolerance (Bunting et al., 2004). These malformed ears are unmarketable in sweet corn sold for fresh consumption. Thus, yield losses from ALS-inhibiting herbicides may be even greater to fresh market sweet corn where yields are based on the number of marketable ears.

The results of this experiment demonstrate the importance of eliminating mutant (i.e., nonfunctional) cyp alleles from commercial sweet corn hybrids, inbreds, and breeding germplasm. There appears to be a substantial risk of injury and subsequent yield loss when mesotrione, nicosulfuron, or possibly other P450-metabolized herbicides are applied to hybrids that are

homozygous for mutant cyp alleles. Hybrids that are heterozygous for a functional and mutant cyp allele appear to have variable responses to mesotrione, nicosulfuron, and possibly to other P450-metabolized herbicides. While the risk of injury and subsequent yield losses is lower for *CYPcyp* hybrids than for *cypcyp* hybrids, *CYPcyp* hybrids appear to be at greater risk than *CYPCYP* hybrids which typically are uninjured and sustain no yield reductions when these herbicides are applied at recommended rates. Despite the risk of injury, *CYPcyp* or *cypcyp* hybrids may still be grown because of their superior agronomic or horticultural traits. Herbicides and application timing should be selected carefully to avoid injury when these hybrids are grown. For example, certain HPPD-inhibiting herbicides applied alone, such as topramezone, appear safe for all CYP genotypes at labeled rates (Williams and Pataky, 2010). Crop safeners, such as isoxadifen-ethyl, can enhance P450 metabolism of herbicides and reduce injury to hybrids with at least one functional CYP allele (Williams and Pataky 2008, 2010). However, crop safeners do not appear to enhance P450 metabolism in hybrids that lack a functional CYP allele (Williams and Pataky, 2010).

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