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Significance of application timing, formulation, and cytochrome P450 genotypic class on sweet corn response to dicamba

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Research Article

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Abstract

Sweet corn (*Zea mays* L.) tolerance to dicamba and several other herbicides is due to cytochrome P450 (CYP)-mediated metabolism and is conferred by a single gene (*Nsf1*). Tolerance varies by CYP genotypic class, with hybrids homozygous for functional CYP (*Nsf1Nsf1*) being the most tolerant and hybrids homozygous for mutant CYP alleles (*nsf1nsf1*) being the least tolerant. The herbicide safener cyprosulfamide (CSA) increases tolerance to dicamba by stimulating the expression of several CYPs. However, the extent to which CSA improves the tolerance of different sweet corn CYP genotypic classes to dicamba is poorly understood. Additionally, the effect of growth stage on sweet corn sensitivity to dicamba is inadequately described. The objective of this work was to quantify the significance of application timing, formulation, and CYP genotypic class on sweet corn response to dicamba. Hybrids representing each of the three CYP genotypes (*Nsf1Nsf1*, *Nsf1nsf1*, *nsf1nsf1*), were treated with dicamba or dicamba + CSA at one of three growth stages: V3, V6, or V9. Across all timings, the *nsf1nsf1* hybrid was the least tolerant to dicamba, displaying 16% higher crop injury levels 2 wk after treatment and 2,130 kg ha^{-1} lower ear mass yields compared with the *Nsf1Nsf1* hybrid. The V9 growth stage was the most susceptible time for dicamba injury regardless of genotypic class, with 1.89 and 1,750 kg ha^{-1} lower ear mass yields compared with the V3 and V6 application timings, respectively. The addition of CSA to dicamba V9 applications reduced the injury from dicamba for all three genotypic classes; however, it did not eliminate the injury. The use of *Nsf1Nsf1* or *Nsf1nsf1* sweet corn hybrids along with herbicide safeners will reduce the frequency and severity of injury from dicamba and other CYP-metabolized herbicides.

Introduction

Since commercialization of dicamba in the 1960s, use of this synthetic auxin herbicide for broadleaf weed control has increased. In 1979, <10% of all U.S. corn (*Zea mays* L.) hectares were treated with dicamba (Hartzler 2017). By 2018, dicamba was applied to 17% of U.S. corn, totaling 1,330,000 kg of product (USDA-NASS 2021). The development and recent adoption of dicamba-tolerant (DT) soybean [*Glycine max* (L.) Merr.] and cotton (*Gossypium hirsutum* L.) further increased dicamba usage. DT soybean cultivars account for 43% of U.S. soybean production, with roughly one-half of this area receiving at least one dicamba application per season (Wechsler et al. 2019). Similarly, DT cotton comprises an estimated 70% of U.S. production, with 40% of the area treated with dicamba (USDA-NASS 2021; USEPA 2020).

Dicamba continues to be used in certain types of corn. Previously, an EPA-approved label for a formulation of dicamba + diflufenzopyr listed sweet corn as a labeled crop. This formulation allowed for applications on sweet corn up to 91 cm or up to 15 d before tasseling (Anonymous 2014). Currently, dicamba is registered for use in field corn and popcorn. Some of the newest dicamba products for field and popcorn (e.g., Diflexx®) have a wide range of postemergence application timings; from spike through V10 (Anonymous 2020). Labels of these new dicamba products warn of potential injury to sensitive seed corn or popcorn lines and recommend that growers verify the selectivity of their hybrids to avoid this injury.

Field corn hybrids are naturally tolerant to dicamba and certain other herbicides due to rapid metabolism via cytochrome P450 (CYP) enzymes (Grossmann et al. 2002). The understanding of the genetic basis of corn sensitivity to CYP-metabolized herbicides has improved in recent years. The *Nsf1* gene, first reported as the gene conditioning nicosulfuron tolerance in field corn (Kang 1993), was mapped to the short arm of chromosome 5 (Williams et al. 2006). They found the *Nsf1* gene was one of four closely linked genes with homologies to CYP genes. Nordby et al. (2008) showed the response of sweet corn hybrids to dicamba + diflufenzopyr, carfentrazone,

mesotrione, nicosulfuron, and certain other herbicides was affected principally by the presence of functional or mutant CYP alleles at, or linked to, *Nsf1*. Sweet corn hybrids homozygous for functional CYP alleles (i.e., *Nsf1Nsf1*) are most tolerant to the herbicides and usually are asymptomatic at recommended use rates. Hybrids homozygous for mutant CYP alleles (i.e., *nsf1nsf1*) are most sensitive to the CYP-metabolized herbicides and can be severely injured or killed depending on the herbicide product. Hybrids heterozygous for a functional and a mutant CYP allele (i.e., *Nsf1nsf1*) metabolize the herbicides at an intermediate rate and often have a phenotype in between tolerant and sensitive hybrids depending on environmental factors (Pataky et al. 2008). A mechanistic understanding of CYP-mediated herbicide metabolism in plants may lead to the development of crops with higher herbicide tolerance via traditional breeding or by transgenic and genome-editing techniques (Dimaano and Iwakami 2021).

Recently Choe and Williams (2020) confirmed that *nsf1* is the gene responsible for sweet corn sensitivity to nicosulfuron. The protein coding sequence of *Nsf1* produces CYP81A9; however, the molecular polymorphisms of *Nsf1* differ in field corn and sweet corn. Field corn lines sensitive to nicosulfuron contain either a 392-bp insertion mutation in their *nsf1* coding sequence, resulting in a truncated, nonfunctional CYP (Williams et al. 2006), or two deletion mutations on CYP81A9 (Liu et al. 2019). While CYP81A9s from tolerant sweet corn inbreds binds with nicosulfuron, CYP81A9s from sensitive inbreds are inactive, the degree to which is due to the type of amino acid substitutions in CYP81A9 (Choe and Williams 2020).

Herbicide safeners overcome some risk of herbicide-induced crop injury. Herbicide safeners help protect grass crops from herbicide injury without reducing herbicide efficacy on target weed species (Brazier-Hicks et al. 2018; Hatzios and Burgos 2004; Riechers et al. 2010). Safeners act by enhancing the expression of genes coding detoxifying enzymes such as CYPs and glutathione transferases (Riechers et al. 2005, 2010; Ye et al. 2019). One of the more recently released safeners, cyprosulfamide (CSA), protects field corn from injury caused by various acetolactate synthase– and hydroxyphenylpyruvate dioxygenase–inhibiting herbicides as well as dicamba (Anonymous 2020; Kraehmer et al. 2014). CSA has safening activity in both preemergence and postemergence applications (Jablonkai 2013). In field corn, CSA enhances the expression of CYP81A9 as well as other CYPs (Giannakopoulos et al. 2020). There appear to be no reports of the combined effects of CSA and CYP genotypic class on corn response to CYP-metabolized herbicides.

Given the current knowledge of field corn sensitivity to CYP-metabolized herbicides, sweet corn provides a unique opportunity to quantify the extent to which CSA can reduce crop injury from dicamba by testing lines from each CYP genotypic class. Moreover, the influence of crop growth stage on sensitivity to dicamba in specialty corns is poorly understood. Therefore, the objective of this research was to quantify the significance of application timing, formulation, and CYP genotypic class on sweet corn response to dicamba. We hypothesize CSA will reduce the risk of crop injury from dicamba, especially for the most sensitive CYP genotypes, *Nsf1nsf1* and *nsf1nsf1*.

Materials and Methods

Field experiments were conducted using a single protocol in 2019 and 2020 at the University of Illinois Vegetable Crop Research Farm near Urbana, IL (40.08°N, 88.24°W) and the Western Illinois University Agricultural Field Laboratory near Macomb, IL (40.49°N, 90.69°W). Different fields were used each year. The preceding crop was soybean. The dominant soil at Urbana was a Flanagan silt loam (fine, smectitic, mesic Aquic Argiudolls) averaging 3.3% organic matter and a pH of 5.9. The dominant soil at Macomb was a Sable silty clay loam (fine-silty, mixed, superactive, mesic Typic Endoaquolls) averaging 4.0% organic matter and a pH of 6.0. Sweet corn production practices common to central Illinois, including fertilization, irrigation, and pest control, were used.

Experimental Approach

The experimental design was a split plot with four blocks (replications) each site-year. Levels of the main plot were the three CYP genotypes (*Nsf1Nsf1*, *Nsf1nsf1*, *nsf1nsf1*), with each genotype represented by a commercial sweet corn hybrid. Hybrids, identified for CYP genotypes in previous experiments (Pataky et al. 2008, 2009), included 'Merit' (Seminis Vegetable Seeds Inc, Deforest, WI, USA, 53532) as an *nsf1nsf1* hybrid, 'Argent' (Crookham Seed Company, Caldwell, ID, USA, 83606) as an *Nsf1nsf1* hybrid, and 'GSS1477' (Syngenta Seeds, Downers Grove, IL, USA, 60515) as an *Nsf1Nsf1* hybrid. While not near-isogenic lines, these hybrids were chosen because they were previously shown to have similar injury response levels to those of near-isogenic sensitive and tolerant lines or their crosses when treated with several CYP-metabolized herbicides, including dicamba (Meyer et al. 2010; Nordby et al. 2008; Pataky et al. 2008, 2009). Levels of the subplot factor were one of seven herbicide treatments, including a nontreated control, and dicamba with CSA (Diflexx®, Bayer CropScience, St. Louis, MO, USA, 63167) and without (XtendiMax®, Bayer CropScience, St. Louis, MO, USA, 63167) both applied at the V3, V6, or V9 crop growth stage. Application rate of dicamba was 1,120 g ae ha^{-1}; twice the highest rate labeled for annual weeds in field corn. Herbicide treatments were applied using a hand-held CO_2 backpack sprayer equipped with TeeJet® AI11003-VS (TeeJet Technologies, Springfield, IL, USA, 62703) air-induction nozzles delivering 187 L ha^{-1} of spray solution at 276 kPa. Herbicide treatments were applied when wind speeds were between 1.34 and 4.47 m s^{-1} and air temperatures were <29.4 C to minimize off-site movement. Dates of key events are reported in Table 1.

Sweet corn was planted in 76-cm spaced rows at a target density of 56,800 plants ha^{-1}. Main plots were 4 rows wide by 88.5-m long. Subplots were 4 rows wide by 9.2-m long. Around each subplot, a 3.1-m border planted to sweet corn was maintained to further reduce the risk of particle drift among plots from herbicide treatments. One exception to plot dimensions was in Urbana in 2020. Due to a soil anomaly affecting crop emergence in a portion of the field, plot lengths were reduced by one-half to position the experiment outside the anomaly. Plots were kept weed-free with a preemergence application of 1.8 kg ai ha^{-1} *S*-metolachlor and 2.2 kg ai ha^{-1} atrazine (Bicep II Magnum, Syngenta, Crop Protection, Greensboro, NC, USA, 27409), followed by a single interrow cultivation before canopy closure, and hand hoeing as needed.

Data Collection

Sweet corn injury was scored visually 1 and 2 wk after treatment on a scale of 0 to 100, where 0 was no observable injury, 10 to 30 was mild injury, 40 to 60 was moderate injury, 70 to 90 was severe injury, and 100 was plant death. Primary injury symptoms were

Table 1. Sweet corn planting dates and growing degree days (GDD) accumulation between planting and herbicide application or harvest in Macomb, IL, and Urbana, IL, in 2019 and 2020.

			Cumulative GDD following planting			
Site	Year	Planting	V3	V6	V9	Harvest
Macomb[a]	2019	June 18, 2019	222.4	406.2	526.5	1,056.0–1,080.6
	2020	May 11, 2020	238.3	361.9	543.1	1,029.2
Urbana[b]	2019	May 18, 2019	227.0	384.9	570.1	1,099.7–1,128.5
	2020	May 11, 2020	242.5	388.6	576.9	1,051.6–1,084.9

[a]40.08°N, 88.24°W.
[b]40.49°N, 90.69°W.

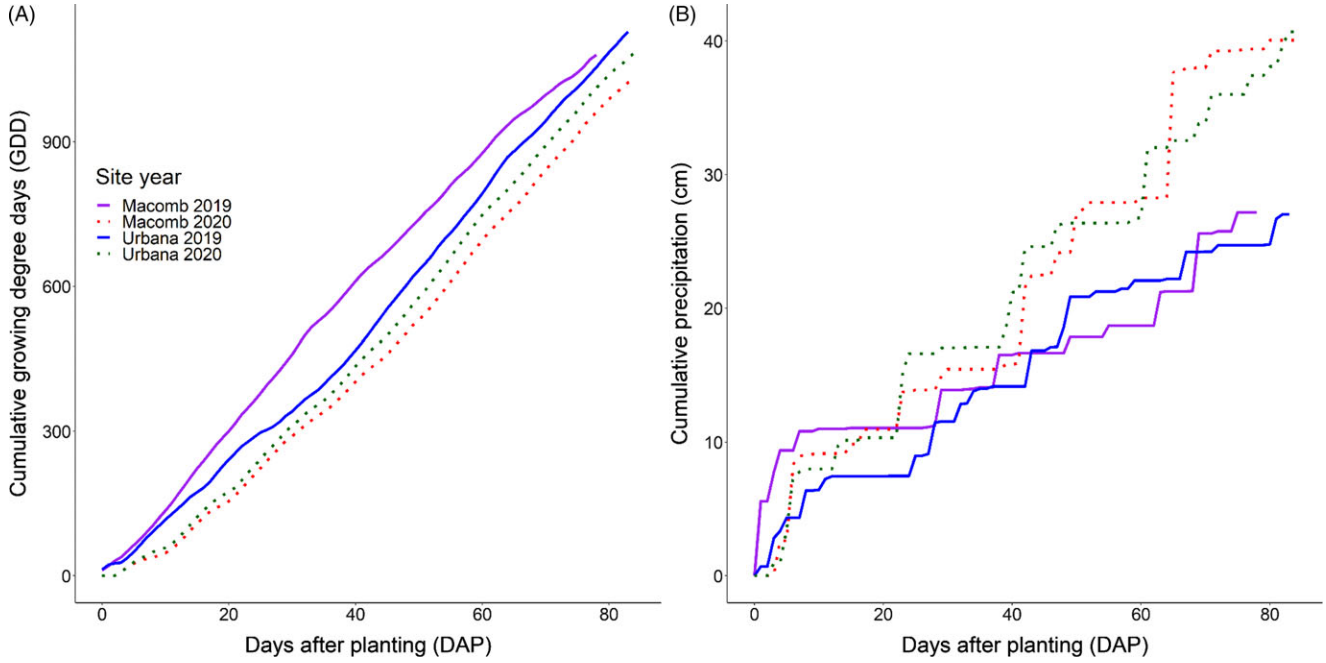

Figure 1. Cumulative growing degree days (A) and precipitation (B) at Macomb, IL, and Urbana, IL, in 2019 and 2020.

fused brace roots, buggy-whipped/wrapped leaves, epinasty, stalk bending, and tassel malformations.

Marketable ears (>4.4 cm in diameter) were hand harvested 18 to 21 d after mid-silk. Sweet corn ears were collected from a 6.1-m length (3.0 m in Urbana in 2020) in the center two rows of each 4-row plot. Ear number, ear mass, and the number of plants were recorded. A random sample of 10 ears per subplot was retained for processing analysis. The number of ears with malformations, including bottle-shaped, pinched, and curved ears, was recorded. Ears were machine husked on a commercial husking bed (A&K Development, Beaver Dam, WI, USA, 53916). The number of ears broken by the husking process was recorded. Husked ear mass, ear length, and filled ear length were recorded. Fresh kernels were cut from the cob with a commercial sweet corn cutter (A&K Development, Beaver Dam, WI, USA, 53916) and cob mass was recorded. A 100-g kernel sample was collected and used to determine kernel moisture. Kernel mass was calculated as the difference between husked mass and cob mass, then corrected to 76% moisture.

Daily rainfall and minimum and maximum air temperatures were obtained from weather stations within 1 km of experimental sites (Illinois State Water Survey, Champaign, IL). Growing degree days (GDD) were determined using daily minimum and maximum air temperatures with a base temperature of 10 C.

Statistical Analysis

Sweet corn injury, yield (ear mass yield, kernel mass yield, and crate yield [50 ears crate^{-1}]), and ear traits (prolificacy, ear mass, ear length, filled ear length, malformed ears, and ear breakage) were analyzed separately using the LME4 package in R (Bates et al. 2015). Sweet corn genotypic class and herbicide treatment, as well as their interactions, were treated as fixed effects, while environment (year by location combinations), interactions between the fixed effects and environments, and replication were treated as random effects. Fixed effect significance was tested using the *F*-test. For response variables without a significant genotypic class by herbicide treatment interaction, mean comparisons were made using Fisher's protected LSD test at $P = 0.05$ with degrees of freedom calculated according to the Kenward-Roger method. For each of the three genotypic classes, orthogonal contrasts were used to compare sweet corn response to dicamba alone versus dicamba + CSA for all injury, yield, and ear trait variables.

Results and Discussion

There were no extreme temperature or water stresses during the experiment. The 2019 Macomb site appeared to accumulate GDDs faster than other site-years (Figure 1A); the result of a 5-

Table 2. Mean and mean separation for crop response variable means and mean separation as a function of sweet corn genotypic class and dicamba formulation and application timing at Macomb, IL, and Urbana, IL, in 2019 and 2020.[a]

Main effects	Corn injury 1 WAT[b]	Corn injury 2 WAT	Ear mass yield	Kernel mass yield	Crate yield	Prolificacy	Ear mass	Ear length	Filled ear length	Malformed ears	Ear breakage
	0–100[c]		kg ha^{-1}		boxes ha^{-1}	ears plant^{-1}	g ear^{-1}	cm		%	
Genotypic class[d]	**	**	**	NS	*	**	**	**	**	**	*
Nsf1Nsf1	21 b	17 b	9,390	4,510	593 a	0.80 a	276	17.6 a	89.8	19.5	2.5
Nsf1nsf1	20 b	17 b	9,420	4,340	603 a	0.80 a	265	17.9 a	91.0	15.2	5.1
nsf1nsf1	37 a	33 a	7,260	4,190	554 b	0.66 b	238	16.4 b	82.7	32.7	3.4
Herbicide treatment	**	**	**	**	*	**	**	**	**	**	**
Nontreated	00 c	00 d	10,130	5,540	628 a	0.77 ab	298	18.9 a	92.9	11.8	1.5
V3 dicamba	25 b	21 c	8,530	4,860	547 b	0.73 ab	263	18.0 abc	90.9	11.0	1.7
V3 dicamba+CSA[e]	23 b	18 c	8,620	4,810	563 b	0.79 a	259	18.2 ab	91.6	11.5	0.9
V6 dicamba	30 b	31 a	8,400	4,050	562 b	0.72 ab	263	17.1 c	90.8	17.3	5.0
V6 dicamba+CSA	28 b	22 c	9,500	4,890	600 ab	0.80 a	273	17.6 bc	92.6	15.6	5.4
V9 dicamba	37 a	28 ab	6,650	2,170	515 b	0.65 b	217	13.9 d	66.5	60.7	7.8
V9 dicamba+CSA	24 b	24 bc	9,040	4,190	626 a	0.79 a	241	17.2 c	85.2	35.2	4.3
Interaction											
G*H	NS	NS	**	**	NS	NS	*	NS	**	**	**

[a]Values shown are means. Main effect means among genotypic class or among herbicide treatment within a column with no common letter are significantly different according to Fisher's protected LSD at $\alpha = 0.05$. Significant at *$P < 0.05$ and **$P < 0.01$.
[b]WAT, weeks after treatment.
[c]0 was no observable injury, and 100 was crop death.
[d]Nsf1Nsf1, homozygous tolerant; Nsf1nsf1, heterozygous; nsf1nsf1, homozygous sensitive.
[e]Cyprosulfamide.

Table 3. Sweet corn ear mass yield and kernel mass yields in response to dicamba formulation and application timing by genotype class at Macomb, IL, and Urbana, IL, in 2019 and 2020.

Response variable	Herbicide treatment[a]	Genotypic class[b]		
		Nsf1Nsf1	*Nsf1nsf1*	*nsf1nsf1*
			1,000 kg ha^{-1}	
Ear mass yield	Nontreated	10.32 ab	9.87 abc	10.59 a
	V3 dicamba	9.48 abcd	8.55 abcd	7.54 abcd
	V3 dicamba + CSA[c]	10.19 ab	9.00 abcd	7.07 cd
	V6 dicamba	8.98 abcd	9.82 abc	6.42 de
	V6 dicamba + CSA	10.48 a	10.39 ab	7.32 bcd
	V9 dicamba	7.59 abcd	8.42 abcd	3.81 e
	V9 dicamba + CSA	9.49 abcd	10.23 a	8.10 abcd
Kernel mass yield	Nontreated	5.45 ab	4.97 b	7.28 a
	V3 dicamba	5.01 b	4.35 bcd	5.21 b
	V3 dicamba + CSA	5.28 b	4.58 bc	4.86 b
	V6 dicamba	4.14 bcd	4.36 bcd	3.66 bcd
	V6 dicamba + CSA	5.10 b	4.95 b	4.63 bc
	V9 dicamba	2.97 cd	2.66 d	0.72 e
	V9 dicamba + CSA	4.28 bcd	4.84 b	4.02 bcd

[a]Cyprosulfamide.
[b]*Nsf1Nsf1*, homozygous tolerant; *Nsf1nsf1*, heterozygous; *nsf1nsf1*, homozygous sensitive. Means within a response variable with no common letter are significantly different according to Fisher's protected LSD at $\alpha = 0.05$. Comparisons for each response variable can be made across herbicide treatments and/or genotypic class.

to 6-wk later planting date. While all site-years had a similar cumulative rainfall the first few weeks after planting, beyond 40 d after planting, 2019 was a drier grower season, as evidenced by 33% lower cumulative seasonal rainfall compared with 2020 (Figure 1B).

Both genotypic class and herbicide treatment were influential in all crop responses to dicamba. Across herbicide treatments, the *nsf1nsf1* hybrid exhibited the most injury and produced fewer and shorter ears compared with other genotypic classes (Table 2). Across genotypic classes, the V9 dicamba treatment without CSA was among the most injurious, as evidenced by the highest injury; furthermore, it produced the fewest ears, and those ears were shorter than ears from all other treatments. Genotypic class and herbicide treatment had an interactive effect on all other yield responses and ear traits.

Ear mass yield and kernel mass yield of the *nsf1nsf1* hybrid were numerically lower with all dicamba treatments but were significantly lower following the V9 application without CSA (Table 3). The V9 application of dicamba without CSA also significantly reduced kernel mass of *Nsf1Nsf1* and *Nsf1nsf1* hybrids relative to the nontreated control. Individual ear mass followed a similar trend to kernel mass yield, with herbicide treatments injurious to the *nsf1nsf1* hybrid, but only V9 applications of dicamba without CSA being injurious to the *Nsf1Nsf1* hybrid relative to the nontreated control (Table 4).

Table 4. Sweet corn ear mass, filled ear length, malformed ears, and ear breakage in response to dicamba formulation and application timing by genotype class at Macomb, IL, and Urbana, IL, in 2019 and 2020.

Ear traits measured	Herbicide treatment[a]	Genotypic class[b]		
		Nsf1Nsf1	*Nsf1nsf1*	*nsf1nsf1*
			g ear^{-1}	
Ear mass	Nontreated	301 ab	276 abc	299 ab
	V3 dicamba	308 a	252 abcd	234 cdef
	V3 dicamba + CSA	281 abc	281 abc	229 cdef
	V6 dicamba	273 abc	277 abc	226 cdef
	V6 dicamba + CSA	274 abc	279 abc	234 cdef
	V9 dicamba	208 def	240 bcde	177 f
	V9 dicamba + CSA	253 abcd	264 abcd	187 ef
			%	
Filled ear length	Nontreated	93.5 a	92.1 a	92.9 a
	V3 dicamba	92.7 a	89.4 a	90.5 a
	V3 dicamba + CSA	91.6 a	93.0 a	90.2 a
	V6 dicamba	93.0 a	93.5 a	85.9 ab
	V6 dicamba + CSA	92.9 a	93.6 a	91.3 a
	V9 dicamba	75.5 c	84.4 abc	46.3 d
	V9 dicamba + CSA	89.2 a	89.9 a	77.0 bc
			%	
Malformed ears	Nontreated	13.1 cde	5.6 e	11.3 cde
	V3 dicamba	10.6 cde	8.8 de	13.8 cde
	V3 dicamba + CSA	11.3 cde	9.4 cde	13.8 cde
	V6 dicamba	5.0 e	15.0 cde	31.9 bcd
	V6 dicamba + CSA	7.5 de	4.4 e	30.8 bcd
	V9 dicamba	55.0 ab	45.6 b	71.9 a
	V9 dicamba + CSA	33.1 bc	17.5 cde	55.0 ab
			%	
Ear breakage	Nontreated	0.6 b	1.9 b	1.9 b
	V3 dicamba	0.0 b	4.4 ab	0.6 b
	V3 dicamba + CSA	0.6 b	1.9 b	0.0 b
	V6 dicamba	1.9 b	5.8 ab	7.5 ab
	V6 dicamba + CSA	0.0 b	11.9 a	4.0 ab
	V9 dicamba	8.1 ab	6.7 ab	7.5 ab
	V9 dicamba + CSA	6.9 ab	3.4 b	2.5 b

[a]Cyprosulfamide.
[b]*Nsf1Nsf1*, homozygous tolerant; *Nsf1nsf1*, heterozygous; *nsf1nsf1*, homozygous sensitive. Means within each measured ear trait with no common letter are significantly different according to Fisher's protected LSD at $\alpha = 0.05$. Comparisons for each response variable can be made across herbicide treatments and/or genotypic class.

Dicamba + diflufenzopyr was previously shown to cause higher injury levels in *nsf1nsf1* sweet corn lines compared with *Nsf1Nsf1* and *Nsf1nsf1* lines (Nordby et al. 2008; Pataky et al. 2006). Furthermore, dicamba + diflufenzopyr injury to *nsf1nsf1* lines was highly correlated with injury caused by carfentrazone, mesotrione, foramsulfuron, and bentazon, because a single gene is responsible for conferring tolerance or susceptibility to these herbicides (Pataky et al. 2006). Higher sensitivity of *nsf1nsf1* sweet corn lines to halosulfuron, mesotrione, nicosulfuron, tembotrione, and topramezone was also previously reported (Williams et al. 2005, 2006, 2008; Williams and Pataky 2008, 2010). Furthermore, sensitivity to nicosulfuron and primisulfuron was shown in *nsf1nsf1* field corn lines (Bradshaw et al. 1994; Hinz et al. 1997; Kang 1993). Over time, sweet corn, field corn, and popcorn breeders should replace mutant *nsf1* alleles with functional *Nsf1* alleles, thereby reducing occurrence of crop injury from CYP-metabolized herbicides (Williams and Pataky 2010).

Sweet corn sensitivity to dicamba and other CYP-metabolized herbicides also has been shown to vary by growth stage at the time of application. In glyphosate-tolerant popcorn, there was no significant difference in injury, biomass, or plant height between dicamba applied at V5 or V8; however, glyphosate and 2,4-D caused greater injury at V5 than at V8 (Barnes et al. 2020). Similar results in sweet corn showed mesotrione and nicosulfuron decreased yield more when applied between V3 and V5 compared with between V5 and V7 (Meyer et al. 2010). Conversely, in field corn, foramsulfuron caused the greatest yield reductions and ear malformations when applied later in the season (V8 or V12) (Bunting et al. 2004). In the present study, tassel malformations (i.e., "tassel-ears" or "crazy top") were observed from the V9 applied treatments (data not shown). We speculate that higher injury to the reproductive growth at V9 may be due to issues in pollen viability or pollen germination caused by injury to a rapidly developing and growing tassel and newly visible ear shoots (Bell 2018).

A low level of ear malformation was observed in nontreated plots, providing evidence that we were unable to use ear malformation alone to differentiate dicamba injury from other types of biotic or abiotic stresses (Table 4). Nonetheless, for all three genotypic classes, the V9 dicamba without CSA treatment showed the largest increase in ear malformation compared with the nontreated plots. Bunting et al. (2004) reported that the CYP-metabolized herbicide foramsulfuron applied at V8 or V12 caused ear malformations on up to 40% of treated field corn plants. Higher levels of malformed ears in the present study were likely due to a combination of inadequate time to metabolize the herbicide before ear development and damage to developing tassels, resulting in reduced pollen viability and sporadic kernel fill (Castner 2021; Meyer et al. 2010). In contrast, ear breakage from the husking bed was generally low (i.e., mean of 3.7%), with few differences observed among treatments. Based on the observed high level of overall injury with

Table 5. Sweet corn injury potential difference averaged across the V3, V6, and V9 timings of the dicamba + cyprosulfamide (CSA) values and dicamba-alone values in the genotypic classes in trials conducted at Macomb, IL, and Urbana, IL, in 2019 and 2020.

Genotypic class[a]	Corn injury 1 WAT[b]	Corn injury 2 WAT	Ear mass yield	Kernel mass yield	Crate yield	Prolificacy	Ear mass	Ear length	Filled ear length	Malformed ears	Ear breakage
	Dicamba + CSA:dicamba[c]										
	0–100[d]		kg ha^{-1}		boxes ha^{-1}	ears plant^{-1}	g ear^{-1}	cm		%	
Nsf1Nsf1	−2	−2	1,370*	850**	15	0.07*	5.9	0.9**	4.2*	−6.3	−3.0*
Nsf1nsf1	−4	−5	940	1,000**	17	0.02	18.8*	1.8**	3.1	−12.7**	−0.1
nsf1nsf1	−10*	−10*	1,570*	1,310**	90*	0.16*	7.7	1.2**	11.9**	−6.0	−0.8

[a]*Nsf1Nsf1*, homozygous tolerant; *Nsf1nsf1*, heterozygous; *nsf1nsf1*, homozygous sensitive.
[b]WAT, weeks after treatment.
[c]Significant difference between dicamba + CSA and dicamba alone at *P < 0.05 and **P < 0.01.
[d]0 was no observable injury, and 100 was crop death.

applications at later growth stages compared with the nontreated plots, especially on the *nsf1nsf1* hybrid, dicamba should not be applied to or near sweet corn after V6.

As hypothesized, CSA reduced the risk of crop injury from dicamba; however, CSA did not eliminate such risk. This observation largely held true regardless of genotypic class (Table 5). In sweet corn, CSA reduces injury and reduces yield loss from preemergence and postemergence isoxaflutole application (Robinson et al. 2013). Giannakopoulos et al. (2020) showed that CSA protects field corn from thiencarbazone-methyl injury by enhancing CYP metabolism. Additionally, Sun et al. (2016) found that CSA effectively reduced the phytotoxic effect of nicosulfuron when applied at V5 in waxy corn. Results from the current research show that, in addition to safening sweet corn from other CYP-metabolized herbicides, CSA effectively reduces but does not preclude risk from applications of dicamba to sweet corn.

As the hybrids used in this study were not near-isogenic lines differing only in the alleles of the *Nsf1* gene, there is the potential for confounding effects from the different genetic backgrounds of the hybrids. However, the hybrids used in this study have repeatedly shown similar injury responses compared with the near-isogenic lines when treated with dicamba or other CYP-metabolized herbicides (Meyer et al. 2010; Nordby et al. 2008; Pataky et al. 2008, 2009). As such, it is highly likely that the results shown in this study were mainly due to the differences in the *Nsf1* alleles.

Due to dicamba's continued use on other crops in the United States, understanding how sweet corn hybrids from different genotypic classes are affected by dicamba is essential to minimizing injury. Results from this study showed that while the *nsf1nsf1* hybrid was the most sensitive to dicamba, dicamba exposure during the V9 growth stage can cause significant injury in all three genotypic classes. Furthermore, the addition of CSA to the dicamba applications alleviated some of this injury, but it did not eliminate risk. As such, *Nsf1Nsf1* or *Nsf1nsf1* sweet corn hybrids should be used in conjunction with herbicide safeners to reduce crop injury from dicamba and other CYP-metabolized herbicides.

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