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Cyprosulfamide Safens Isoxaflutole in Sweet Corn (*Zea mays* L.)

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***Abstract*. There is little published information on the sensitivity of sweet corn to the PRE and POST application of isoxaflutole + cyprosulfamide. Four field trials were conducted during 2010 and 2011 in Ontario, Canada, to determine the sensitivity of 'Merit', 'GH 4927', 'BSS 5362', and 'GG 741' sweet corn hybrids to the PRE and POST application of isoxaflutole alone or in combination with cyprosulfamide. Isoxaflutole applied PRE or POST at 105 and 210 g a.i./ha caused as much as 12% visual injury, 18% reduction in height. and 24% reduction in marketable yield of some sweet corn hybrids evaluated. Isoxaflutole + cyprosulfamide applied PRE or POST at 105 and 210 g·ha^{-1} caused up to 7% initial injury in some sweet corn hybrids but the injury was transient with no effect on sweet corn height, cob size, and yield. Isoxaflutole applied POST was more injurious to sweet corn than when applied PRE; however, there was no differences in sweet corn injury between the PRE and POST applications of isoxaflutole + cyprosulfamide. Based on these results, there is potential for use of isoxaflutole + cyprosulfamide applied at 105 g a.i./ha in 'Merit', 'GH 4927', 'BSS 5362', and 'GG 741' sweet corn hybrids.**

Sweet corn (*Zea mays* L.) production is important to the economy of Ontario where nearly 113,000 t of sweet corn are produced on nearly 10,000 ha annually [Ontario Ministry of Agriculture, Food, and Rural Affairs (OMAFRA), 2013]. In 2012, sweet corn had a farm-gate value of $33 million and ranked as the second largest field-grown vegetable crop after tomato in Ontario in terms of farm-gate value (OMAFRA, 2013). Effective weed control is important for sweet corn production. The number of herbicides labeled for sweet corn lags behind that of field corn in Ontario mainly as a result of tolerance concerns for some sweet corn hybrids. More research is needed to identify preemergence (PRE) and postemergence (POST) herbicides that provide broad-spectrum weed control in sweet corn production.

Isoxaflutole is a soil and postemergence-applied isoxazole herbicide in field corn that inhibits p-hydroxyphenyl pyruvate dioxygenase (HPPD), an important enzyme involved in the biosynthesis of carotene pigments in plants (Senseman, 2007). Isoxaflutole provides residual control of grass and broadleaf weeds such as wild proso millet (*Panicum miliaceum* L.), giant foxtail (*Setaria faberii* Herrm.), green foxtail [*Setaria viridis* (L.) Beauv.], yellow foxtail [*Setaria glauca* (L.) Beauv.], barnyard grass [*Echinochloa crus-galli* (L.) Beauv.], fall panicum (*Panicum dichotomiflorum* Michx.), common lambsquarters (*Chenopodium album* L.), redroot pigweed (*Amaranthus retroflexus* L.), common ragweed (*Ambrosia artemisiifolia* L.), smartweed (*Polygonum* spp.), annual nightshades (*Solanum* spp.), and velvetleaf (*Abutilon theophrasti* Medic.) (Bhowmik et al., 1996, 1999; Clarke et al., 2001; Luscombe et al., 1995; OMAFRA, 2012; Taylor-Lovell and Wax, 2001; Vrabel et al., 1997, 1998).

Earlier studies with isoxaflutole have shown unacceptable crop injury in some sweet corn hybrids in Ontario and other geographic areas (O'Sullivan et al., 2001; Van Wychen et al., 1999; Wicks et al., 2007). The manufacturer of isoxaflutole (Bayer® Inc.) has recently introduced isoxaflutole in combination with a safener, cyprosulfamide, thus allowing PRE and early POST application through the V2 stage (two leaves with visible leaf collars) of field corn (Owen, 2011). The registration of isoxaflutole plus cyprosulfamide would provide Ontario sweet corn producers with a new, broad-spectrum herbicide that controls selected annual grass and broadleaf weed species.

Determining sensitivity of sweet corn hybrids to new herbicides is critical to sweet corn growers to minimize the potential for crop injury and yield losses (Morton and Harvey, 1992). Sweet corn hybrid sensitivity has been documented for isoxaflutole (O'Sullivan et al., 2001), but this work was done before the safener was available. Before isoxaflutole plus cyprosulfamide can be registered for use in sweet corn, hybrid sensitivity must be determined. On the basis of work by O'Sullivan et al. (2001) and Pataky et al. (2008), the hybrids in our study were categorized as sensitive ('Merit'), as having intermediate sensitivity ('GH 4927' and 'BSS 5362'), and tolerant ('GG 741'). We hypothesized that cyprosulfamide improves tolerance of sweet corn to isoxaflutole (PRE and POST) in the hybrids with intermediate sensitivity but not in the sensitive or tolerant hybrids. The objective of this study was to determine the sensitivity of four commonly grown sweet corn hybrids to isoxaflutole alone or in combination with cyprosulfamide.

Materials and Methods

Field experiments were conducted at the University of Guelph, Ridgetown Campus, Ridgetown, Ontario, and the Huron Research Station, Exeter, Ontario, in 2010 and 2011. The soil at the Ridgetown location was a Watford/Brady very fine sandy loam composed of 78% sand, 14% silt, 8% clay, and 4.4% organic matter with a pH of 6.7 in 2010 and a clay loam composed of 38% sand, 32% silt, 31% clay, and 6.1% organic matter with a pH of 6.0 in 2011. The soil at the Exeter location was a Brookston clay loam composed of 31% sand, 38% silt, 31% clay, and 4.6% organic matter with a pH of 8.0 in 2010 and 21% sand, 42% silt, 37% clay, and 3.7% organic matter with a pH of 7.4 in 2011. Seedbed preparation consisted of moldboard plowing in the fall and two passes with a field cultivator with rolling basket harrows in the spring.

The experiments were arranged in a split-split plot design with four replications. The main plots were application timing (PRE and POST). The subplots were treatments, which included a non-treated control, isoxaflutole at 105 and 210 g a.i./ha alone, and in pre-mixed combination with cyprosulfamide (Tables 1 to 3). The sub-subplots were sweet corn hybrids and consisted of four rows of 'Merit', 'GH 4927', 'BSS 5362', and 'GG 741' hybrids spaced 75 cm apart in rows that were 8 m long at Ridgetown and 10 m long at Exeter. The sweet corn was thinned to 50,000 plants/ha 1 week after emergence. All plots were kept weed-free using interrow cultivation and hand hoeing as needed.

Herbicide treatments were applied PRE (4 to 8 d after planting) and POST (V2 stage, two-leaf corn). Herbicide applications were made with a CO_2-pressurized backpack sprayer calibrated to deliver 200 L·ha^{-1} of spray solution at a pressure of 200/241 kPa using low-drift nozzles (ULD120-02; Spraying Systems Co., Wheaton, IL). The boom was 2.5 m wide with six nozzles spaced 0.5 m apart.

Sweet corn injury was visually estimated on a scale of 0 (no injury) to 100% (complete plant death) at 1, 2, and 4 weeks after crop emergence for PRE treatments or after herbicide application for POST treatments (WAE/T). Corn height was measured for 10 plants per plot at 3 WAE/T and averaged. Sweet corn was harvested by hand based on the maturity of each hybrid and the total cob

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Table 1. Injury of four sweet corn varieties as a function of herbicide treatment.[z]

Herbicide (g a.i./ha) by variable	Sweet corn variety (%) Merit			GH 4927			BSS 5362			GG 741		
1 WAE/T												
Isoxaflutole (105)	5	ab	Y	7	b	Y	5	b	Y	4	b	Z
Isoxaflutole (210)	11	c	Y	10	c	Y	10	c	Y	7	c	Z
Isoxaflutole + safener (105)	4	a	X	3	a	X	2	a	Y	1	a	Z
Isoxaflutole + safener (210)	7	b	X	5	ab	Y	4	b	Y	2	a	Z
2 WAE/T												
Isoxaflutole (105)	5	ab	YZ	7	b	Y	6	b	YZ	4	b	Z
Isoxaflutole (210)	11	c	YZ	12	c	Y	11	c	YZ	9	c	Z
Isoxaflutole + safener (105)	3	a	Y	3	a	Y	2	a	YZ	1	a	Z
Isoxaflutole + safener (210)	6	b	X	4	ab	Y	3	ab	Y	2	ab	Z
4 WAE/T												
Isoxaflutole (105)	3	ab	Z	4	b	Z	4	b	Z	3	b	Z
Isoxaflutole (210)	6	c	Z	9	c	Y	7	c	YZ	6	c	Z
Isoxaflutole + safener (105)	1	a	Z	2	a	Z	2	a	Z	1	a	Z
Isoxaflutole + safener (210)	4	bc	Y	3	ab	Y	3	ab	Y	2	ab	Z

[z]Means followed by the same letter within a column (a–c) or row (X–Z) for each section are not significantly different according to Fisher's protected least significant difference at $P < 0.05$.
WAE/T = weeks after emergence or application; safener = cyprosulfamide.

Table 2. Injury of sweet corn at two application timings as a function of herbicide treatment.[z]

Herbicide (g a.i./ha) by variable	Application timing (%) PRE			POST		
1 WAE/T						
Isoxaflutole (105)	0	a	Z	14	c	Y
Isoxaflutole (210)	1	a	Z	24	d	Y
Isoxaflutole + safener (105)	0	a	Z	6	a	Z
Isoxaflutole + safener (210)	1	a	Z	10	b	Z
2 WAE/T						
Isoxaflutole (105)	1	a	Z	14	b	Y
Isoxaflutole (210)	3	a	Z	24	c	Y
Isoxaflutole + safener (105)	0	a	Z	4	a	Z
Isoxaflutole + safener (210)	1	a	Z	7	a	Z

[z]Means followed by the same letter within a column (a–e) or row (Y–Z) for each section are not significantly different according to Fisher's protected least significant difference at $P < 0.05$.
WAE/T = weeks after emergence or application; POST = postemergence; PRE = preemergence; safener = cyprosulfamide.

weight at maturity and number were recorded as were the marketable cob weight and number after sorting.

Data were analyzed as a three-way factorial using PROC MIXED in SAS 9.2 (SAS, 2008). Sweet corn hybrid, herbicide treatment, and application timing as well as their interactions were considered fixed effects, whereas environment (year–location combinations), interactions between environments and the fixed effects, and replicate nested within environment were considered random effects. Significance of fixed effects were tested using F-tests and random effects were tested using a Z-test of the variance estimate. Environments were combined for a given variable if the environment by variety by herbicide by timing interaction was not significant. The UNIVARIATE procedure was used to test data for normality and homogeneity of variance. For all injury ratings, the untreated control (assigned a value of zero) was excluded from the analysis. However, all values were compared independently to zero to evaluate treatment differences with the untreated control. To satisfy the assumptions of the variance analyses, injury 1, 2, and 4 WAE/T were square root-transformed. Treatment comparisons were made using Fisher's protected least significant difference at a level of $P < 0.05$. Additionally, an orthogonal contrast was constructed to compare sweet corn response to isoxaflutole alone vs. isoxaflutole plus safener for those variables where significant interaction effects were not observed and main effects were significant [i.e., for height, cob weight, and number (total and marketable) and yield (total and marketable)]. Data compared on the transformed scale were converted back to the original scale for presentation of results.

Results and Discussion

Statistical analysis of the data showed that environment by variety by treatment by timing interactions were not significant for any variable; therefore, data sets were analyzed together. We observed two significant two-way treatment interactions for visible injury: 1) a variety by herbicide treatment interaction (Table 1); and 2) a herbicide treatment by timing interaction (Table 2). Only main effects of sweet corn hybrid and herbicide treatment were significant for sweet corn height, cob weight, cob number and yield.

Visible injury

Sweet corn hybrid by herbicide treatment interaction. Visible injury symptoms in all hybrid classes, which included leaf and stem bleaching, were reduced by the addition of cyprosulfamide. At 1 WAE/T, isoxaflutole caused as much as 11%, 10%, 10%, and 7% injury and isoxaflutole + cyprosulfamide caused 7%, 5%, 4%, and 2% injury in 'Merit', 'GH 4927', 'BSS 5362', and 'GG 741' sweet corn hybrids, respectively (Table 1). This ranking for visible injury among hybrids was also observed at 2 WAE/T; the addition of cyprosulfamide to isoxaflutole reduced visible injury in all four hybrids at this observation time. At 4 WAE/T, isoxaflutole (210 g a.i./ha) alone caused 6%, 9%, 7%, and 6% injury, whereas isoxaflutole + cyprosulfamide caused 4%, 3%, 3%, and 2% injury in 'Merit', 'GH 4927', 'BSS 5362', and 'GG 741' sweet corn hybrids, respectively. Although visible injury varied among the four hybrids examined, cyprosulfamide reduced visible injury at all three observation times, which was similar to observations made by Malidža et al. (2010).

Herbicide treatment by application timing interaction. The addition of cyprosulfamide safened the POST treatments of isoxaflutole (Table 2). PRE applications of isoxaflutole alone or with cyprosulfamide did not cause greater than 3% visible injury; however, the POST isoxaflutole treatment (210 g a.i./ha) caused 24% visible injury where the herbicide had been applied without a safener at both 1 and 2 WAE/T. The addition of the safener to POST applications of isoxaflutole reduced visible injury to 10% and 7% at 1 and 2 WAE/T, respectively. Visible injury caused by isoxaflutole was reduced by the addition of cyprosulfamide only in the POST application timing. Similar safening effects of isoxadifen on tembotrione applied POST to sweet corn (Williams and Pataky, 2010) have been observed.

Plant height

Plant height reductions caused by isoxaflutole were alleviated in all sweet corn hybrids when cyprosulfamide was applied along with isoxaflutole in the PRE and POST application timings (Table 3). At 3 WAE/T, among sweet corn hybrids evaluated, 'Merit' had the greatest height followed by 'GG 741', 'GH 4927', and then 'BSS 5362'. Isoxaflutole reduced sweet corn height as much as 12% and 18% in some hybrids when applied at 105 and 210 g a.i./ha, respectively. These reductions are comparable to those measured by O'Sullivan et al. (2001) and Van Wychen et al. (1999). Isoxaflutole + cyprosulfamide did not reduce sweet corn height at either rate. Contrast indicated height was on average 10% lower across all hybrids when isoxaflutole was applied alone vs. when it was applied with cyprosulfamide (Table 3). These results compare well with those of Malidža et al. (2010), who showed that sweet corn hybrids treated with isoxaflutole + cyprosulfamide applied PRE at 96 and 192 g a.i./ha were as much as 8 and 16 cm taller compared with isoxaflutole alone applied at the same rates, respectively.

Cob weight and number

'GH4927' produced the smallest cobs—total and marketable—of the four hybrids examined. Among the sweet corn hybrids evaluated, 'GG 741' had the greatest total number of cobs followed by 'BSS 5362' followed by 'Merit' and then 'GH 4927' (Table 3). However, there was no difference in marketable cob number of various sweet corn hybrids evaluated. Marketable cob weight was reduced where isoxaflutole

Table 3. Significance of main effects and interactions for percent visual injury, height, cob number, and yield of four sweet corn varieties treated with isoxaflutole and isoxaflutole + cyprosulfamide.[z]

		Cob wt		Cob number		Yield	
		Total	Marketable	Total	Marketable	Total	Marketable
Main effects[y]	Ht (cm)	(g/cob)		(no. 10^3/ha)		(t·ha^{-1})	
Sweet corn variety	**	*	**	**	NS	**	NS
Merit	70 a	201 a	277 a	107 c	52	20.2 a	13.9
GH 4927	55 c	172 c	248 b	96 d	45	16.0 b	11.6
BSS 5362	52 d	190 b	267 a	120 b	52	21.8 a	14.2
GG 741	60 b	171 c	265 a	135 a	48	21.2 a	12.7
Herbicide (g a.i./ha)	*	NS	*	NS	NS	*	*
Untreated	65 a	184	277 a	115	55	20.3 a	14.7 a
I (105)	57 bc	179	267 a	117	47	19.8 ab	12.6 ab
I (210)	53 c	173	231 b	115	43	18.8 b	11.1 b
I + S (105)	62 ab	190	280 a	115	52	20.4 a	14.0 a
I + S (210)	59 abc	188	285 a	113	48	19.7 ab	13.0 ab
Application timing	NS	NS	NS	NS	NS	NS	NS
PRE	68	193	264	115	53	20.3	14.3
POST	70	173	254	115	45	19.2	11.8
Contrast: I vs. I + S	55 vs. 61*	177 vs. 190	245 vs. 283*	116 vs. 114	45 vs. 50	19.3 vs. 20.0*	11.8 vs. 13.5*

[z]Means followed by the same letter within a column are not significantly different according to Fisher's protected least significant difference at $P < 0.05$. Means for a main effect were separated only if there were no significant interactions involving that main effect.
[y]Significance at $P < 0.05$ and $P < 0.01$ levels denoted by * and **, respectively.
WAE/T = weeks after emergence or application; H = herbicide treatment; I = isoxaflutole; POST = postemergence; PRE = preemergence; T = application timing; NS = non-significant at $P = 0.05$ level; S = safener (cyprosulfamide) added to isoxaflutole; V = sweet corn variety.

was applied alone at 210 g a.i./ha, but the isoxaflutole + cyprosulfamide treatment did not reduce marketable cob weight. Herbicide treatment did not have any detrimental effect on total or marketable cob number regardless of the herbicide application rate or timing (Table 3). Other studies have shown that cob weight of susceptible hybrids can be reduced up to 67% (Soltani et al., 2005). Contrasts indicated that cob weight, but not cob number, was less where isoxaflutole had been applied without cyprosulfamide (Table 3).

Yield

Isoxaflutole alone applied at 105 g a.i./ha did not reduce sweet corn total and marketable yield but when applied at 210 g a.i./ha reduced total and marketable yield 7% and 24%, respectively, compared with untreated control. Isoxaflutole + cyprosulfamide caused no reduction in sweet corn total and marketable yield at either rate evaluated. Contrasts comparing the overall response to isoxaflutole alone versus isoxaflutole + cyprosulfamide indicated that sweet corn total yield was reduced 4% and marketable yield was reduced 13% with isoxaflutole alone compared with isoxaflutole + cyprosulfamide (Table 3). There were no significant differences in total and marketable yield between PRE and POST applications. In other studies, isoxaflutole alone applied at 105 and 210 g a.i./ha caused as much as 37% and 99% reduction in sweet corn yield compared with the untreated control, respectively (O'Sullivan et al., 2001). In contrast, isoxaflutole applied at rates up to 158 g a.i./ha caused no significant reduction in grain yield of field corn (Bhowmik et al., 1996, 1999; Luscombe et al., 1995; Vrabel et al., 1997, 1998). In Serbia, Malidža et al. (2010) reported that isoxaflutole (192 g a.i./ha) reduced yield of some sweet corn hybrids as much as 26% compared with isoxaflutole + cyprosulfamide, which had a comparable yield with the weed-free untreated control. Sweet corn hybrid sensitivity varies among the various HPPD-inhibiting herbicides (Williams and Pataky, 2010). Some HPPD-inhibiting herbicides such as topramezone (Soltani et al., 2007; Williams and Pataky, 2010) have been shown to cause no adverse effect on yield when applied at the label rate. Although others such as mesotrione and tembotrione (O'Sullivan et al., 2002; Williams and Pataky, 2010) have caused a significant reduction in some sweet corn hybrids.

Conclusions

The safening effect of cyprosulfamide may not always be detectable by estimating visible injury, because sweet corn response to isoxaflutole applied PRE varies with soil coarseness (Van Wychen et al., 1999) and rainfall immediately after application (O'Sullivan et al., 2001). Van Wychen et al. (1999) evaluating isoxaflutole applied preemergence at 210 g a.i./ha found as little as 7% and as much as 60% visible injury in 'Jubilee' sweet corn on a silt loam soil in Arlington, WI (27% sand content) and a sandy loam near LaSauer, MN (61% sand content), respectively. In another study, isoxaflutole alone applied PRE at 210 g a.i./ha caused between 0% and 78% visible injury where 48.5 and 13.8 mm of rainfall occurred within 10 d after application on the same sweet corn hybrids (O'Sullivan et al., 2001). Soil type and precipitation soon after application caused similar variability in field corn visible injury response to isoxaflutole (Luscombe et al., 1995). Malidža et al. (2010) showed that the addition of cyprosulfamide reduced phytotoxicity of some sweet corn hybrids to PRE applications of isoxaflutole in Serbia. We observed very low levels of visible injury in the PRE applications of isoxaflutole in all sweet corn hybrids, which may account for why we did not detect a safening effect of cyprosulfamide in our visible injury ratings.

Despite the variable effect of adding cyprosulfamide to isoxaflutole on visible injury, sweet corn height and yield (both total and marketable) were consistently less where isoxaflutole was applied alone than when it was applied with cyprosulfamide. This safening effect was observed at both rates of isoxaflutole. Similar observations were made by Malidža et al. (2010) with cyprosulfamide on isoxaflutole applied PRE to sweet corn. Our study is the first study to show a safening effect of cyprosulfamide for both PRE and POST applications of isoxaflutole in sweet corn.

Cyprosulfamide safened isoxaflutole in all sweet corn hybrids evaluated. 'Merit', 'GH 4927', 'BSS 5362', and 'GG 741' sweet corn were sensitive to isoxaflutole applied PRE or POST at 105 and 210 g a.i./ha. The injury was consistent, especially at the 2× rate and reduced sweet corn height along with total and marketable yield. Conversely, 'Merit', 'GH 4927', 'BSS 5362', and 'GG 741' were tolerant to isoxaflutole + cyprosulfamide applied PRE or POST at 105 and 210 g·ha^{-1}. Initial injury was transient and had no negative effect on sweet corn height, total cob number, marketable cob number, and total and marketable yield. As the rate of isoxaflutole + cyprosulfamide was increased from 1× to 2× of the labeled field corn rate, there was generally no negative effect on any sweet corn hybrids.

There was more injury with isoxaflutole POST compared to PRE; however, there were no differences in sweet corn injury between PRE and POST applications of isoxaflutole + cyprosulfamide. Based on these results, there is not an adequate margin of crop safety in sweet corn hybrids evaluated to isoxaflutole alone applied PRE and POST at the proposed rate of 105 g a.i./ha, which confirms earlier research (O'Sullivan et al., 2001). However, there is an adequate margin of crop safety in the sweet corn hybrids evaluated to isoxaflutole + cyprosulfamide applied PRE or POST at the proposed rate of 105 g a.i./ha. Availability of isoxaflutole + cyprosulfamide will provide sweet corn producers with an effective weed management option for the control of troublesome weeds in Ontario.

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