Initial Field Evaluation of Sulfonylurea Herbicide Resistant Sugarbeet from Somatic Cell Selection

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ABSTRACT

Field studies were conducted with a sugarbeet *(Beta vulgaris* L.) breeding line segregating for monogenic dominant sulfonylurea herbicide resistance conditioned by the *Sur_l* allele and obtained from somatic cell selection. Sulfonylurea herbicide resistant and susceptible sublines were compared to each other and to the commercially available susceptible cultivar MONO-HY E4 in regards to root yield, sugar content, and processing purity. In addition, the response of MONO-HY E4 and the sulfonylurea resistant and susceptible counterparts to simulated carryover sulfonylurea residues in soil and to postemergence (POST) applications of selected sulfonylurea herbicides was evaluated. In the absence of herbicides, counterpart resistant and susceptible sugarbeets produced similar root yield, sugar content, and clear juice purity at both locations. Nicosulfuron applied preplant incorporated (PPI) at 9 g ai ha^{-1} to simulate carryover in soil had no effect on the growth of sugarbeets from the resistant population or from the susceptible MONO-HY E4 cultivar seeded immediately after application. Primisulfuron and chlorimuron applied PPI at 10 and 3 g ai ha^{-1}, respectively, caused over 95% visible injury to the susceptible MONO-HY E4 sugarbeet 6 weeks after treatment (WAT), but had no adverse effect on the growth of resistant sugarbeet. POST application of primisulfuron at 40 and 80 g ai ha^{-1}, and thifensulfuron at 4 and 8 g ha^{-1} (one and two times the normal field use rate for corn and soybean, respectively), caused less than 15% visible injury to the resistant sugarbeet 4 WAT, but caused severe injury to the susceptible MONO-HY E4 sugarbeet. The sulfonylurea resistant sugarbeet was tolerant to POST applications of primisulfuron at four times and thifensulfuron at two times the field use rate. This magnitude of resistance is great enough for effective use of primisulfuron and thifensulfuron for weed control in sulfonylurea resistant sugarbeet.

Additional Key Words: *Beta vulgaris* L., herbicide carryover, herbicide resistant crop.

A major disadvantage of sulfonylurea herbicides in sugarbeet production areas has been their long persistence in alkaline soils and injury to sensitive rotational crops (Brewster and Appleby, 1983; Renner and Powell, 1991). The development of sulfonylurea resistant sugarbeet cultivars provides a potential solution to this problem. Resistant cultivars also could increase chemical weed control options for farmers. A sulfonylurea resistant sugarbeet clone (CR1-B) has been generated successfully via somatic cell selection against chlorsulfuron in tissue culture (Saunders, et al., 1992). In greenhouse studies, CR1-B was resistant to primisulfuron and thifensulfuron applied POST at rates exceeding normal field use rates, and was slightly cross-resistant to nicosulfuron and chlorimuron (Hart, et al., 1993). However, the field response of resistant sugarbeet to herbicide residues in soil and to POST applications of sulfonylurea herbicides in the field has not been evaluated. The effect of the sulfonylurea resistance trait on the agronomic performance of sugarbeet in the absence of herbicides also has not been determined. This research was conducted to assess the effect of the sulfonylurea resistance trait on the agronomic performance of sugarbeet, and to evaluate the response of sulfonylurea resistant sugarbeet to simulated herbicide residues in soil, and to POST application of primisulfuron and thifensulfuron at or exceeding normal field use rates.

MATERIALS AND METHODS

Nomenclature. Chlorimuron, 2-[[[[(4-chloro-6-methoxy-2-pyrimidinyl) amino]carbonyl]amino]sulfonyl]benzoic acid; chlorsulfuron (2-chloro-*N*-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]benzenesulfonamide); nicosulfuron, 2-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-*N,N*-dimethyl-3-pyridinecarboxamide; primisulfuron,2-[[[[[4,6-bis(difluoromethoxy)-2-pyrimidinyl]amino]carbonyl]amino]sulfonyl] benzoic acid; thifensulfuron, 3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]-amino]sulfonyl]-2-thiophenecarboxylic acid; sugarbeet, *Beta vulgaris* L. 'CR1-B', 'MONO-HY E4', 'TR-504'.

Plant material. The following procedure was used to obtain a sufficient amount of comparable resistant and susceptible seed for field studies. An initial cross was made in the greenhouse between the susceptible biennial breeding clone TR-504 and the clone CR1-B, heterozygous for both resistance and annualism (the *B* locus) (Abegg, 1936). F_1 progeny were grown in the greenhouse, and resistant or susceptible segregates were identified by a non-destructive tissue culture leaf disk test. The 5th and 6th leaves were removed, surface sterilized for 20 minutes with a dilute (1:5 v/v) commercial (5.25% w/v) bleach solution containing 100 mg L^{-1} sodium laurylsulfate, and cut into 10-mm diameter leaf disks. Two leaf disks were placed in each 100 X 20 mm petri dish containing an agar-solidified growth medium for rapid sugarbeet leaf disk expansion and callus induction (Doley and Saunders, 1989). The growth medium contained 0 or 140 nM chlorsulfuron and the plates were incubated in the dark for 14 d at 31°C. Plants were considered to be resistant F_1 segregates if their leaf disks exhibited vigorous tissue expansion on 140 nM. The ratio of resistant to susceptible segregates was 54:46 and agreed closely with expected 1:1 progeny segregation ratio ($\chi^2 = 0.52$). Biennial F_1 segregates were kept at 4°C for 10 weeks and then placed in the greenhouse and exposed to continuous incandescent light to induce flowering. Eighteen resistant and 20 susceptible F_1 segregates were isolated in two separate locations and allowed to self and cross pollinate freely among themselves. F_2 seed from resistant and susceptible plants was bulked separately, and used for field studies. All F_2 plants from the susceptible parents were expected to be susceptible, whereas only 75% of the F_2 plants from resistant parents were expected to be resistant because all resistant F_1 plants were heterozygous.

Agronomic evaluation. Field studies were conducted in 1991 at the Saginaw Valley Bean and Beet Farm, which had a clay soil with 3.2% organic matter and pH 8.1, and at Bay City, on the grounds of the Monitor Sugar Co., with sandy clay loam soil with 2.5% organic matter and pH 8.0. Plots were 3 X 8.5 m, with a 76-cm row spacing; each plot had two MONO-HY E4 border rows and two middle rows of the entry under evaluation. Plots were arranged in a randomized complete block with four replicates. F_2 seed was multigerm. Sugarbeets were hand-planted in May and thinned to one plant per 20 cm of row after emergence, except one entry of the resistant F_2 that was treated just before thinning with 10 g ha^{-1} primisulfuron plus X-77 to eliminate susceptible segregates comprising an expected 25% of the population. [X-77 = a nonionic surfactant composed of a mixture of alkylarylpolyoxyethylene glycols, free fatty acids, and isopropanol; Chevron Chemical Co., Richmond CA.] Plots were hand-weeded throughout the growing season and harvested in September for root yields. Clear juice purities and sugar percentages were measured from a random sample of 20 sugarbeet roots per plot (Dexter, et al., 1967). Treatment means were subjected to analysis of variance as a two factor design (location X variety) and treatment means were separated by the least significant difference test at the 5% probability level.

Herbicide evaluations. Studies were conducted at the Saginaw site with the same soil properties described above. Plots were 3 X 3.6 m, with 76-cm row spacing. Each plot had two MONO-HY E4 border rows, an F_2 resistant row, and a MONO-HY E4 row. The susceptible F_2 line was not planted. All herbicide treatments were applied with a tractor mounted compressed air sprayer in 205 L ha^{-1} at 200 kPa. Nicosulfuron, chlorimuron, and primisulfuron were applied PPI at 9, 3, and 10 g ha^{-1}, respectively, by incorporating them to a depth of 8 to 10 cm with a Danish S tine field cultivator travelling at 13 kph. Primisulfuron was applied POST at rates of 40, 80, and 160 g ha^{-1} and thifensulfuron at 4 to 8 g ha^{-1} when sugarbeets had four true leaves. All treatments included X-77 at 0.25% (v/v). PPI and POST treatments were visually evaluated for sugarbeet injury at 6 and 4 WAT, respectively. Treatment means were subjected to a two factor analysis of variance (variety X herbicide treatment) and separated by the least significant difference test at the 5% probability level.

RESULTS AND DISCUSSION

Agronomic evaluation. A significant location X variety interaction ($P \leq 0.001$) was observed for sugarbeet root yield and sugar content (Table 1). This interaction was due to differentially lower root yields

Table 1. Agronomic performance of sulfonylurea resistant and susceptible sugarbeet lines in the absence of herbicides.

Location	Entry	Root Yield	Sugar Content	CJP[†]
		kg/ha	%	
Saginaw	MONO HY E4 (Susceptible)	40200	18.5	95
	TR504 X CR1-B F_2-S (Susceptible)	35600	17.0	93
	TR504 x CR1-B F_2-R (Resistant)	33400	16.9	94
	TR504 X CR1-B F_2-R (Resistant)[‡]	35000	16.5	93
	LSD (0.05)	3800	0.9	2
Bay City[§]	MONO HY E4 (Susceptible)	21500	15.2	95
	TR504 X CR1-B F_2-S (Susceptible)	11500	14.0	93
	TR504 X CR1-B F_2-R (Resistant)	12700	14.3	94
	TR504 X CR1-B F_2-R (Resistant)	14600	13.9	94
	LSD (0.05)	2600	0.4	2

[†] CJP = Clear juice purity

[‡] Plots sprayed with 10 g ha^{-1} of primisulfuron before thinning to eliminate susceptible segregates.

[§] Nematode infested site.

and sugar content, thought to be caused by nematodes, at the Bay City location.

Root yield, sugar content, and clear juice purity did not differ between the susceptible and resistant F_2 population at Saginaw (Table 1). Root yield and sugar content of the commercial hybrid check entry MONO-HY E4 was greater than that of herbicide susceptible and resistant F_2 sugarbeets at both locations. However, root yield of the resistant F_2 treated with primisulfuron was greater than that of the susceptible F_2 at Bay City.

These initial results comparing sulfonylurea resistant sugarbeets with susceptible sugarbeets with a similar genetic background suggest that both possess the same yield potential and sugar content. The resistance to sulfonylurea herbicides conferred by the Sur_1 allele in sugarbeet is due to an acetolactate synthase enzyme less sensitive to the herbicide (Hart, et al., 1993). Imidazolinone herbicide resistant corn hybrids developed from a form of resistance obtained from somatic cell selection and characterized by a similar mechanism of resistance were found to have the same yield potential as herbicide susceptible corn hybrids in the absence of herbicides (Newhouse, et al., 1991).

The F_2 "resistant" line from F_1 resistant segregates in theory contained 25% susceptible segregates as well as 25% homozygous resistant segregates. The primisulfuron treatment of the "resistant" F_2 line should have eliminated the susceptible segregates and provided a 100% resistant entry. While the comparison of resistant and susceptible lines does not enjoy the benefits of near isogenicity, this comparison involves a common genetic background assuming equivalent recombination in the F_1 plants from resistant and susceptible parents between the monogenic sulfonylurea resistant gene and any other major genes affecting agronomic characters. We know of no linkages to the resistant locus, including the *B* locus conferring annual vs. biennial behaviors.

Herbicide evaluations. The susceptible MONO-HY E4 and the resistant F_2 sugarbeet line were unaffected by nicosulfuron applied PPI at 22.5% field use rate (Table 2). In contrast, MONO-HY E4 was injured severely (98% or greater) by PPI primisulfuron and chlorimuron (15 and 25% field use rate, respectively), while the resistant F_2 was not injured. MONO-HY E4 germinated but failed to develop beyond the cotyledonary growth stage in plots treated with the latter two herbicides.

The susceptible MONO-HY E4 was injured severely (95% or greater) by all POST applications of primisulfuron and thifensulfuron (Table 3). Sulfonylurea resistant sugarbeets were unaffected by primisulfuron at 40 and 80 g ha^{-1} four WAT, and only slightly injured by thifensulfuron applied at 4 and 8 g ha^{-1}. Although the resistant sugarbeet showed 21% visible injury from primisulfuron at 160 g ha^{-1} 4 WAT, visual injury was not evident at 8 WAT.

From the research reported here we conclude that the sulfonylurea resistance trait had no adverse effect on agronomic performance of sugarbeet in the absence of the herbicides. Comparative performance of sulfonylurea resistant sugarbeet was similar to that observed with imidazolinone resistant corn (Carlson and Weis, 1990; Newhouse, et al., 1991; Roeth and Martin, 1990). The PPI herbicide study demonstrated that sulfonylurea resistant sugarbeets were resistant to

Table 2. Response of sulfonylurea resistant (TR504 X CR1-B F_2-R) and susceptible (MONO-HY E4) sugarbeet to PPI treatments of sulfonylurea herbicides.

Treatment	Rate	Visible Injury 6 WAT† MONO-HY E4	Visible Injury 6 WAT† Resistant
	g ha^{-1}	%	
Nicosulfuron	9	0	0
Primisulfuron	10	98	0
Chlorimuron	3	100	5
LSD (0.05)		6	

† WAT = weeks after treatment

Table 3. Response of resistant (TR504 X CR1-B F_2-R) and susceptible (MONO-HY E4) sugarbeet to post applications of primisulfuron and thifensulfuron.

Treatment‡	Rate	Visible Injury 4 WAT† MONO-HY E4	Visible Injury 4 WAT† Resistant
	g ha^{-1}	%	
Primisulfuron	40	95	0
	80	100	5
	160	100	21
Thifensulfuron	4	99	10
	8	100	14
LSD (0.05)		8	

† WAT = weeks after treatment

‡ All herbicides applied with NIS at 0.25% (v/v).

primisulfuron and chlorimuron soil residues that killed the susceptible sugarbeet. Sulfonylurea resistant sugarbeet was resistant to POST applications of primisulfuron and thifensulfruon at field use rates, raising the possibility of direct use of these herbicides for weed control in resistant sugarbeet.

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