CHLORSULFURON EFFECTS ON LEAF EXTENSION, ACETOLACTATE SYNTHASE ACTIVITY AND BRANCHED CHAIN AMINO ACID CONTENT OF A SUSCEPTIBLE WHEAT CULTIVAR

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SUMMARY

The effect of chlorsulfuron (15 g/ha) on leaf extension, acetolactate synthase (ALS) activity and branched chain amino acid (BCAA) content of wheat (*Triticum aestivum* L. cv. Rongotea) was examined during the first week after spraying to determine if inhibition of ALS is the primary mode of action of the herbicide. Chlorsulfuron caused decreases in leaf extension, ALS activity and BCAA content of wheat within one day of spraying. It is concluded that inhibition of ALS activity is likely to be the primary mode of action of the herbicide in this cultivar.

Keywords: *Triticum aestivum* L., sulfonylurea herbicide, chlorsulfuron, acetolactate synthase, branched chain amino acids.

INTRODUCTION

Although used widely as a selective, post-emergence herbicide on wheat (*Triticum aestivum* L.) crops in New Zealand and Australia, chlorsulfuron can reduce the ability of some cultivars to respond to additional nitrogen (N) (Bowran and Blacklow 1987; Dastgheib *et al.* 1992). The primary mode of action of the herbicide in a range of broadleaf species and maize (*Zea mays* L.) is inhibition of acetolactate synthase (ALS), the enzyme which catalyses the first common reaction in the synthesis of the branched chain amino acids (BCAA) valine, leucine and isoleucine (Ray 1989). Recently, chlorsulfuron was found to increase total free amino acid content and decrease BCAA content in wheat and maize, but the concentration required to produce these effects was two to three orders of magnitude greater for wheat (Royuela *et al.* 1991).

For the chlorsulfuron sensitive wheat cultivar Rongotea, decreased growth of sprayed plants on high nitrate (NO_3^-) supply was associated with increases in NO_3^- and reduced-N content of root and shoot (Dastgheib *et al.* 1992). It was concluded that decreased growth with chlorsulfuron was due to an inability to utilise increased reduced-N to produce dry matter. With regard to mode of action of the herbicide, increased reduced-N content at high NO_3^- may have been due to amino acid accumulation caused by decreased BCAA production. The main objective of the present study was to assess if inhibition of ALS activity is the primary mode of action of chlorsulfuron in Rongotea.

METHODS

The experiment was carried out under controlled environment conditions as described previously (Dastgheib *et al.* 1992). Seeds of Rongotea were germinated on moist paper towels in the growth cabinet. Seedlings with a shoot length of approximately 30 mm were transferred to pots 800 cm³ in volume (one plant/pot) containing a vermiculite/perlite (1:1, v/v) mixture soaked in basal nutrient solution (Andrews *et al.* 1989) containing 1 mol/m³ NO_3^- (low NO_3^-) as potassium nitrate. Plants were supplied with low NO_3^- until 1 week prior to chlorsulfuron application then transferred to nutrient solution containing either 5 mol/m³ NO_3^- (high NO_3^-) or low NO_3^- plus 4

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mol/m³ BCAA (1.33 mol/m³ of each of valine, leucine and isoleucine). The pretreatment on low NO₃⁻ ensured that plants supplied high NO₃⁻ or BCAA were at a similar stage of development at spraying. Potassium concentration in the BCAA treatment was made equal to that in the 5 mol/m³ NO₃⁻ treatment by the addition of potassium sulphate. All pots were flushed with the appropriate nutrient solution every 2 days. At the three-leaf stage, chlorsulfuron was applied at 0 or 15 g/ha as described previously (Dastgheib *et al.* 1992). Length of the youngest expanding leaf (leaf 4) was measured (Andrews *et al.* 1991) every 2 days from 1 day prior to spraying to 7 days after spraying (DAS). Acetolactate synthase activity of shoots was determined 1 and 7 DAS as described by Ray (1984) with minor modifications and expressed on a protein (Bradford 1976) basis. The content of 12 amino acids (valine, leucine, isoleucine, aspartic acid, glutamic acid, glycine, alanine, lysine, arginine, phenylalanine, tyrosine and methionine) was determined in shoots 1 and 4 DAS using HPLC. Shoot fresh weight and shoot dry weight were determined 7 DAS and 28 DAS respectively. The experimental design was a randomised complete block with five replicates. All measurements were carried out on all five replicates except for amino acid content which was determined on three replicates, two of which comprised pooled material from two replicates. The experiment was done twice. An analysis of variance was carried out on all data. All effects discussed have a probability of $P < 0.01$ and were also obtained in the repeat experiment.

RESULTS AND DISCUSSION

The objective of the present study was to assess if inhibition of ALS activity is the primary mode of action of chlorsulfuron in Rongotea wheat. In sensitive plants, chlorsulfuron can cause decreased ALS activity within one day of spraying (Ray 1989). Thus, in the present study, detailed measurements were taken from 1 DAS onwards. Leaf extension was used as a rapid indicator of the chlorsulfuron effect on growth (Bowran and Blacklow 1987).

TABLE 1: Effect of chlorsulfuron on length of leaf four 1, 3, 5, and 7 days after spraying (DAS) Rongotea wheat supplied high nitrate (NO₃⁻) or low NO₃⁻ plus branched chain amino acids (BCAA).

Nitrogen supply	Chlorsulfuron	Leaf length (mm)				
		-1 DAS	1 DAS	3 DAS	5 DAS	7 DAS
NO ₃ ⁻	Untreated	20	78	134	186	220
	Sprayed	18	60	96	134	160
BCAA	Untreated	19	76	130	182	215
	Sprayed	15	56	84	116	140
LSD		6	9	18	24	27

TABLE 2: Effect of chlorsulfuron on shoot fresh weight 7 days after spraying (DAS) and shoot dry weight 28 DAS Rongotea wheat supplied high nitrate (NO₃⁻) or low NO₃⁻ plus branched chain amino acids (BCAA).

Nitrogen supply	Fresh weight (g)		Dry weight (g)	
	Untreated	Sprayed	Untreated	Sprayed
NO ₃ ⁻	1.27	0.69	1.44	0.44
BCAA	1.04	0.70	1.35	0.65
LSD	0.24	0.10	0.46	0.14

For untreated plants during the first 7 DAS, length of leaf 4 was similar with NO₃⁻ or BCAA as main N source (Table 1). Regardless of N treatment, chlorsulfuron caused a decrease in leaf length 1 DAS. For sprayed plants during the first 7 DAS, leaf length

was as great with NO_3^- or BCAA. Also, for sprayed plants, shoot fresh weight 7 DAS was as great with NO_3^- or BCAA (Table 2). Thus in the short term, addition of BCAA to the rooting medium did not give greater protection against the herbicide (cf. Devine *et al.* 1990). In the longer term (28 DAS), shoot DW was greater with BCAA than with NO_3^- (Table 2). This effect was associated with excessive NO_3^- accumulation in plants supplied NO_3^- (Andrews *et al.* 1993).

For untreated plants 1 and 7 DAS, ALS activity was similar for plants supplied NO_3^- or BCAA (Table 3). Regardless of N treatment, ALS activity was 80-90% lower in sprayed plants than in unsprayed plants 1 DAS. Seven DAS, ALS activity was 55-75% lower in sprayed than in unsprayed plants. For untreated and sprayed plants 1 and 4 DAS, BCAA content of shoots was similar for plants supplied NO_3^- or BCAA (Table 4). Regardless of N treatment, chlorsulfuron caused an increase in total amino acid content and a decrease in valine content of shoots 1 DAS. Similar data were obtained for broadleaf plants and maize (Scheel and Casida 1985; Royuela *et al.* 1991). The finding that leaf extension and shoot ALS activity and valine content all decrease within one day of spraying with chlorsulfuron, in conjunction with previous data for other species, is strong evidence that inhibition of ALS activity is the primary mode of action of the herbicide in Rongotea. Branched chain amino acids added to the rooting medium are not translocated to the shoot in high enough quantities to counter this effect.

TABLE 3: Effect of chlorsulfuron on acetolactate synthase (ALS) activity in shoots 1 and 7 days after spraying (DAS) Rongotea wheat supplied high nitrate (NO_3^-) or low NO_3^- plus branched chain amino acids (BCAA).

Nitrogen supply	ALS activity (nmol/mg protein/h)			
	1 DAS		7 DAS	
	Untreated	Sprayed	Untreated	Sprayed
NO_3^-	191	34	318	84
BCAA	155	19	305	133
LSD	73	13	73	36

TABLE 4: Effect of chlorsulfuron on total amino acid content and valine content of shoots 1 and 4 days after spraying (DAS) Rongotea wheat supplied high nitrate (NO_3^-) or low NO_3^- plus branched chain amino acids (BCAA).

Nitrogen supply	Chlorsulfuron	Amino acid content ($\mu\text{mol/g}$ dry weight)			
		1 DAS		4 DAS	
		Total	Valine	Total	Valine
NO_3^-	Untreated	43.2	2.2	52.8	2.6
	Sprayed	59.3	1.6	58.0	1.8
BCAA	Untreated	40.1	2.5	41.4	2.4
	Sprayed	52.0	1.8	47.5	1.6
LSD		8.4	0.6	5.7	0.6

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