CHLORSULFURON EFFECTS ON NITRATE ASSIMILATION IN A SUSCEPTIBLE WHEAT CULTIVAR

F. DASTGHEIB¹, M.F. BARNES², J.D. MORTON² and M. ANDREWS¹

¹Plant Science Department and ²Department of Biochemistry and Microbiology,
P.O. Box 84, Lincoln University, Canterbury, New Zealand.

SUMMARY

Chlorsulfuron (15 g/ha) limited the capacity of wheat (*Triticum aestivum* L. cv. Rongotea) to respond to nitrate (NO₃⁻). Addition of branched chain amino acids (BCAA) which are associated with the mode of action of the herbicide partially overcame the restriction in growth. For NO₃⁻ and BCAA treatments, decreased growth with chlorsulfuron was associated with increased N content. The amino acids glutamine and glutamate were as effective as BCAA in overcoming the chlorsulfuron restriction in growth. It is concluded that (a) decreased growth with chlorsulfuron was due to an inability to utilise increased N to produce dry matter and (b) increased tolerance of wheat to chlorsulfuron with BCAA was not a specific BCAA effect.

Keywords: *Triticum aestivum* L., wheat, sulfonylurea herbicide, chlorsulfuron, nitrate assimilation.

INTRODUCTION

Chlorsulfuron is a highly selective, post-emergence herbicide of the sulfonylurea group which is used to control broadleaf weeds in cereal crops (Beyer *et al* 1988). The primary mode of action of the herbicide in a range of broadleaf species is inhibition of acetolactate synthase, an enzyme involved in the synthesis of the branched chain amino acids (BCAA) valine, leucine and isoleucine (Ray 1989). Tolerance of chlorsulfuron by cereals appears to be related to its rapid metabolism to non-toxic products (Sweetser *et al* 1982). Although used widely on wheat (*Triticum aestivum* L.) crops in New Zealand and Australia, chlorsulfuron has been shown to reduce the ability of some cultivars to respond to additional nitrogen (N) (Bowran and Blacklow 1987; Dastgheib *et al* 1989). In the case of the susceptible cultivar Rongotea, addition of valine, leucine and isoleucine to the rooting medium partially overcame this restriction in growth (Dastgheib *et al* 1989).

The objectives of the present study were to determine if chlorsulfuron effects on the response of Rongotea to additional N are due to reduced nitrate (NO₃⁻) uptake and/or assimilation and if effects of valine, leucine and isoleucine are specific BCAA effects.

METHODS

Two experiments are described. Both were carried out under controlled environment conditions. The photoperiod was 12 h and day/night temperatures 20/10°C. Photon flux density was approximately 300 µmol photons/m²/s at the pot surface. In Experiment 1, 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 800 ml volume pots (one plant/pot) containing a vermiculite/perlite (1:1) mixture soaked in basal nutrient solution (Andrews *et al* 1989) containing the appropriate N treatment. There were four N treatments initially: low (1 mol/m³) and high (5 mol/m³) NO₃⁻ with 0 or 1 mol/m³ of each of the three BCAA valine, leucine and isoleucine.

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The nitrate was added as potassium nitrate. In all treatments, potassium was made equal to that at high NO_3^- by the addition of potassium sulphate. All pots were flushed with the appropriate nutrient solution every 2 days. At the $3\frac{1}{2}$ leaf stage, chlorsulfuron was applied at 0 or 15 g/ha using a CO_2 pressurised sprayer system fitted with "Teejet" 8001 nozzles which gave 250 litres/ha spray solution at a pressure of 275 kPa. Citowett at 0.025% was added to the spray solution. Immediately after spraying, some plants supplied with low NO_3^- were transferred to high NO_3^- , giving a fifth N treatment. Plants were harvested 45 days after spraying, divided into root and shoot then dried at 70°C for 2 days. Dried material was weighed then ground and an aqueous extract of a 20–50 mg sample analysed for NO_3^- and nitrite (NO_2^-) as described in Mackereth *et al* (1978) and ammonium (NH_4^+) as described in Baethgen and Alley (1989). Total N was determined in 100 mg samples by Kjeldahl digestion. Reduced-N was determined as the difference between total N and $\text{NO}_3^- + \text{NO}_2^-$ -N.

In Experiment 2, plants supplied low NO_3^- were taken to the $3\frac{1}{2}$ leaf stage and sprayed with 0 or 15 g/ha chlorsulfuron as in Experiment 1. Four N treatments were introduced 1 day prior to spraying. These were low NO_3^- , high NO_3^- , low NO_3^- plus 4 mol/m³ N as BCAA (1.33 mol/m³ of each) or low NO_3^- plus 4 mol/m³ N as glutamine and glutamate (GLN/GLU, 1.33 mol/m³ of each). Shoot dry weight was determined as above, 37 days after spraying.

Both experiments were of randomised complete block design. Five replicates from all treatments were used for all measurements. All experiments were carried out twice. All effects discussed have a probability of $P < 0.01$ and were obtained in repeat experiments.

RESULTS AND DISCUSSION

For all N treatments in Experiment 1, total plant dry weight was greater for unsprayed plants than for sprayed plants (Table 1). As reported previously (Dastgheib *et al* 1989), chlorsulfuron limited the ability of *Rongotea* to respond to NO_3^- . Plants transferred from low to high NO_3^- at spraying were similar to plants given high NO_3^- throughout. This shows that low NO_3^- prior to spraying does not result in increased tolerance of the herbicide. Addition of BCAA gave greater growth of sprayed plants at low or high NO_3^- but did not give complete protection against the herbicide (Table 1). The objectives of Experiment 1 were to determine if chlorsulfuron effects on the response of *Rongotea* to additional N are due to reduced NO_3^- uptake and/or assimilation. At high NO_3^- , total plant N was approximately 30% lower in sprayed than in unsprayed plants (Table 1), thus, less NO_3^- was taken up. Total plant reduced -N at high NO_3^- was approximately 40% lower in sprayed plants than in unsprayed plants (Table 1). For all N treatments, NO_2^- was undetectable and NH_4^+ constituted 1–2% of reduced -N in root and shoot (level was unaffected by chlorsulfuron) therefore, at high NO_3^- , chlorsulfuron caused a 40% decrease in NO_3^- assimilation. This decrease is likely to have been part of the cause of the decrease in dry weight at high NO_3^- . However, for sprayed plants, total plant reduced -N was similar with the high NO_3^- and low $\text{NO}_3^- + \text{BCAA}$ treatments but total plant dry weight was twice as great with the latter (Table 1). Therefore the main factor limiting growth of sprayed plants at high NO_3^- was not decreased NO_3^- uptake or assimilation but was an inability to utilise increased reduced-N to produce dry matter. This resulted in increased reduced-N content (mg/g dry weight) in both root and shoot (Table 2). With regard to mode of action of the herbicide, increased reduced-N content at high NO_3^- in comparison with low $\text{NO}_3^- + \text{BCAA}$ may have been due to amino acid accumulation caused by decreased BCAA production and hence decreased protein synthesis. Chlorsulfuron also caused an increase in reduced-N content of root and shoot of plants supplied BCAA (Tables 1, 2). This may have been due to inadequate BCAA reaching the tissues or other effects of the herbicide such as decreased cell division and hence decreased sink activity, or inhibition of translocation processes between mature tissues and growing regions (Beyer *et al* 1988; Bestman *et al* 1990).

TABLE 1: Effect of nitrate (NO₃⁻) supply and branched chain amino acids (BCAA) on dry weight, total nitrogen (N) and reduced-N per plant of *Rongotea* wheat sprayed with chlorsulfuron. Values in parenthesis are S.E.M.

N treatment	Dry weight (g)		Total N (mg)		Reduced-N (mg)	
	Control	Sprayed	Control	Sprayed	Control	Sprayed
Low NO ₃ ⁻	2.24 (0.09)	1.44 (0.04)	22.2 (1.1)	20.6 (0.5)	21.8 (0.9)	20.4 (0.5)
High NO ₃ ⁻	5.10 (0.44)	2.10 (0.30)	114.4 (5.2)	78.0 (5.4)	112.6 (5.7)	67.2 (8.7)
¹ Low → High NO ₃ ⁻	5.15 (0.18)	1.47 (0.23)	—	—	—	—
Low NO ₃ ⁻ + BCAA	5.72 (0.23)	4.63 (0.37)	83.9 (5.0)	76.3 (5.0)	83.7 (5.0)	76.2 (5.0)
High NO ₃ ⁻ + BCAA	8.31 (0.54)	3.67 (0.45)	171.0 (8.6)	130.3 (7.7)	169.8 (8.9)	113.0 (10.3)

¹Switchover from low to high NO₃⁻ occurred immediately after spraying. Least significant differences for any comparisons may be calculated as: [$\sqrt{\text{SEM}_1^2 + \text{SEM}_2^2}$].

TABLE 2: Effect of nitrate (NO₃⁻) supply and branched chain amino acids (BCAA) on reduced-nitrogen (N) and NO₃⁻ content of shoot and root of *Rongotea* wheat sprayed with chlorsulfuron. Values in parenthesis are S.E.M.

N treatment	Reduced-N mg/g dry weight)		NO ₃ ⁻ -N (mg/g dry weight)	
	Control	Sprayed	Control	Sprayed
Shoot				
Low NO ₃ ⁻	11.2 (0.3)	17.6 (0.7)	0.05 (0.01)	0.05 (0.01)
High NO ₃ ⁻	28.4 (0.7)	37.8 (1.4)	0.26 (0.12)	9.49 (2.95)
Low NO ₃ ⁻ + BCAA	18.1 (1.1)	21.3 (0.8)	0.05 (0.02)	0.02 (0.01)
High NO ₃ ⁻ + BCAA	26.7 (1.3)	40.0 (2.1)	0.12 (0.04)	5.88 (1.73)
Root				
Low NO ₃ ⁻	8.6 (0.1)	11.3 (0.4)	0.03 (0.01)	0.17 (0.02)
High NO ₃ ⁻	13.9 (0.7)	15.9 (0.6)	0.60 (0.21)	8.24 (2.39)
Low NO ₃ ⁻ + BCAA	10.3 (0.5)	11.2 (0.8)	0.05 (0.02)	0.03 (0.02)
High NO ₃ ⁻ + BCAA	11.1 (0.2)	15.6 (0.7)	0.20 (0.12)	4.61 (0.94)

For LSD calculation see Table 1.

Within the plant, NO₃⁻ is reduced to NH₄⁺ before being assimilated into amino acids: GLN and GLU are considered to be the primary products of NH₄⁺ assimilation (Andrews 1986, and references therein). If increased tolerance of chlorsulfuron with additional valine, leucine and isoleucine in comparison with NO₃⁻ is a specific BCAA effect, then addition of GLN/GLU to sprayed plants should have no effect. This was tested in Experiment 2. For all N treatments in Experiment 2, shoot dry weight was greater for unsprayed plants than for sprayed plants (Table 3). Relative to controls, the effect of chlorsulfuron on shoot dry weight was greater at high NO₃⁻ than at low NO₃⁻ or low NO₃⁻ + BCAA as in Experiment 1. For control and sprayed plants, shoot dry weight was as great with GLN/GLU as with BCAA. This indicates that increased tolerance of *Rongotea* to chlorsulfuron with additional BCAA is not a specific BCAA effect. If this is the case, it must then be asked why reduced-N produced via NO₃⁻ assimilation cannot be utilised to produce dry matter in sprayed plants. One possible reason is that NO₃⁻ accumulation (Table 2) inhibits growth (Andrews *et al* 1992). Further work is required to determine the relationships between external and internal NO₃⁻ concentration and growth of *Rongotea*. It is also important to determine if decreased growth of *Rongotea* with chlorsulfuron correlates with decreased acetolactase synthase activity and decreased levels of BCAA, and if BCAA supplied via the nutrient medium are translocated to the shoot.

TABLE 3: Effect of nitrate (NO₃⁻) supply, branched chain amino acids (BCAA) and glutamine plus glutamate (GLN/GLU) on dry weight of shoot of Rongotea wheat sprayed with chlorsulfuron. Values in parenthesis are S.E.M.

N treatment	Dry weight (g)	
	Control	Sprayed
Low NO ₃ ⁻	0.93 (0.06)	0.75 (0.09)
High NO ₃ ⁻	2.24 (0.33)	0.67 (0.13)
Low NO ₃ ⁻ + BCAA	2.40 (0.08)	1.50 (0.21)
Low NO ₃ ⁻ + GLN/GLU	2.75 (0.08)	1.61 (0.12)

For LSD calculation see Table 1.

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