Effects of isoxaflutole on the growth and nodulation of chickpea (*Cicer arietinum*) under different soil nitrogen rates

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Summary The growth and nodulation response of one isoxaflutole tolerant and one sensitive chickpea cultivar was assessed with differing soil nitrate levels and isoxaflutole rates. The sensitive cultivar was more susceptible to isoxaflutole damage with increasing herbicide rate and nitrate levels but detrimental effects of isoxaflutole occurred with both cultivars. Some damage was observed with the tolerant cultivar at the highest herbicide rate and nitrate levels. In general, isoxaflutole decreased shoot and root dry weight, nodule number, and nodule dry weight of the sensitive cultivar. Herbicide applied at the recommended rate reduced nodule dry weight of chickpea by 51% at 6mM nitrate level. Nodule number of the tolerant cultivar declined by 30% at the recommended herbicide rate. Nodule dry weight declined with increasing nitrate levels without herbicide application, and addition of herbicide caused a further reduction.

Keywords Isoxaflutole, soil nitrate, *Cicer arietinum*, phytotoxicity, growth, nodulation.

INTRODUCTION

In Australia, isoxaflutole at 75 g a.i. ha^{-1} is registered for the control of several broadleaf weeds in chickpea. However, there have been records of chickpea crop damage due to isoxaflutole at an application rate of less than 75 g a.i. ha^{-1} that may result in a yield penalty (Felton *et al.* 2004).

Residual levels of some herbicides have also been found to inhibit nodulation and N fixation by legumes and may have negative effects on the N balance of legume-cereal rotations. While the symbiotic nitrogen (N) fixation in legumes associated with rhizobia is inhibited by nitrate N applied to the soil (Daimon and Yoshioka 2001), the effects of different soil nitrate levels influencing the degree of isoxaflutole injury to chickpea growth and nodulation are not well documented. Therefore, the objectives of this research were to assess the effect of isoxaflutole on the growth and nodulation of two chickpea cultivars (isoxaflutole tolerant and sensitive) as well as possible interactions at varying nitrate levels.

MATERIALS AND METHODS

The experiment was conducted under a glasshouse conditions (25°C day and 15°C night) at the University of New England, Armidale, New South Wales, Australia and was arranged as a complete randomised block design with 5 × 2 × 3 factorial combinations: five NO_3^- concentrations (0, 0.75, 1.5, 3.0 and 6.0mM), two chickpea cultivars (the isoxaflutole tolerant cultivar – Kyabra, and the sensitive cultivar – Yorker) and three isoxaflutole rates (0, 75, and 300 g a.i. ha^{-1}). There were four replications.

Five seeds of the chickpea cultivar were grown in a free-draining sand system without any N content in a 14 cm diameter plastic pot. The seeds were inoculated with the *Rhizobium* culture before sowing. Isoxaflutole was applied at one day after sowing (DAS) using a gas operated boom-sprayer. At 7 DAS, plants were thinned to three per pot. Pots were flushed with 150 mL of distilled water or 150 mL of nutrient solution on alternate days after sowing so that regular flushing of solution through the pots occurred. The nutrient solution contained 0, 0.75, 1.5, 3.0 and 6.0mM NO_3^- as KNO_3. Other macro and micro nutrients used as a basal treatment were: 0.25mM $CaCl_2$, 0.125mM K_2SO_4, 0.5mM $MgSO_4.7H_2O$, 0.13mM KH_2PO_4, 0.13mM K_2HPO_4, 22.4µM Fe-EDDHA, 1.2µM $MnSO_4.H_2O$, 0.08µM $ZnSO_4.7\ H_2O$, 0.05µM $CuSO_4.5\ H_2O$, 0.002µM $CoSO_4.7H_2O$, and 0.02µM $Na_2MoO.2H_2O$.

Plants were harvested 35 days after herbicide treatment and nodule number and dry weights of shoots, roots and nodules were measured. From the ANOVA, standard errors were calculated in order to compare means. The treatment combination means presented for a variable are based on the highest order of factorial combination that is significant in the ANOVA. Where this is less than the maximum factorial combination, the tables have been generated by pooling the data across the non significant factors.

RESULTS

The addition of nitrate did not significantly affect shoot dry weight of the chickpea. Isoxaflutole at the recommended rate significantly decreased chickpea shoot dry weight by 18% in the tolerant cultivar (Kyabra) and 29% in the sensitive cultivar (Yorker) (Table 1). Root dry weight of Yorker was reduced by 48% with the recommended herbicide rate while Kyabra had a 24% reduction (Table 2). Root dry weight of Kyabra was greater under 0 and 0.75mM nitrate levels but was reduced significantly with increasing nitrate levels with a 46% reduction at 6.0mM nitrate compared with those grown without nitrate. Root dry weight of Yorker was unaffected with the increasing level of nitrate (up to 3.0mM level) but was also significantly reduced (29%) with 6.0mM nitrate level as compared with the nil nitrate treatment.

Nodule number was similarly reduced with the recommended herbicide rate with Kyabra (30%) and with Yorker (32%) though at 300 g a.i. ha^{-1} herbicide rate nodule number was reduced more in Yorker than Kyabra (Table 3). Nodule number across chickpea culitvars generally declined with increasing nitrate but not significantly until nitrate level reached 1.5mM. Chickpea nodule number was decreased by 43 and 61% with 3.0 and 6.0mM nitrate levels respectively compared with the zero nitrate treatment. The addition of increasing concentrations of nitrate significantly reduced nodule dry weight of both chickpea cultivars by similar percentages (Table 4). Isoxaflutole at the recommended rate reduced nodule dry weight of chickpea by 37% (without nitrate), 43% (0.75mM), 40% (1.5mM), 43% (3.0mM) and 51% (6.0mM). Nitrate and herbicide addition combined reduced nodule dry weight more than either factor alone.

DISCUSSION

The rate of herbicide metabolism and deactivation is likely to have been a major factor in determining the differential tolerances of chickpea cultivars. Pallett *et al.* (1998) identified isoxaflutole metabolism as the primary basis for differential selectivity between the tolerant species *Zea mays* and a susceptible species *Abutilon theophrasti*. More shoot dry weight was reduced in the sensitive cultivar compared with the

Table 1. Effect of isoxaflutole on the shoot dry weight of a tolerant (Kyabra) and sensitive (Yorker) chickpea cultivar.

Isoxaflutole (g a.i. ha^{-1})	Shoot dry weight (g pot^{-1}) Kyabra	Yorker
0	0.41	0.38
75	0.34	0.27
300	0.32	0.21
SE	0.018	

Table 2. Effect of isoxaflutole and nitrate on the root dry weight of a tolerant (Kyabra) and sensitive (Yorker) chickpea cultivar.

	Root dry weight (g pot^{-1})	
	Kyabra	Yorker
Isoxaflutole (g a.i. ha^{-1})		
0	0.21	0.19
75	0.16	0.10
300	0.14	0.08
SE	0.011	
Nitrate (mM)		
0	0.22	0.14
0.75	0.20	0.14
1.5	0.17	0.13
3.0	0.15	0.11
6.0	0.12	0.10
SE	0.015	

Table 3. Effect of isoxaflutole and nitrate on the nodule number of chickpea.

	Nodule number	
	Kyabra	Yorker
Isoxaflutole (g a.i. ha^{-1})		
0	26.5	26.1
75	18.9	17.9
300	16.9	10.9
SE	1.73	
Nitrate (mM)		
0	26.0	
0.75	24.0	
1.5	22.0	
3.0	15.0	
6.0	10.3	
SE	1.58	

Table 4. Effect of nitrate and isoxaflutole on the nodule dry weight of chickpea.

	Nodule dry weight (mg pot^{-1})				
	Nitrate (mM)				
	0	0.75	1.5	3.0	6.0
Cultivar					
Kyabra	29.7	22.2	18.4	7.6	3.2
Yorker	20.2	13.9	11.8	5.7	3.0
SE			1.57		
Isoxaflutole (g a.i. ha^{-1})					
0	35.5	28.1	21.3	10.1	5.1
75	22.4	16.1	12.6	5.8	2.5
300	17.0	9.9	11.3	4.0	1.7
SE			1.92		

tolerant cultivar with increasing herbicide rate. We also found more root dry weight reduction than shoot dry weight under increased nitrate levels and herbicide rates. Roots came into direct contact with the soil-applied isoxaflutole and therefore probably exhibited effects earlier than the shoots. The addition of increased levels of nitrate failed to alleviate the shoot dry weight loss experienced from the application of isoxaflutole. The reduction in root dry weight in the presence of isoxaflutole may have decreased the ability of chickpea to absorb nutrients and contributed to the lack of response to nitrate in the presence of isoxaflutole. The reduction in root dry weight in response to the presence of isoxaflutole was enhanced when nitrate was added at higher concentrations. This further reduction may be due to an accumulation of carbohydrates and free amino acids, along with an increase in fermentation and a decrease in respiration, leading to growth inhibition (Gaston *et al.* 2002). Anderson *et al.* (2004) found a reduction in chickpea root dry weight from fertiliser N and another soil applied herbicide, chlorsulfuron.

Isoxaflutole reduced the number of nodules of both cultivars. Increasing levels of nitrate also decreased the overall nodule numbers in chickpea. Nodule dry weight of both cultivars was decreased with increasing nitrate levels, with or without isoxaflutole. The lower number of nodules in the presence of isoxaflutole suggests an impedance of nodule formation, whereas the low nodule weight points to an effect on nodule development or maintenance. Anderson *et al.* (2004) found that the addition of N fertiliser reduced the nodule weight of chickpea and the magnitude of the reduction was greater with the presence of chlorsulfuron in the soil. Kumar *et al.* (1981) observed a drastic reduction in nodulation of chickpea when simazine was applied to the soil surface at 1.6 and 3.2 kg ha^{-1}. Nodulation of chickpeas is known to decrease with increasing levels of soil inorganic N (Jessop *et al.* 1984) and nodulation *per se* can be impaired in the presence of nitrate.

Wheat (*Triticum aestivum*) cropping systems in Australia rely strongly on legume fixed N for sustained yields. If grain legumes are to replace a part of the pasture ley phase, chickpeas must prove their capacity to enhance soil N status under a range of inherent soil N levels. The results of this study suggest that higher nitrate levels coupled with isoxaflutole had a detrimental effect on the general growth and nodulation of both chickpea cultivars. But the response of the isoxaflutole tolerant cultivar was better than the sensitive one for at least some growth parameters. For effective weed control in chickpea using isoxaflutole under different soil nitrate levels, tolerant genotypes will need to be selected to incur less crop injury and better N economy for the succeeding cereal crops.

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REFERENCES

Anderson, A., Baldock, J.A., Rogers, S.L., Bellotti, W. and Gill, G.S. (2004). Influence of chlorsulfuron on rhizobial growth, nodule formation, and nitrogen fixation with chickpea. *Australian Journal of Agricultural Research* 55, 1059-70.

Daimon, H. and Yoshioka, M. (2001). Response of root nodule formation and nitrogen fixation activity to nitrate in a split-root system in pea nut (*Arachis hypogaea* L.). *Journal of Agronomy and Crop Science* 187, 89-95.

Felton, W.L., Knights, T.D., Haigh, B.M. and Hardsen, S. (2004). Tolerance of chickpea to isoxaflutole. Proceedings of the 14th Australian Weeds Conference, eds B.M. Sindel and S.B. Johnson, pp. 257-60. (Weed Management Society of South Australia, Adelaide).

Gaston, S., Zabalza, A., Gonzalez, E.M., Arrese-Igor, C., Aparicio-Tejo, P.M. and Royuela, M. (2002). Imazethapyr, an inhibitor to the branched-chain amino acid biosynthesis, induces aerobic fermentation in pea plants. *Physiologia Plantarum* 114, 524-32.

Jessop, R.S., Hetherington, S.J. and Hoult, E.H. (1984). The effect of soil nitrate on the growth, nodulation and nitrogen fixation of chickpeas (*Cicer arietinum*). *Plant and Soil* 82, 205-14.

Kumar, S., Pahwa, S.K., Promila, K. and Sharma, H.R. (1981). Effect of simazine and prometryne on the growth and nodulation of chickpea (*Cicer arietinum* L.). *Journal of Agricultural Science* 97, 663-8.

Pallett, K.E., Little, J.P., Sheekey, M. and Veerasekharan, P. (1998). The mode of action of isoxaflutole I. Physiological effects, metabolism, and selectivity. *Pesticide Biochemistry and Physiology* 62, 113-24.