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Investigating the growth of imidazolinone-tolerant chickpea

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Key points

- This study assessed the growth of an imidazolonine (imi)-tolerant chickpea line being developed by Chickpea Breeding Australia (CBA) under controlled conditions at Tamworth in 2020.
- Growth and root nodulation of imi-tolerant chickpea was similar to that of PBA Seamer⁽ᴰ⁾ in control soil.
- PBA Seamer⁽ᴰ⁾ growth was significantly reduced in the presence of imazapic, whereas imi-tolerant chickpea growth was only significantly reduced where plants were grown in soil where imazapic was applied at the high rate of 72 g/ha.
- Results from this experiment suggested that the imi-tolerant chickpea line was tolerant of imazapic when the herbicide was applied at the recommended rate of 36 g/ha.

Introduction

Chickpea is a key break/rotational crop in the cereal-dominated northern grains region (NGR) of northern NSW and southern/central QLD (Aslam et al. 2003). The decision to grow a break crop is based on a number of factors including the profitability of the crop, cereal disease pressure and herbicide resistance, with chickpea capable of providing a number of benefits in crop rotation (Herridge 2009). In recent years, the demand for chickpea from the sub-continent has seen an increasing number of growers in the NGR sow chickpea (mainly desi type). Chickpea production has steadily risen, with a record production year in 2016. Chickpea production fell between 2017 and 2019, due to drought conditions.

Group 2 herbicides inhibit the acetolactate synthase (ALS) enzyme, which depletes the key branched-chain amino acids required for plant growth (Cobb and Reade 2010).

Group 2 herbicides are classed into 4 groups:

1. imidazolinones
2. sulfonylureas
3. pyrimidinylthiobenzoates
4. triazolopyrimidines.

Group 2 herbicide use is increasing across the NGR to manage problem grasses and broadleaf weeds. For example, applying Group 2 herbicides such as Flame®, an imi herbicide, in fallows is now common practice to help control annual summer grass weeds such as feathertop Rhodes grass and awnless barnyard grass. The plant-back period for Flame® is approximately 4 months for chickpea, with recent research suggesting this could be closer to 8 months.

Plantback periods can be lengthened or shortened by:

- soil type (e.g. clay or sand, acid or alkaline)
- environmental factors (e.g. moisture and temperature)
- microbial activity.

Soil type is a key driver of herbicide persistence, with imidazolinone herbicides generally more persistent in acidic soils. Growers must pay close attention to these soil parameters as Group 2 herbicide residues can be extremely damaging to sensitive crops, including chickpea.

The South Australian Research and Development Institute (SARDI) conducted initial evaluations of chickpea lines with desirable herbicide-tolerant traits. Following the identification of these traits, CBA is currently developing imi-tolerant desi chickpea lines. This 2020 pilot study contributed towards early studies that assessed the growth of select (derived) material – CBA2061 (Croser et al. 2021). The aim of this experiment was to assess the growth of this material line in the presence of imazapic compared with the commercial variety PBA Seamer⫽. The results from this work were to ideally generate proof of concept data that would contribute to commercialising the imi-tolerant material. The key characteristics assessed included root nodulation and plant biomass when plants were grown in soil where imazapic was applied at a number of rates. The chickpea plants were grown for 15 weeks under controlled conditions.

Methodology

Pot experiment set up and treatments

Imi-tolerant chickpea seed was obtained from CBA, Tamworth, NSW.

All seed was inoculated with *Mezorhizobium ciceri* (CC1192), the commercial Group N rhizobial strain.

The imidazaolinone herbicide Flame®, 240 g/L imazapic, was used.

PBA Seamer⫽ and the imi-tolerant chickpea material had 4 different treatments applied:

1. control, no herbicide applied to the soil
2. imazapic applied to the soil at 36 g/ha, added at the start of the experiment
3. imazapic applied to the soil at 72 g/ha, added at the start of the experiment
4. no herbicide applied to the soil at the start of the experiment, imazapic applied to the soil at 36 g/ha 5 weeks post-planting (Table 1).

Treatment 4 was an unconventional application method for imazapic and was used as an experimental treatment to test how tolerant the imi-tolerant chickpea material was to an over the top (OTT) spray treatment. Imazapic is not registered for use in this manner.

Table 1 Treatments established at Tamworth.

Treatment	Product (Flame®) application rate, (mL/ha)	Concentration of active (imazapic) applied (g/ha)	Chickpea
1	0	0	PBA Seamer
			Imi-tolerant CBA2061
2	150 commercially recommended rate	36	PBA Seamer
			Imi-tolerant CBA2061
3	300	72	PBA Seamer
			Imi-tolerant CBA2061
4	150	36 OTT, over the top	PBA Seamer
			Imi-tolerant CBA2061

- Plants were grown in 175 mm squat plant pots of approximately 2.1 L in volume.
- One plant per pot, four replicates of each treatment.
- Pots were maintained for 15 weeks within a glasshouse facility at the Tamworth Agricultural Institute (31° 15′ 00.38″ S, 150° 96′ 61.47″ E), Tamworth NSW 2340, with a maximum temperature of 25 °C.
- For treatment 2 and treatment 3, herbicide was applied once at the start of the experiment. Pots were watered to capacity with a volume of imazapic that the equivalent area of soil would receive in the paddock to obtain the targeted application rate.
- For treatment 4, imazapic was applied at 5 weeks post-planting using a boom spray over the plants. Plants were at the 5–6 node growth stage.
- All pots were hand-watered weekly to maintain growth.

Soil type and nutrition

Dark earth (vertosol).

The soil used in the pots was collected from the NSW DPI Liverpool Plains Field Station at Breeza. The soil represented a 'typical' cropping soil from the district and consisted predominantly of surface soil (0–10 cm). The collected soil was passed through a 2 mm sieve and analysed for starting nutrition (Table 2).

Table 2 Characteristics of soil used in pot experiment.

Soil characteristic	Value
Ammonium nitrogen (mg/kg)	8
Nitrate nitrogen (mg/kg)	54
Phosphorus, Colwell (mg/kg)	40
Organic carbon (%)	1.24
Potassium, Colwell (mg/kg)	509
pH_{Ca}	7.5

Assessment

Root nodulation

- Eight weeks post-planting, plant roots were evaluated for nodulation using scores of between 0 (no nodulation) and 8 (extremely abundant nodulation).
- Scores measured nodule number, size, location and activity (Howieson and Dilworth 2016). Scores were used as an indicator of the interaction between the chickpea plants and rhizobia.
- Additional pots were established at the start of the experiment that were destructively harvested for root nodulation.

Plant biomass

- Fifteen weeks post-planting, individual plants were removed from each pot and excess soil washed off the roots. Plant material was then dried at 40 °C for 72 hours and weighed to establish plant biomass, which was used as an indicator of plant health.

Herbicide levels in soil

- Triple quadrapole mass spectrometry was used to analyse herbicide levels in the soil.
- A day (24 hours) after pots were established, a 5 g soil sample was taken from each pot and immediately stored at –20 °C before analysis.
- Samples were taken using a mini soil corer 100 mm long.
- Soil samples taken from pots of the same treatment were combined into a single sample for analysis.
- Herbicide levels from the treatment 4 soil were also further analysed a day after the OTT herbicide application. Here, all samples (from both PBA Seamer⁽ᶦ⁾ and imi-tolerant pots) were combined into a single sample for analysis.

Analysis

- Plant biomass was analysed via analysis of variance (ANOVA) using Genstat for Windows (Genstat 2018).
- Treatment means were compared using least significant difference (l.s.d.) with significant differences accepted at *P*<0.05.

Results

Imazapic levels in soils

- The imazapic concentration in soil increased with increasing herbicide rates (Table 3).
- Imazapic was not detected in the control soil.

- An average concentration of 57.5 ng/g imazapic was recorded where herbicide was applied at the recommended commercial rate of 150 mL/ha at the start of the experiment (treatment 2).
- The OTT imazapic application (treatment 4) resulted in the highest level of herbicide from any treatment. This type of imazapic application most likely resulted in the herbicide not being evenly distributed throughout the soil, compared with the application method used in treatments 2 and 3.

Table 3 Imazapic concentration in soils from different treatments.

Treatment	Imazapic application rate (g/ha)	Chickpea	Imazapic concentration in soil (ng/g)
1	0	PBA Seamer	0.0
		Imi-tolerant CBA2061	0.0
2	36	PBA Seamer	56.0
		Imi-tolerant CBA2061	58.9
3	72	PBA Seamer	194.0
		Imi-tolerant CBA2061	175.7
4	36, OTT	PBA Seamer	0.0 (Herbicide level tested before the OTT application)
		Imi-tolerant CBA2061	0.6 (Herbicide level tested before OTT application)
		PBA Seamer Imi-tolerant CBA2061	291.0 (Herbicide level tested after OTT application)

Root nodulation

- Root nodules were recorded on all plants except for the PBA Seamer$^{\Phi}$ plants grown in soil treated with imazapic at 36 g/ha and 72 g/ha (Table 4).
- The highest nodulation score recorded was 3.
- Root nodulation of the imi-tolerant plants was equal to or higher than PBA Seamer$^{\Phi}$ root nodulation plants in every treatment.

Table 4 Chickpea root nodulation for each treatment.

Treatment	Imazapic application rate (g/ha)	Chickpea	Nodulation score
1	0	PBA Seamer	3
		Imi-tolerant CBA2061	3
2	36	PBA Seamer	0
		Imi-tolerant CBA2061	3
3	72	PBA Seamer	0
		Imi-tolerant CBA2061	2
4	36, OTT	PBA Seamer	2
		Imi-tolerant CBA2061	3

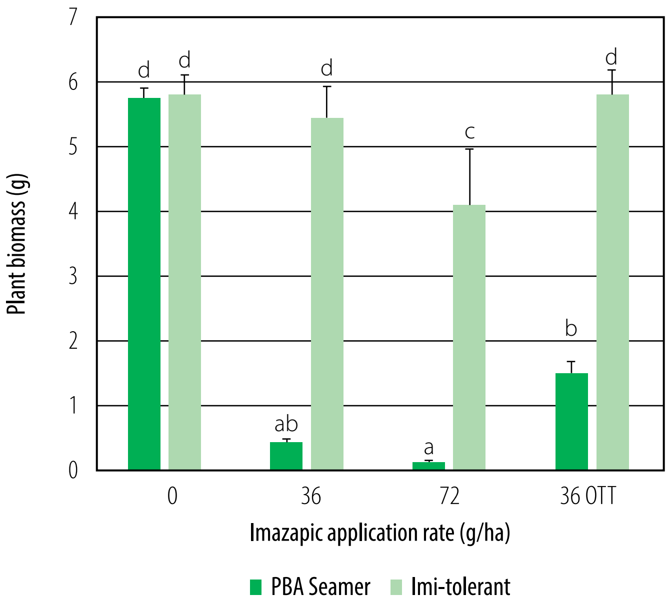
A score of 0 represents no nodulation and a score of 4 represents adequate nodulation.

Plant growth

- Visual differences in plant shoot biomass were obvious between treatments in early plant growth, particularly with PBA Seamer⁽ᵇ⁾.
- Eight weeks post-planting, there was a clear biomass reduction in PBA Seamer⁽ᵇ⁾ plants grown in soil treated with imazapic (treatments 2 and 3) compared with the control plants (Figure 1).
- Differences in biomass were not obvious 8 weeks post-planting when comparing imi-tolerant plants grown across all treatments (Figure 2).
- Fifteen weeks post-planting, the average biomass of PBA Seamer⁽ᵇ⁾ and imi-tolerant plants grown in the control soil (treatment 1) was not significantly different (Figure 3).
- A significant reduction in biomass was recorded when PBA Seamer⁽ᵇ⁾ plants were grown in soil where imazapic was applied at any rate and at any stage (treatments 2, 3 and 4).
- A significant reduction in imi-tolerant plant biomass was only recorded in Treatment 3 where imazapic was applied to the soil at 72 g/ha (Figure 3).

Figure 1 Eight-week-old PBA Seamer⁽ᵇ⁾ plants grown in soil treated with 0 g/ha (A), 36 g/ha (B) and 72 g/ha (C) of imazapic. Also pictured (D) is a plant grown in unamended soil for 5 weeks before an OTT imazapic at 36 g/ha application.

Figure 2 Eight-week-old imi-tolerant plants grown in soil treated with 0 g/ha (A), 36 g/ha (B) and 72 g/ha (C) of imazapic. Also pictured (D) is a plant grown in unamended soil for 5 weeks before an OTT imazapic at 36 g/ha application.



Letters indicate significant differences between treatments (P<0.05, l.s.d. = 1.201).

Figure 3 Average plant weight of PBA Seamer⁽ᵇ⁾ and imi-tolerant chickpea after 15 weeks of growth.

Winter crops

Conclusions and future work

The aim of this pot experiment was to investigate the growth of imi-tolerant chickpea material being developed by CBA. The experiment analysed the imi-tolerant material growth compared with PBA Seamer⁽ᶲ⁾, as well as imi-tolerant material growth in the presence of imazapic at specific rates. Plant biomass was the main measurement used to determine plant growth. The experiment was conducted under controlled conditions.

After 15 weeks of growth, the imi-tolerant plants produced biomass similar to that of PBA Seamer⁽ᶲ⁾ (Figure 3). Significant plant biomass reductions were measured where PBA Seamer⁽ᶲ⁾ was grown in soil where imazapic was applied, either at the start of the experiment or with an OTT application after plant emergence. Imazapic impeded PBA Seamer⁽ᶲ⁾ growth; imazapic inhibits key amino acid production, which is necessary for protein synthesis and growth. There was a significant growth reduction in the imi-tolerant material where plants were grown in soil where imazapic was applied at double the commercially recommended rate (72 g/ha, treatment 3).

The experiment showed that there is no negative growth effect on plants containing the imi-tolerant trait when compared with conventional PBA Seamer⁽ᶲ⁾ growth, under the controlled conditions used in this experiment. The results from this pot experiment suggests that the imi-tolerant material tolerated imazapic up to the commercially recommended rate of 36 g/ha, equivalent to 150 mL/ha Flame®.

The root nodulation analysis also indicated that the imi-tolerant trait has no effect on the symbiotic relationship that roots form with rhizobia. Imi-tolerant and PBA Seamer⁽ᶲ⁾ root nodulation were similar. Nodulations scores in the control soil were slightly below average for PBA Seamer⁽ᶲ⁾ and imi-tolerant chickpea, but the soil used in this study did have relatively high starting nitrate levels.

Root nodulation was reduced in the presence of imazapic, particularly in PBA Seamer⁽ᶲ⁾. This reduction in nodulation corresponded to the reduced below-ground biomass where plants were grown in imazapic-treated soil (data not shown). The herbicide itself could have potentially reduced rhizobial numbers in the soil as well.

This experiment was conducted under controlled conditions over a limited period. Though valuable data was collected as part of the initial imi-tolerant material studies, future studies should include a suitably measured imi-tolerant material growth under field conditions over an entire cropping season. The potential tolerance to other Group 2 herbicides should also be tested.

CBA is continuing to study the imi-tolerant material. Such material has the potential to be integrated into farming systems in the NGR and could provide growers with increased chickpea sowing options, particularly around using Group 2 herbicides that provide growers with an important tool to help control problem weeds such as feathertop Rhodes grass.

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