Improving herbicide tolerance in pulses to support the diversification of Australian crop rotations

Dili Mao [1], Jeff Paull [2], Chris Preston [2], Chris Dyson [3], Shi Ying Yang [2], Larn McMurray [1]

[1] South Australian Research and Development Institute, 9 Old North Road Clare, SA, 5453
[2] University of Adelaide, Waite Campus Glen Osmond, SA, 5064
[3] South Australian Research and Development Institute, Waite Campus Glen Osmond, SA, 5064

Abstract
Pulse crops are an essential component of sustainable intensive cropping systems in Australia, offering rotational benefits such as pest and disease breaks, improved soil health and reduced reliance on synthetic nitrogen sources. Broadleaf weeds are a major limitation to pulse production as there are limited safe or suitable herbicide control options available. Metribuzin is a broad-spectrum herbicide, which offers control of a number of key broadleaf and grass weeds in a range of pulse crops. However, metribuzin activity is variable under different soil and seasonal conditions and can cause significant yield loss at registered rates and timings in pulse crops, resulting in the usage of lower rates and reduced weed control. Studies in other crops have shown variation in tolerance exists to metribuzin, and genotypic variation has been identified in pulse herbicide tolerance trials in Australia. Identification of lines with improved levels of tolerance would improve crop safety and weed control. However, metribuzin can be difficult to screen under field and glasshouse conditions due to its variable activity. This paper reports on the development of new rapid, repeatable, high-throughput and inexpensive screening methods for identifying metribuzin tolerance in lentil, field pea and faba bean, as well as their comparison to field results. A high level of agreement between the new methods and field results was observed, and over 200 diverse accessions of each crop were screened for tolerance. These methods are now being used to incorporate improved levels of metribuzin tolerance into Pulse Breeding Australia (PBA) breeding programs.

Key words
Legumes, Metribuzin, germplasm, resistance, method, validation

Introduction
Pulse crops are an important component of Australian farming systems and provide a number of rotational benefits, such as disease and pest breaks, reduced energy inputs through nitrogen fixation and improved soil fertility (Rubiales and Mikic 2015). With an increasing need for greater temporal diversity in cropping sequences, the benefits of pulse crops will continue to play an important role in the future of sustainable systems (Drinkwater, Wagoner et al. 1998). However, one of the major limitations to Australian pulse production is weed competition, due to the lack of herbicide control options (Siddique, Johansen et al. 2012). Herbicide control is the main method of weed control in modern-day no-till farming systems, however pulses have limited suitable or safe herbicide control options available and many of the registered options in pulses, such as metribuzin, have low safety margins between phytotoxicity to the weed and to the crop (Brand 2012). Furthermore, there is an increasing need to maximise the use of available products, with the decreasing number of new herbicides being introduced, the deregulation of older herbicides, and the reduced efficacy of some current products due to the increasing number of herbicide resistant weed species.

Metribuzin is a broad-spectrum Group C herbicide which works by blocking the electron transport system in photosynthesis, (Trebst and Wietoska 1975). Metribuzin has both foliar and root uptake, however its activity is highly variable depending on soil type and environmental conditions such as light intensity, temperature, soil moisture and humidity (Phatak and Stephenson 1973, Peter and Weber 1985). Metribuzin is commonly used in pulse crops as a post-sowing-pre-emergent application, or in some cases as a post-emergent; however can often result in crop damage (Brand 2012) or the use of lower than recommended rates.

The development of pulse germplasm with improved tolerance to metribuzin will help to reduce yield loss from crop damage as well as support alternative application rates and timings, improving weed control and grower confidence in pulse production systems (Yadav, McNeil et al. 2007). Variation in tolerance to metribuzin has been observed in pulse varieties through Pulse Breeding Australia (PBA) and National Variety Trials (NVT) herbicide tolerance trials (Brand 2012); however field screening can be unpredictable from season to season, and is slow, expensive, and low throughput. Controlled environments, such as glasshouse and growth rooms, have been used in the past for screening for metribuzin tolerance, however methods can be limited by access to spray facilities and variations in soil media.

This paper reports on the development of new rapid, repeatable, high-throughput and inexpensive screening methods for identifying metribuzin tolerance in pea, lentil and faba bean, as well as the direct comparison of the results of these methods with field trials. In addition, over 200 diverse accessions of each crop were screened for variation in tolerance using the methods developed with the aim of identifying lines with improved tolerance over current Australian varieties.

Methods

Faba bean, lentil and field pea lines previously identified to vary in metribuzin tolerance were compared in hydroponic sand experiments and field trials. Hydroponic sand methods were conducted in controlled environment rooms at the Waite Research Precinct, SA during 2011 and 2012 (Table 1). Experimental design was a RCB with four replicates. Pots 4.5cm in diameter and 9cm in depth for lentils, and 8 x 10 x 8 cm for faba bean and field peas, were filled using Waikerie sand with a 1cm base of washed blue metal stone. Pots were suspended in fabricated racks which allowed the complete drainage of each pot without inter-pot contamination. Faba bean was sown 2 seeds per pot, while field pea and lentil were sown 4 seeds per pot, and all pots were watered with ¼ strength Hoagland nutrient solution every 2-3 days. Pots were thinned to one faba bean plant and two field pea and lentil plants at a uniform growth-stage of 2-3 nodes prior to treatment. To ensure uniform uptake, pots were pre-watered to field capacity 2 hours prior to treatment. Preliminary trials (data not shown) were conducted to determine herbicide rates for each crop (Table 1), and all treatments were applied at 12 days after sowing (DAS) directly to the surface of each pot, flooding the soil surface in a "drenching" method and carefully avoiding any leaf contact. Pots were flushed using ¼ strength Hoagland nutrient solution at twice the quantity of treatment 24 hours after treatment. Plants were assessed 2 weeks after treatment for plant damage as percentage of necrosis.

Post-emergent metribuzin rate response field trials were conducted at Turretfield (2011) and Kybunga (2010 and 2012), in the Mid North of South Australia (calcareous clay loams). In South Australia, 2010 was a dryer than average season with only 49mm of rainfall recorded in June and July, compared to 97-110mm in 2011/2012. A RCB design with four replicates was used for each trial with a plot size of 1.5m by 5m. The metribuzin treatment rates were based on field label rates, with 180g/ha the lowest recommended rate for sandy soils, and applied using a hand held boom at the 5 node growth stage (Table 2). Best management practice was used throughout the seasons to control insects, pests and diseases and no other in-crop herbicides were applied. Hand weeding occurred when required to remove weed competition. Visual plant damage (percentage of necrosis) and grain yield were measured. All data from hydroponic sand experiments and field experiments was analysed using ANOVA in Genstat.

Table 1: Hydroponic sand experiment details, including lines and metribuzin treatment rates

	Location	Lines	Rate (ppm)
Faba bean	Temperature controlled glasshouse at 20°C	AF3109, Nura, Farah, 1952/1	50ml solution of: 0, 20, 40 and 100
Field pea	Growthroom 20°C/10°C day/night,16-h photoperiod	PBA Oura, Yarrum, Kaspa, Sturt	50ml solution of: 0, 4, 8, 12, 16, 24 and 48
Lentils	Growthroom 20°C/10°C day/night,16-h photoperiod	99-088L, 96-047L	30ml solution of: 0, 0.3, 0.6, 0.8, 1.0, 1.2 and 2.4

Table 2: Field experiment details, including lines and metribuzin treatment rates

	Site / Year	Lines	Rates (g/ha)
Faba bean	Kybunga 2012,	AF3109, Nura, Farah, 1952/1	0, 180, 360, 720
Field pea	Turretfield 2011,	PBA Oura, Yarrum, Kaspa, Sturt	0 , 180, 360, 720
Lentils	Kybunga 2010,	99-088L, 96-047L	0, 180, 380

In addition, approximately 200 diverse accessions of lentil, faba bean and field pea were screened for metribuzin tolerance using the hydroponic sand methods above. Two to four reps of each accession were screened in RCBD with check lines spatially replicated 8-20 times throughout, and results were analysed using ANOVA in Genstat.

Results & Discussion

Hydroponic screening method results and field results showed the same significant trends when comparing both plant damage scores and yield results across methods (Figure 1). In faba bean, AF3109 performed significantly better than 1952/1, with roughly 60% less plant damage at rates of 40 ppm in hydroponic studies as well as 60% higher yield at rates of 360g/ha in field studies. Commercial bean line Farah also suffered significantly more plant damage and yield loss when compared to AF3109, while Nura showed statistically the same level of tolerance across both methods. In the field peas, PBA Oura consistently had an improved level of tolerance to metribuzin compared to Sturt across both methods; with 50% less plant damage at rates of 8ppm in hydroponic sand studies and over 60% higher yield at rates of 360g/ha in field studies. In addition, field pea line Yarrum suffered significantly less damage and yield loss when compared to Kaspa at all rates. The lentils also showed consistency between hydroponic screening methods and field results, with 99-088L having significantly less plant damage and yield loss compared to 96-047L at rates of 0.6ppm in hydroponic studies and 180g/ha in field studies. It is noted in Figure 1 substantial damage to field pea and lentil occurred in the hydroponic methods at the lower end of the range of rates applied, and in future, the range of rates could be restricted.

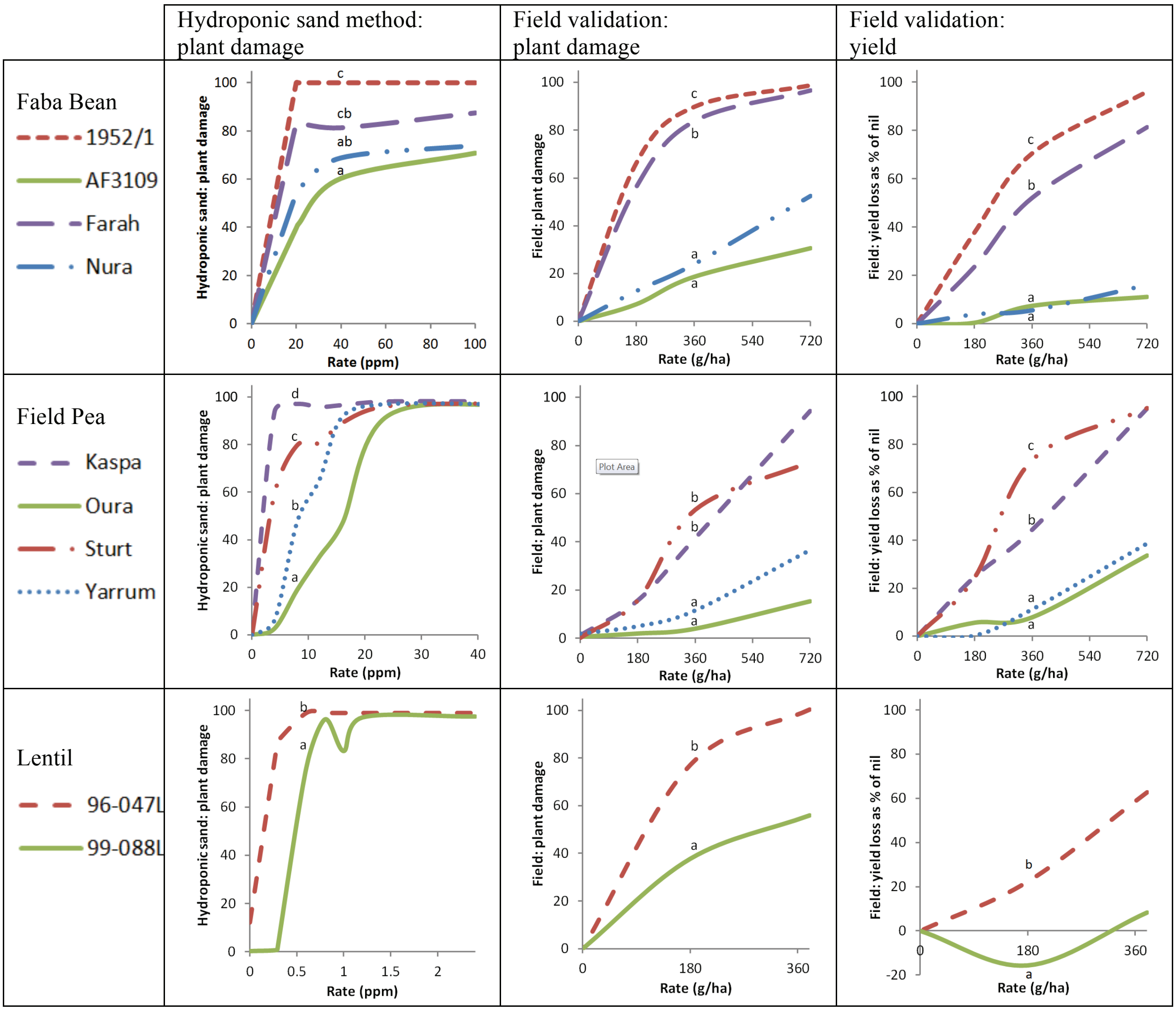

Figure 1: Results of hydroponic sand (plant damage) and field (plant damage and yield) trials in faba bean, field pea and lentil. Letters indicate significance at α=0.05 the major points of difference.

While the method of metribuzin application differed between hydroponic sand methods, which utilize root application, and field methods, which utilize foliar application, the results for all three crops were consistent across both methods for both plant damage and yield measurements. Previous work in this area has shown metribuzin to be similarly toxic regardless of whether absorption was via roots, shoots or seed zone uptake, and the method of application appears to be less crucial than the consistency of application (Fortino and Splittstoesser 1974). The hydroponic sand method allows a consistent application of metribuzin by eliminating inconsistencies in plant response caused by variations in soil and climatic conditions (De Weese, Wax et al. 1989). For example, the use of a sand based medium eliminates variability in herbicide binding to organic matter and clay content, allowing the metribuzin to be more available for plant uptake, and lower rates are required to develop comparable levels of plant damage to field studies. Hydroponic methods have been successfully used to screen for metribuzin tolerance in other crops (De Weese, Wax et al. 1989), however as pulse crops are difficult to grow in controlled environment conditions (Summerfield and Muehlbauer 1982) and are sensitive to waterlogging, methods were developed to ensure optimum plant growth as well as a high level of metribuzin efficacy. Further, as metribuzin is highly soluble, flushing the system 24 hours after treatment allowed a consistent dose of the herbicide and reduced variation from herbicide persistence. The results showed that plant damage scores taken at 14 days after treatment (DAT) in hydroponic sand methods were sufficient at identifying agronomically useful levels of improved metribuzin tolerance in all three crops. The hydroponic sand method takes only 28 days, and can screen thousands of lines for metribuzin tolerance reliably, efficiently and inexpensively all year round.

Screening of 200 diverse accessions of each crop showed that genotypic variation existed for metribuzin tolerance in all 3 crops. About 95% of faba bean lines performed similarly to or significantly worse than commercial line Nura, however of the 5% with improved tolerance, 2 lines showed a significant level of improved tolerance compared to AF3109. Similarly, the screening of 200 diverse accessions of lentil also identified 5% of lines with an improved level of metribuzin tolerance compared to the commercial line PBA Flash. In contrast, none of the 200 diverse accessions of field pea screened showed a significant level of improved tolerance to metribuzin compared with the commercial line PBA Oura, though 3% of lines were identified as having a similar level of improved tolerance over the widely grown variety Kaspa.

Conclusion

These results confirm that variation in tolerance to metribuzin exists within faba bean, field pea and lentil germplasm, and the developed hydroponic sand methods can successfully identify lines with agronomically useful levels of tolerance. These methods could be applied in breeding programs to routinely ensure appropriate levels of tolerance are maintained within the programs. Furthermore, these methods could be applied to other crops as well as other herbicides, particularly those with similar chemical characteristics such as simazine and diuron, which also have low safety margins in pulses. The development of improved herbicide tolerance is critical to improving weed control in pulse crops, and expanding pulse production into new areas to support the diversification of Australian crop rotations.

References

Brand J (2012). Interpreting variable data from herbicide tolerance trials on pulse cultivars for on-farm applicability. Proceedings of 16th Agronomy Conference 2012. I. Yunusa. Armidale, NSW.

De Weese W, Wax L, Carlson W and Ciarletta J (1989). Response of soybean (*Glycine max*) cultivars to metribuzin in the field and greenhouse. Weed Technology 3, 566-572.

Drinkwater LE, Wagoner P and Sarrantonio M (1998). Legume-based cropping systems have reduced carbon and nitrogen losses. Nature 396, 262-265.

Fortino J and Splittstoesser W (1974). Response of tomato to metribuzin. Weed Science 22, 460-463.

Peter CJ and Weber JB (1985). Adsorption, mobility, and efficacy of metribuzin as influenced by soil properties. Weed Science 33, 868-873.

Phatak SC and Stephenson GR (1973). Influence of light and temperature on metribuzin phytotoxicity to tomato. Canadian Journal of Plant Science 53, 843-847.

Rubiales D and Mikic A (2015). Introduction: Legumes in Sustainable Agriculture. Critical Reviews in Plant Sciences 34, 2-3.

Siddique KHM, Johansen C, Turner NC, Jeuffroy MH, Hashem A, Sakar D, Gan Y and Alghamdi SS (2012). Innovations in agronomy for food legumes. A review. Agronomy for Sustainable Development 32, 45-64.

Summerfield RJ and Muehlbauer FJ (1982). Controlled environments as an adjunct to field-research on lentils (*Lens culinaris*). 2. Research strategy. Experimental Agriculture 18, 3-15.

Trebst A and Wietoska H (1975). Mode of action and structure-activity-relationships of aminotriazinone herbicide metribuzin - inhibition of photosynthetic electron-transport in chloroplasts by metribuzin. Zeitschrift Fur Naturforschung C - A Journal of Biosciences 30, 499-504.

Yadav SS, McNeil DL and Stevenson PC (2007). Lentil - An Ancient Crop for Modern Times. Springer.