The impact of acetohydroxyacid synthase inhibiting herbicides on symbiotic nitrogen fixation of grain and pasture legumes

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### Acronyms and abbreviations

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<th>Definition</th>
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<tr>
<td>a.i.</td>
<td>active ingredient</td>
</tr>
<tr>
<td>A500</td>
<td>absorbance at 500nm</td>
</tr>
<tr>
<td>AA</td>
<td>amino acid</td>
</tr>
<tr>
<td>AHAS</td>
<td>acetohydroxyacid synthase</td>
</tr>
<tr>
<td>AHASIH</td>
<td>acetohydroxyacid synthase inhibiting herbicide</td>
</tr>
<tr>
<td>ALS</td>
<td>acetolactate synthase</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>AOX</td>
<td>alternative oxidase</td>
</tr>
<tr>
<td>BCAA</td>
<td>branched chain amino acid</td>
</tr>
<tr>
<td>BNF</td>
<td>biological nitrogen fixation</td>
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<tr>
<td>CFU</td>
<td>colony forming units</td>
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<td>chlorsulfuron</td>
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<tr>
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<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>F</td>
<td>flumetsulam</td>
</tr>
<tr>
<td>FAD</td>
<td>flavin adenine dinucleotide</td>
</tr>
<tr>
<td>GST</td>
<td>glutathione s-transferase</td>
</tr>
<tr>
<td>IM</td>
<td>imazethapyr</td>
</tr>
<tr>
<td>IX</td>
<td>imazamox</td>
</tr>
<tr>
<td>LSD</td>
<td>least significant difference</td>
</tr>
<tr>
<td>MM</td>
<td>metsulfuron methyl</td>
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<tr>
<td>ns</td>
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<tr>
<td>PAL</td>
<td>phenylalanin ammonia-lyase</td>
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<tr>
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</tr>
<tr>
<td>ppm</td>
<td>parts per million</td>
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<tr>
<td>PSPE</td>
<td>post-sowing pre-emergence</td>
</tr>
<tr>
<td>RBP</td>
<td>RuBisCO binding protein</td>
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<tr>
<td>REML</td>
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<td>rpm</td>
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### Scientific and common names

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<tr>
<td><em>Medicago littoralis</em></td>
<td>strand medic</td>
</tr>
<tr>
<td><em>Medicago sativa</em></td>
<td>lucerne, alfalfa</td>
</tr>
<tr>
<td><em>Medicago truncatula</em></td>
<td>barrel medic</td>
</tr>
<tr>
<td><em>Ornithopus compressus</em></td>
<td>serradella</td>
</tr>
<tr>
<td><em>Pisum sativum</em></td>
<td>field pea</td>
</tr>
<tr>
<td><em>Trifolium michelianum</em></td>
<td>balansa clover</td>
</tr>
<tr>
<td><em>Trifolium subterraneum</em></td>
<td>subterraneum clover</td>
</tr>
<tr>
<td><em>Vicia faba</em></td>
<td>faba bean</td>
</tr>
<tr>
<td><em>Vicia sativum</em></td>
<td>vetch</td>
</tr>
<tr>
<td><strong>Other plants</strong></td>
<td></td>
</tr>
<tr>
<td><em>Amsinckia intermedia</em></td>
<td>burrweed</td>
</tr>
<tr>
<td><em>Arachis hypogaea</em></td>
<td>peanuts</td>
</tr>
<tr>
<td><em>Capsella bursa-pastoris</em></td>
<td>shepherd’s purse</td>
</tr>
<tr>
<td><em>Carthamus lanatus</em></td>
<td>thistle</td>
</tr>
<tr>
<td><em>Erodium spp</em></td>
<td>storksbill</td>
</tr>
<tr>
<td><em>Galium tricornutum</em></td>
<td>three-horned bedstraw</td>
</tr>
<tr>
<td><em>Juncus bufonius</em></td>
<td>toad rush</td>
</tr>
<tr>
<td><em>Lathyrus</em></td>
<td>lathyrus</td>
</tr>
<tr>
<td><em>Lemna minor</em></td>
<td>duckweed</td>
</tr>
<tr>
<td><em>Rapistrum rugosum</em></td>
<td>wild turnip, turnip weed</td>
</tr>
<tr>
<td><em>Raphanus raphanistrum</em></td>
<td>wild radish</td>
</tr>
<tr>
<td><em>Sisymbrium orientale</em></td>
<td>Indian hedge mustard</td>
</tr>
<tr>
<td><em>Trigonella foenum-graecum</em></td>
<td>fenugreek</td>
</tr>
<tr>
<td><em>Urtica incisa</em></td>
<td>nettle</td>
</tr>
<tr>
<td><em>Zea mays</em></td>
<td>maize</td>
</tr>
</tbody>
</table>
Abstract

Group B herbicides inhibit the acetohydroxyacid synthase (AHAS - also known as acetolactate synthase) enzyme in the pathway of branched chain amino acid synthesis. These herbicides have gained widespread use in Australia, however potential impacts on nitrogen fixation by legumes have not been comprehensively assessed. Group B herbicides recommended for in-crop application to grain and pasture legume species were assessed for impacts on growth, nodulation and nitrogen fixation. Although it was demonstrated that nitrogen fixation can be affected by these herbicides, the range of responses indicated that multiple mechanisms could be responsible. These could include a reduction nitrogen fixation directly coupled to reduced plant growth; more specific and direct disruption of nitrogen fixation related to the inhibition of nodulation; or other mechanisms yet to be defined that could include affects on the rhizobia. To begin to understand these mechanisms, a herbicide tolerant *Medicago littoralis* cultivar ‘FEH-1’ was compared to Herald. Decreased nodulation, nitrogen fixation and acetylene reduction activity due to herbicide application were primarily related to the susceptibility of the plant to the herbicide. Thus herbicide tolerant legumes have the potential to alleviate suboptimal nitrogen fixation due to group B herbicides. A proteomics study of the response of root tips of model legume *Medicago truncatula* A17 to flumetsulam and metsulfuron methyl was conducted to identify more specifically the herbicide impacts on plant physiology. An increased abundance of stress response proteins and a decline in the abundance of some metabolic proteins was found, including a reduction in the abundance of glutamine synthetase which is expected to have direct consequences for the regulation of nitrogen fixation. Observations of root morphology revealed changes to root hairs and the development of lateral roots related to the disruption of meristems, with likely consequences for infection and nodule development. The results from this thesis confirm the potential for acetohydroxyacid synthase inhibiting herbicides to reduce nitrogen fixation of legumes. In addition to a general effect on nitrogen fixation via coupling to reduced plant growth, more specific biochemical and morphological mechanisms that disrupt nodulation are plausible.
Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying

Ryan Farquharson

July 2009
Acknowledgements

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SARDI seed services provided legume seed.

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Adelaide Creek – I liked it so much I decided to stay.

Jeff Baldock, Ross Ballard, Steve Rogers - the most patient of supervisors. Thanks for your guidance and your generosity with your time and expertise.

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The Prescott group (an outlet for healthy cynicism)
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The Fernandez family for a home away from home.

And last but not least, my family.
To my wife.

They told us to collaborate...

And we did.