

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

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## ***Acronyms and abbreviations***

a.i.	active ingredient
A500	absorbance at 500nm
AA	amino acid
AHAS	acetohydroxyacid synthase
AHASIH	acetohydroxyacid synthase inhibiting herbicide
ALS	acetolactate synthase
ANOVA	analysis of variance
AOX	alternative oxidase
BCAA	branched chain amino acid
BNF	biological nitrogen fixation
CFU	colony forming units
CS	chlorsulfuron
DNA	deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay
F	flumetsulam
FAD	flavin adenine dinucleotide
GST	glutathione s-transferase
IM	imazethapyr
IX	imazamox
LSD	least significant difference
MM	metsulfuron methyl
N	nitrogen
N <sub>2</sub>	molecular nitrogen
[N]	nitrogen concentration
na	not applicable
ns	not significant
PAL	phenylalanin ammonia-lyase
PBS	phosphate buffered saline
ppm	parts per million
PSPE	post-sowing pre-emergence
RBP	RuBisCO binding protein
REML	restricted maximal likelihood
RNA	ribonucleic acid
rpm	revolutions per minute
RuBisCO	ribulose biphosphate carboxylase
SU	sulfonylurea
TAL	tyrosine ammonia-lyase

## Scientific and common names

<b>Legumes</b>	
<i>Cicer arietinum</i>	chickpea
<i>Glycine max</i>	soybean
<i>Lens culinaris</i>	lentil
<i>Lupinus albus</i>	lupin
<i>Medicago littoralis</i>	strand medic
<i>Medicago sativa</i>	lucerne, alfalfa
<i>Medicago truncatula</i>	barrel medic
<i>Ornithopus compressus</i>	serradella
<i>Pisum sativum</i>	field pea
<i>Trifolium michelianum</i>	balansa clover
<i>Trifolium subterraneum</i>	subterraneum clover
<i>Vicia faba</i>	faba bean
<i>Vicia sativum</i>	vetch
<b>Other plants</b>	
<i>Amsinckia intermedia</i>	burrweed
<i>Arachis hypogaea</i>	peanuts
<i>Capsella bursa-pastoris</i>	shepherd's purse
<i>Carthamus lanatus</i>	thistle
<i>Erodium spp</i>	storksbill
<i>Galium tricornutum</i>	three-horned bedstraw
<i>Juncus bufonius</i>	toad rush
<i>Lathyrus</i>	lathyrus
<i>Lemna minor</i>	duckweed
<i>Rapistrum rugosum</i>	wild turnip, turnip weed
<i>Raphanus raphanistrum</i>	wild radish
<i>Sisymbrium orientale</i>	Indian hedge mustard
<i>Trigonella foenum-graecum</i>	fenugreek
<i>Urtica incisa</i>	nettle
<i>Zea mays</i>	maize

## **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

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*And we did.*