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

Ryan L. Farquharson

Thesis submitted for the degree of
Doctor of Philosophy
in the
School of Earth and Environmental Sciences
at the
University of Adelaide

Discipline of Soil and Land Systems

Waite Campus

The University of Adelaide

July 2009

Table of contents

List of figures

List of tables

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_2	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 bisphosphate carboxylase
SU	sulfonylurea
TAL	tyrosine ammonia-lyase

Scientific and common names

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

July 2009

Acknowledgements

Grains Research and Development Corporation, CSIRO Land and Water and the University of Adelaide are gratefully acknowledged for funding and facilitating this project.

Barry Rolfe and the ARC Centre of Excellence for Integrative Legume Research supported the proteomics work. The technical assistance of Peta Holmes in particular is much appreciated.

Technical assistance from Belinda Harris, Liang Guo, Guangmei Zhang, Marie O'Hanlon, Maryam, Felicity Brake and Andrew Patterson made the arduous tasks less arduous. Colin Rivers provided assistance with Kjeldahl digests, the autosampler and general help around the university laboratory and field lab. Neil Schubert provided assistance around the SARDI lab and glasshouse. Andreas Flench and Paul Ingram from SARDI glasshouse services assisted with soil and glasshouse logistics.

Herbicides were provided by Dow Agroscience Australia and Robert Dorigo (Broadstrike, flumetsulam), Dupont Australia (chlorsulfuron and metsulfuron methyl), BASF Australia (imazethapyr as Spinnaker, imazamox as Raptor).
Chris Preston and group for access to the herbicide spraying facility, technical grade chlorsulfuron, advice on ALS assays.
Greg Gemmel and Elizabeth Hartley, ALIRU for providing authenticated, true to type rhizobia strains.
SARDI seed services provided legume seed.
Jake Howie and John Heap are acknowledged for their advice on FEH-1 and together with Ram Nair provided access to the new cultivar. Nigel Charman for input into the FEH-1 experiment.

I thank Annette Anderson for helping get my feet on the ground in a new city, and provision of references and advice at the start of the project.

Elizabeth Drew can't be thanked enough for assistance with acetylene reduction assays, out of hours help, advice and comments on the thesis and emotional support along the way.

Gupta Vadakattu and David Roget are acknowledged for their general advice on herbicides and N_2 fixation, particularly in the field.

Many thanks to Prof. John Howieson for his thorough and constructive examination of my thesis which resulted in a number of improvements.

Steve Wakelin, Naomi Gray, Tanja Jankovic, Adrienne Greg – cohabitants of the Taylor lab and Sally Smith, Rob Murray, Cameron Grant, Debbie Miller, Murray Unkovich and Annie McNeil from the University, thanks for your help and smiling faces along the way.

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.

The special people you meet along the way:

Warwick Dougherty and Therese McBeath, the office buddies now friends for life.
Told you I'd finish.

The Prescott group (an outlet for healthy cynicism)
Various flat mates – the good (Therese, Karen, Jon), the brotherly (Jean-Patrick), the welcoming (Myrna) and the insane.

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.