Current understanding
AHAS-Inhibitor herbicide resistance:
Qin Yu
AHAS inhibitor herbicides

- SU (34 ai)
  - Chlorsulfuron
- IMI (6)
  - Imazethapyr
- TP (7)
  - Penoxsulam
- PTB (6)
  - Bispyribac-sodium
- SCT (3)
  - Thifencarbazon-methyl
- PTB  (6)
  - Bispyribac-sodium
- TP  (7)
  - Penoxsulam
- IMI (6)
  - Imazethapyr
- SU (34 ai)
  - Chlorsulfuron

First introduction in 1982

Now five chemical families, 54 active ingredients

AHAS inhibitor herbicides
Globally, persistently and widely used:

- High efficacy at low use rates
- Low mammalian toxicity
- Soil residue activity
- Wide crop selectivity
- High efficacy at low use rates

AHAS inhibitor herbicides
Resistance evolution

- First case in 1987 in USA (Mallory-Smith et al., 1990)
- Now 129 R biotypes of weed species, world wide
- Random surveys in some cropping areas show resistance is now more common than susceptibility in some major weed species!
Lolium rigidum, Western Australia, 98% R pops (Owen et al., 2013, poster)
Raphanus raphanistrum, WA, 84% R pops (Owen et al. 2013, poster)
Kochia scoparia, Western Canada: 90% R pops (Beckie et al. 2011)

Courtesy, Hugh Beckie
Amaranthus tuberculatus, Illinois: >50% R pops (Tranel et al. 2011)
Resistance mechanisms

Target site based

Mutation

Over production

Non-target site based

Other

Metabolism

Translocation

Uptake
Target-site AHAS resistance mutations

- First identified in Kochia scoparia and Lactuca serriola (Guttieri et al., 1992)
- Now 26 resistance-endowing amino acid substitutions, at 8 positions of AHAS gene
- More new mutations?
Target-site AHAS resistance mutations

Some mutations are more frequent than others!

◊ Mutations at Pro-197 are most frequent at Pro-197-Ser: >20 weed species.

◊ Selection pressure from frequent, selective herbicide use patterns (Su, SU+IMI) and frequent, selective herbicide use patterns (Su, SU+IMI) and Trp-574-Leu are most frequent than other!

◊ The frequency of mutations requiring only one nucleotide change is higher than those requiring two nucleotide changes.

Pro-197-Asn/Ile/Met/Lys/Trp: once
Cross-resistance patterns: according to position ?

<table>
<thead>
<tr>
<th>SU</th>
<th>IMI</th>
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<tbody>
<tr>
<td>R</td>
<td>R</td>
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<tr>
<td>R</td>
<td>S</td>
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<td>R</td>
<td>S</td>
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• Target-site AHAS resistance mutations
Target-site AHAS resistance mutations

- Cross-resistance patterns: case by case!

### Plant species:

- *Raphanus*
- *Amaranthus*

### Herbicide molecules:

- Asp-376-Glu
- R to imazethapyr (IMI)
- Asp-376-Glu
- S to imazapyr (IMI)

### Amino acid substitutions:

- Ala-122-Tyr (HR to SU+IMI)
- Ala-122-Val (R to SU+IMI)
- Ala-122-Thr (R to IMI)

(Kryshel et al. 2011; Beckie and Tardif, 2012; Han et al. 2012; Yu et al. 2012; Riar et al. 2013)

Beckie and Tardif, 2012; Han et al. 2012; Yu et al. 2012; Riar et al. 2013)
AHAS resistance mutations are diverse!

- **Population level:**
  -Allele level:
  -Individual level:
  -Population level:

**Target site AHAS resistance mutations are diverse!**
Target-site AHAS resistance mutations

(You et al., 2011; Han et al., 2011)

Chlorsulfuron (20g/ha)

Some mutations strong, some weak!

Susceptible

Some mutations strong, some weak!
PCR-based diagnostic tools: a great help!

Target-site AHAS resistance mutations

Medium/high throughput
Robustness
Accuracy

Pyrosequencing
SNAPSHOT multiplex
TaqMan assay
TILLIN
Allele-specific PCR
CAPS/DCAPS

Diploid/polyploid

Resistance evolution
Population dynamics
Fitness studies
Target-site resistance surveys
Large scale genotyping for

Corbett and Tradif, 2006; Marshall et al., 2012; Burgos et al., 2013
Fitness cost is generally negligible for known AHAS resistance mutations, except for Trp-574-Leu mutations, except for Trp-574-Leu.
Structure basis of AHAS resistance mutations

Herbicide binding sites in AtAHAS

not in the active site

but within the substrate access channel

block substrate access to the active site

(McCourt et al. 2006; Duggleby et al. 2008)
Herbicide binding on substrate access channel

Courtesy Fran Lopez Ruiz

Please note this is NOT a real model of AHAS!
Structure basis of AHAS resistance mutations

- 18 amino acid residues involved in herbicide binding
- SU and IMI binding site overlapping but not identical
Highly resistant and catalytically efficient mutant AHAS (i.e. 574, 122) (in contrast to EPSPS 106 mutations)

Lack of major fitness cost at the AHAS level (i.e. 574, 122) (in contrast to EPSPS 106 mutations)

Highly resistant and catalytically efficient mutant AHAS

Structure basis of AHAS resistance mutations

• Predict, validate resistance mutations by modelling

• Cross-resistance patterns

• Structure basis due to substantial structural changes upon herbicide binding
Polyploid weed species (allopolyploids or allohexaploids)

Target-site AHAS resistance mutations in polyploids
Multicopies and introns

Lindernia spp. (Uchino and Watanabe, 2002)

Schenoplectus spp. (Uchino et al., 2007; Scarabel et al., 2010)

Target-site AHAS resistance mutations: Polyploids

Heterozygosity

Double peak!

No RR genotype!

SR
Gene copy-specific PCR-markers:

Polyplloid

Diploid

Target-site AHAS resistance mutations: Polyplloid
Assume equal expression, the higher the ploidy, the higher the dilution 

Dilution effect by multiple S alleles

Diploid

Tetraploid

Hexaploid

Target-site AHAS resistance mutations: Polyploids

(Ru et al. 2012, Panozzo et al. 2013)
**Implications in Resistance Evolution?**

- Epigenetic regulations (Scarabel et al., 2010)
- Differentially expressed genes (Iwakami et al., 2012)
- Silenced genes (Iwakami et al., 2012)
- Pseudogenes (Ohsako and Tominaga, 2007)

Expression of multiple gene copies

**Target-site AHAS Resistance Mutations: Polyploids**
Keywords: Acrase mutations; Acrase, Herbicide resistance; hexaploid, resistance evolution

Herbicide-resistant online publication, 10 October 2012: doi:10.1088/0031-9384/2012/1-2

Hexaploid species are found in many wild and cultivated grass species with a hexaploid genome. Hexaploids are known to show herbicide resistance evolution in response to herbicide pressure. In hexaploid species, the evolution of resistance to herbicides is often complex and involves multiple mechanisms, including gene mutations and the expression of resistance-related genes. Understanding the genetic basis of herbicide resistance in hexaploid species is crucial for the development of sustainable herbicide-resistant crop varieties.

In this study, hexaploid wild oat (Avena fatua) was used as a model species to investigate the molecular mechanisms underlying herbicide resistance. The results showed that hexaploid wild oat contains a set of genes that confer resistance to different herbicides. The expression of these genes is regulated by complex genetic interactions, which can be influenced by environmental factors such as temperature and soil pH.

The findings of this study suggest that hexaploid wild oat species can be used as a valuable model system for understanding the evolution of herbicide resistance in complex genomes. Further research is needed to identify the specific genes and molecular pathways involved in herbicide resistance in hexaploid wild oat and other hexaploid species.

Overall, the results of this study highlight the importance of understanding the molecular mechanisms underlying herbicide resistance in hexaploid species, as this knowledge can be used to develop strategies for managing herbicide-resistant weeds in agriculture.
Non-target-site resistance (NTSR)

- Cross resistance to certain herbicide chemistries
- Involves cytochrome P450s, GSTs
- Enhanced rates of herbicide metabolism
- Mimics herbicide tolerant crops

† Metabolism
X Translocation
X Uptake
Chlorsulfuron metabolism in tolerant crops
Chlorsulfuron metabolism in Lolium rigidum (Christopher et al. 1991).

Major metabolite: flax (dicot)
Chlorsulfuron metabolites in L. rigidum (Cotterman and Saari, 1992)
At least two P450s!
Ethametsulfuron-methyl metabolism in Sinapis arvensis (dicot)

(Lichtner et al. 1995; Van Eerd and Hall, 2000; Veldhuis et al. 2000)

At least two P450s!
Propoxycarbzone - sodium (SCT) in Bromus tectorum (Park et al., 2004; Yasuor et al., 2009)

Enhanced metabolism involving P450s

Penoxsulam (TP) in Echinochloa phyllopogon

Propoxycarbzone-sodium (SCT) in Bromus tectorum
Genetic control

◊ Nuclear-encoded
◊ Semi-to-dominant
◊ Polygenic (between 1-3 genes)
◊ Quantitative (interaction with environment)
◊ Fitness

Genes involved largely unknown!

Genes, or even in different individuals of a given population.

For a given herbicide, different P450/GT may be involved, in

Different weed species, in different populations of a given

Plants have high number P450 genes (200-400)

Non-target-site resistance (NTSR)

(Preston 2003; Petit et al. 2010; Busi et al. 2011, Han et al. 2013, unpub.)
Rapid progress in P450/GT gene discovery is expected!

L. rigidum: AHRI-Bayer CropScience, Germany
A. myosuroides: Delve-Bayer CropScience, Germany
E. phyllopogon: Japan

Non-target-site resistance (NTSR)
Worse case scenario:

Target-site + non-target-site resistance

Consequence of using a low chlorsulfuron rate for 6 years on L. rigidum

This resistant population has six AHAS gene mutations as well as all plants possessing enhanced metabolism based resistance (Christopherson et al. 1992; Yu et al. 2008)

Implications

Risk using low rates and metabolisable herbicides complicates in resistance management
Target site
- AHAS resistance is mostly studied

- Many AHAS resistance mutations can occur and be rapidly enriched because:
  - High initial resistance gene frequency (Preston and Powles, 2002)
  - Nuclear-encoded, pollen-transmitted, dominant
  - No major fitness cost

Summary
Non-target-site metabolic resistance is important, but masked, and understudied.

- Positive identification of metabolic resistance is a possibility (e.g., malathion).

- Synergists to overcome metabolic resistance is a diagnostic tool.

- Metabolic resistance gene discovery.

- Summary
Thank you!