AHAS-inhibitor herbicide resistance: Current understanding

Qin Yu

THE UNIVERSITY OF WESTERN AUSTRALIA
Achieve International Excellence

AHRI
AUSTRALIAN HERBICIDE RESISTANCE INITIATIVE

GRDC
Grains Research & Development Corporation

AHAS inhibitor herbicides

First introduction in 1982

Now five chemical families, 54 active ingredients

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

AHAS inhibitor herbicides

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

Globally, persistently and widely used!

Resistance evolution

- First case in 1987 in USA (Malllory-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)

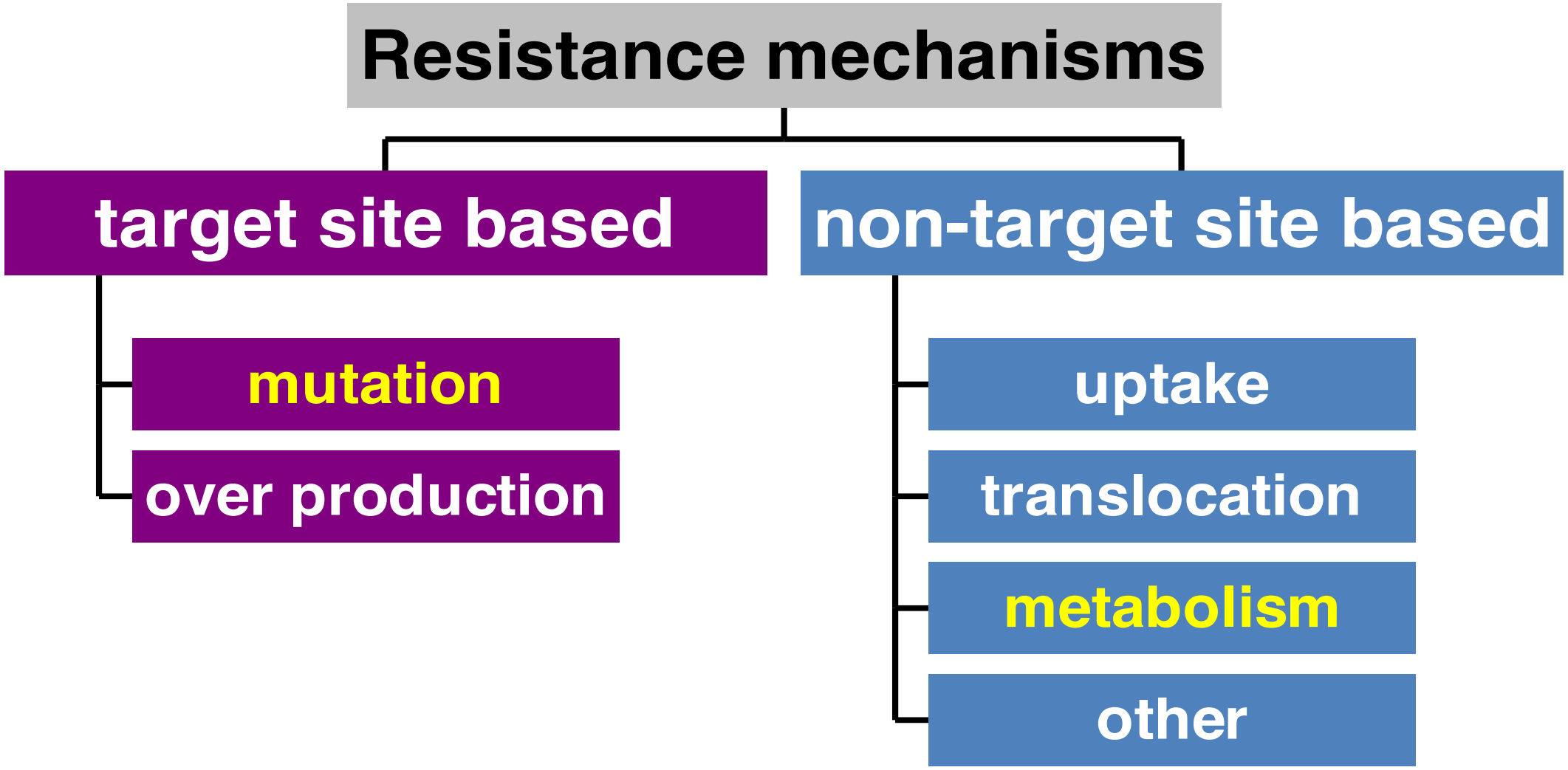
Kochia scoparia, Western Canada: 90% R pops

(Beckie et al. 2011)

Courtesy, Hugh Beckie

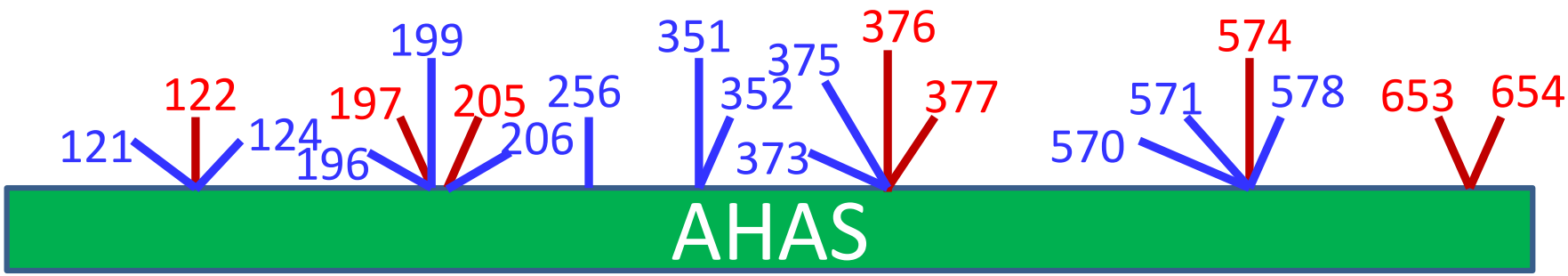
Amaranthus tuberculatus, Illinois: >50% R pops

(Tranel et al. 2011)


Resistance mechanisms
target site based
non-target site based
mutation
over production
uptake
translocation
metabolism
other

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 and the Trp-574-Leu are most frequent, reflecting herbicide use patterns (Su, SU+IMI) and selection pressure

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

  Pro-197-Ser: >20 weed species

  Pro-197-Asn/Ile/Met/Lys/Trp: once

Targe-site AHAS resistance mutations

- Cross-resistance patterns: according to position ?

	SU	IMI
122	S	R
197	R	S
574	R	R

Targe-site AHAS resistance mutations

- **Cross-resistance patterns: case by case!**

Amino acid substitutions:
- Ala-122-Thr (R to IMI)
- Ala-122-Val (R to SU+IMI)
- Ala-122-Tyr (HR to SU+IMI)

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

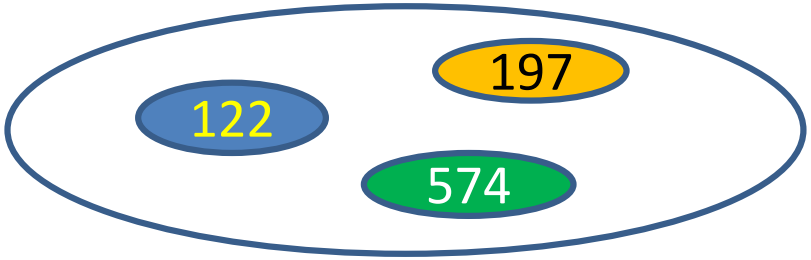
Plant species:
Asp-376-Glu
- R (*Amaranthus*)
- r (*Raphanus*)

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

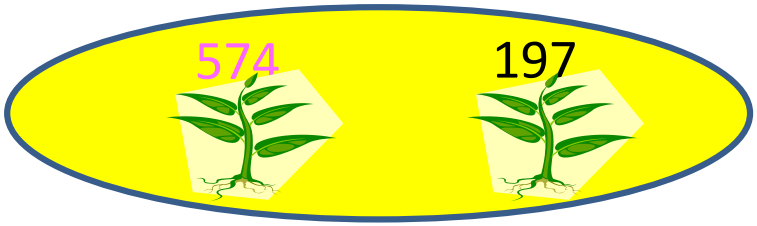
Targe-site AHAS resistance mutations

- **AHAS resistance mutations are diverse!**

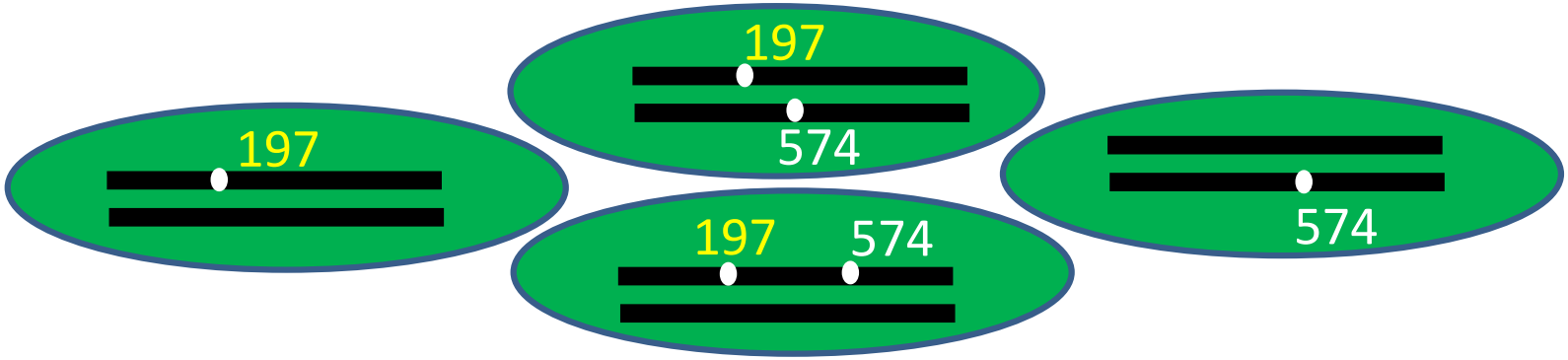
Population level:



Individual level:

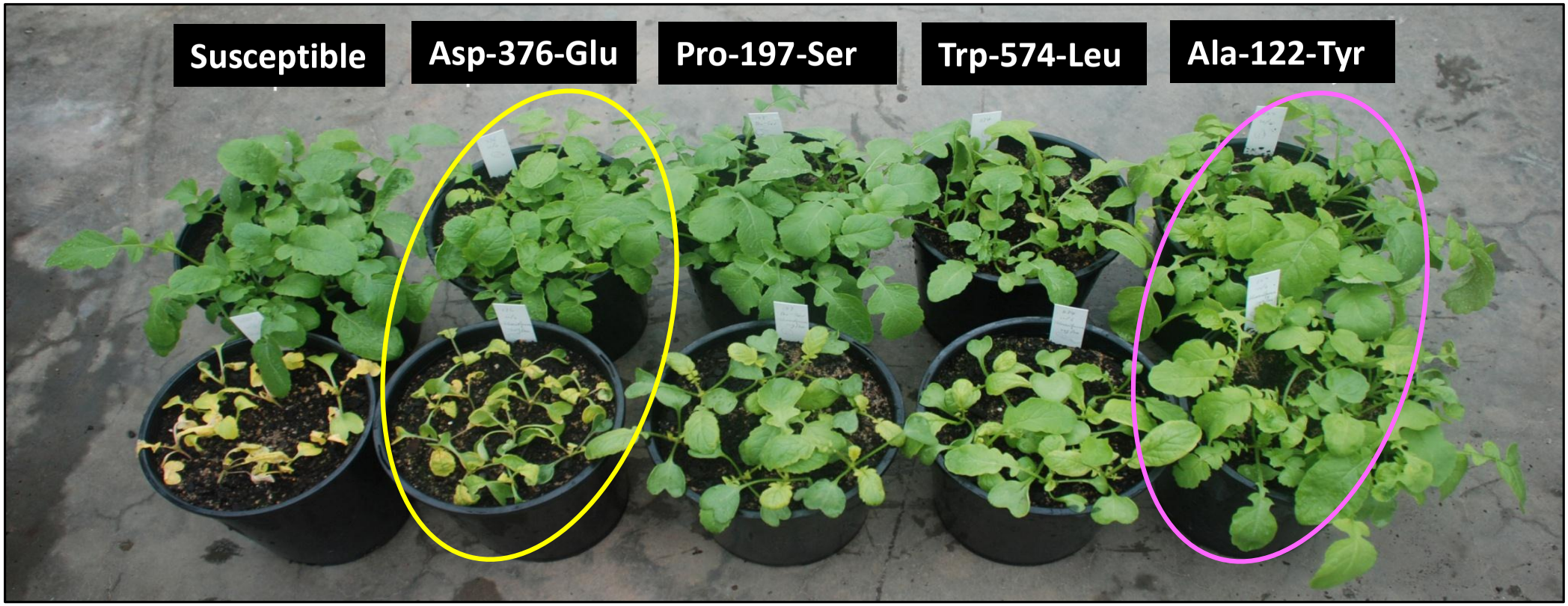


Allele level:



Target-site AHAS resistance mutations

- **Some mutations strong, some weak!**



Chlorsulfuron (20g/ha)

(Yu et al. 2011, Han et al. 2011)

Targe-site AHAS resistance mutations

- **PCR-based diagnostic tools: a great help!**

CAPS/dCAPS

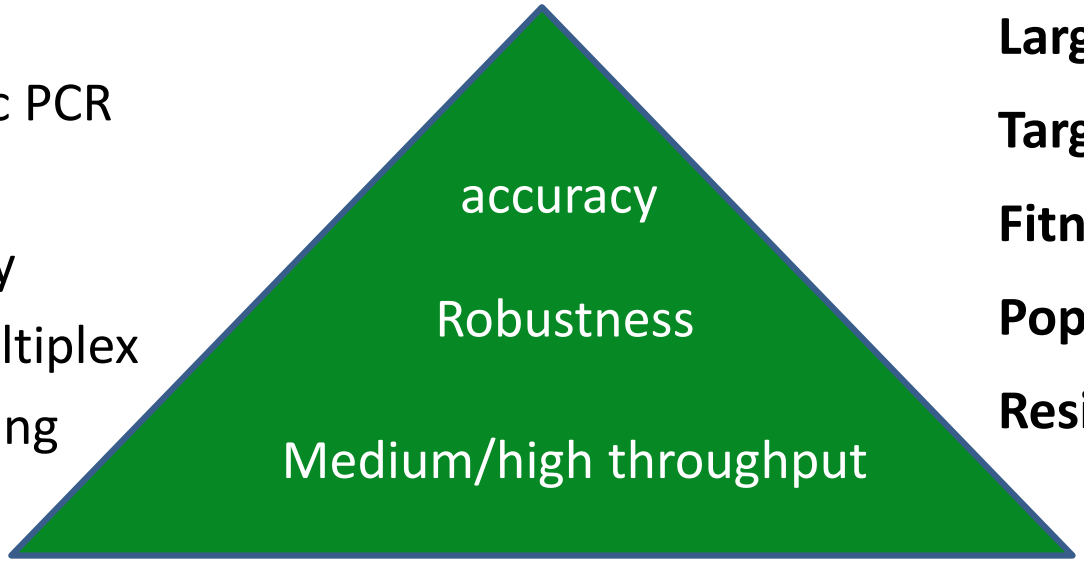
Allele Specific PCR

TILLIN

TaqMan assay

SNaPshot multiplex

Pyrosequencing



Large scale genotyping for

Target-site resistance surveys

Fitness studies

Population dynamics

Resistance evolution

Diploid/polyploid?

(Corbett and Tradif, 2006; Marshall et a. 2012; Burgos et al. 2013)

Targe-site AHAS resistance mutations

- **Fitness cost is generally negligible for known AHAS resistance mutations, except for** Trp-574-Leu

With fitness cost : *A. powellii* *(Tardif et al. 2006)*

No fitness cost : *L. rigidum* *(Yu et al. 2010)*
B. subalternans *(Lamego et al. 2011)*
R. raphanistrum *(Li et al. 2012)*
K. scoparia *(Legere et al. 2012)*

◊ Resistance AHAS alleles, weak or strong, once selected will not decline in the absence of herbicide selection pressure.

Structure basis of AHAS resistance mutations

- Herbicide binding sites in *At*AHAS

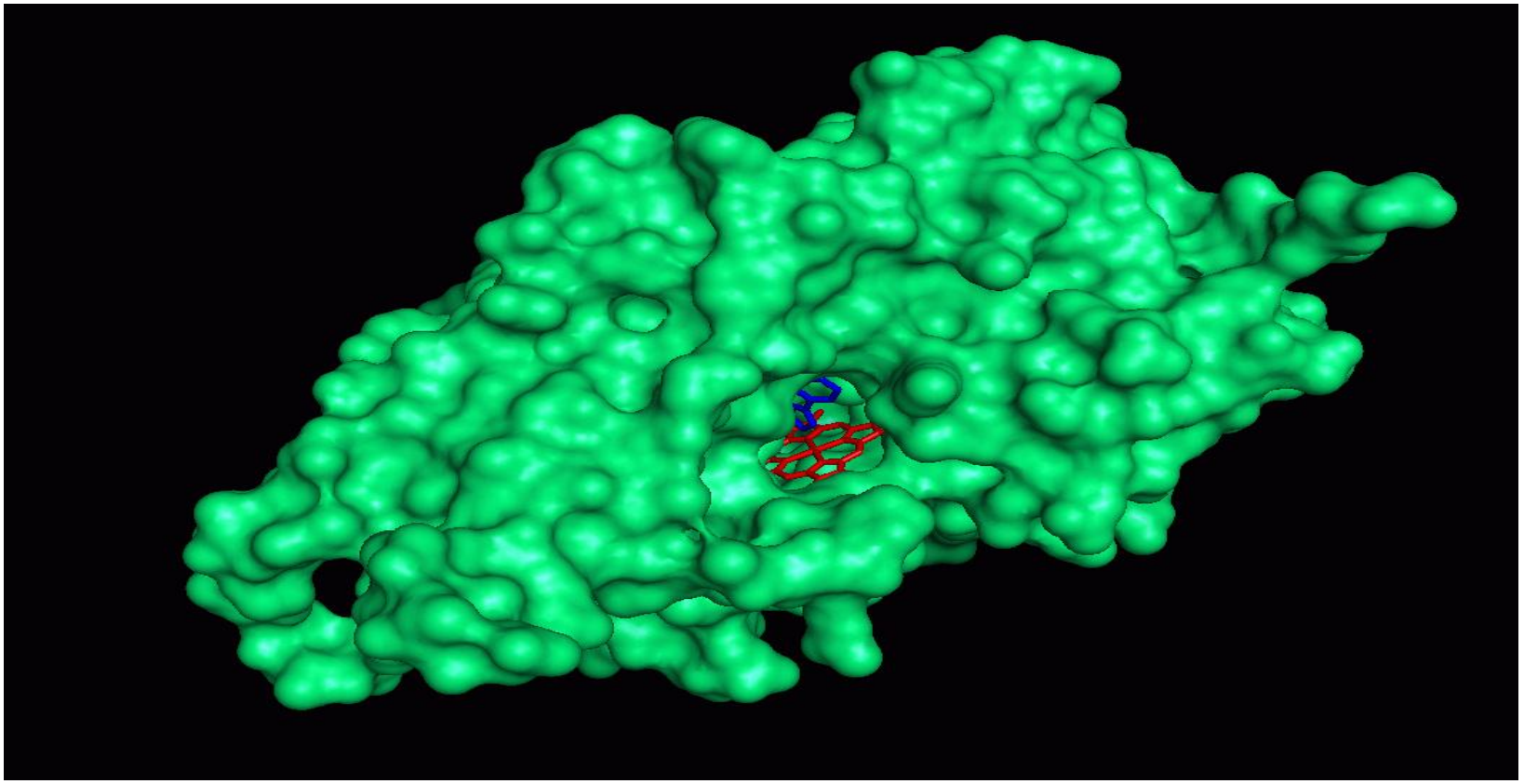
  not in the active site

  but within the substrate access channel

  block substrate access to the active site

(McCourt et al. 2006; Duggleby et al. 2008)

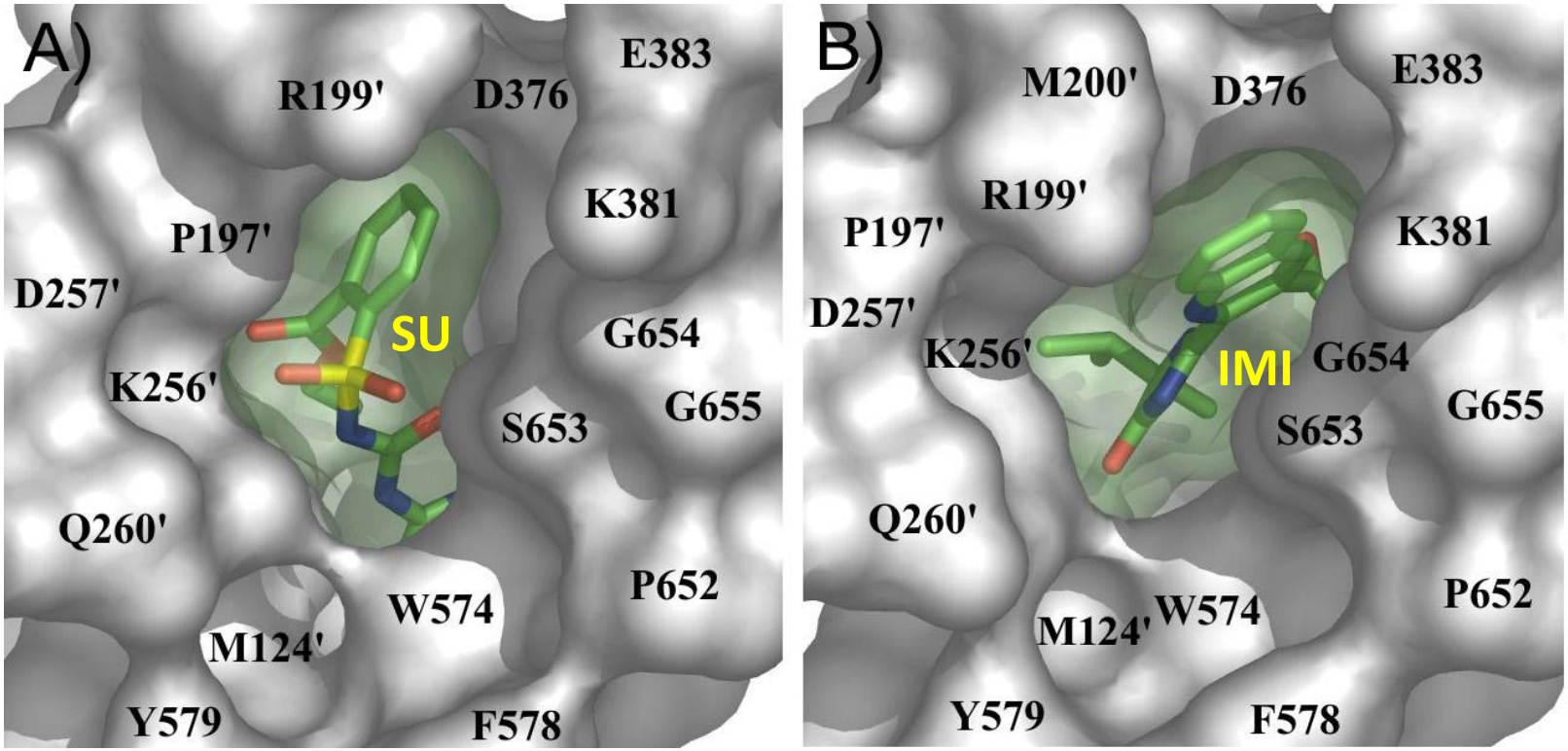
Herbicide binding on substrate access channel



Please note this is NOT a real model of AHAS!

Courtesy Fran Lopez Ruiz

Structure basis of AHAS resistance mutations



- SU and IMI binding site overlapping but not identical
- 18 amino acid residues involved in herbicide binding

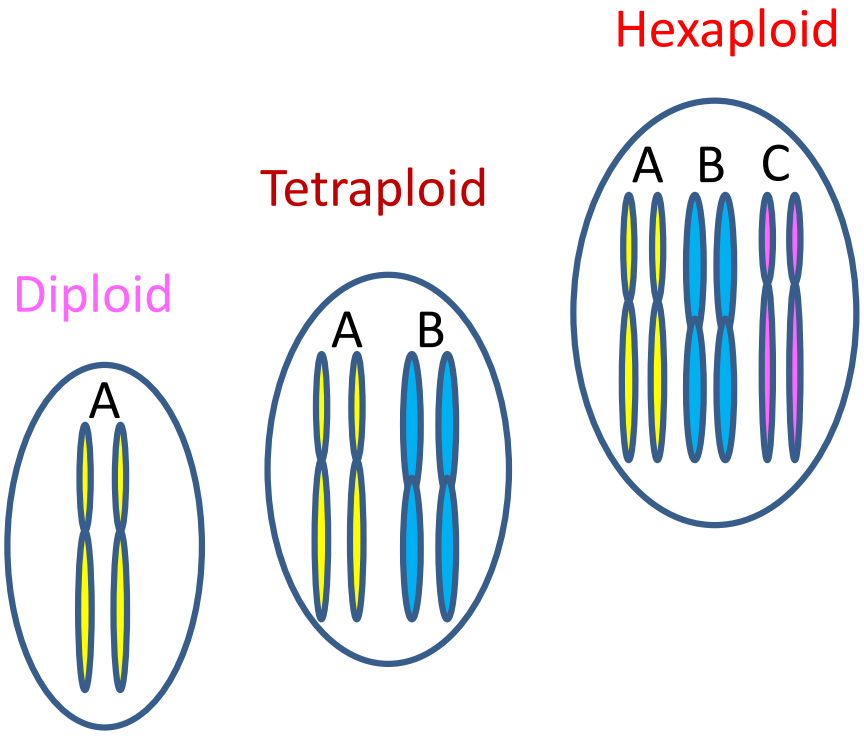
Structure basis of AHAS resistance mutations

- Highly resistant and catalytic efficient mutant AHAS (i.e. 574, 122) (in contrast to EPSPS 106 mutations)
- Lack of major fitness cost at the AHAS level
- Cross-resistance patterns
- Predict, validate resistance mutations by modelling (but speculative due to substantial structure changes upon herbicide binding)

Targe-site AHAS resistance mutations in polyploids

- **Polyploid weed species (allotetraploids or allohexaploids)**

Avena fatua
Bidens subalternans
Cyperus difformis
Echinochloa spp
Lindernia spp
Monochoria vaginalis
Poa annua
Polygonum convolvulus
Salsola tragus
Schenoplectus spp
Sorghum halepense



Target-site AHAS resistance mutations: Polyploids

◊ Multicopies and introns

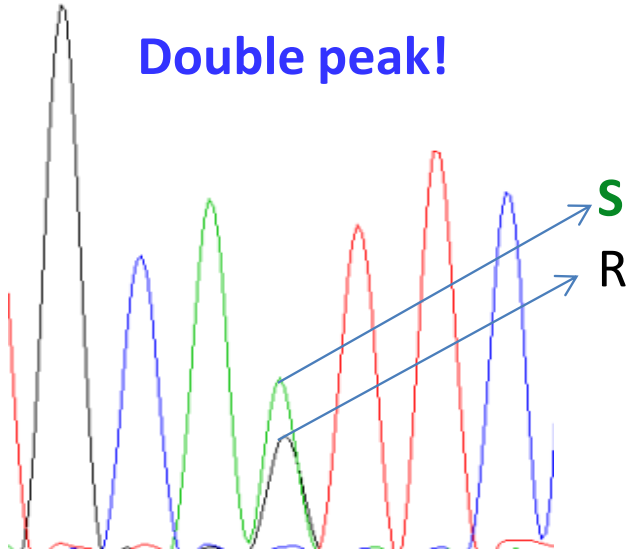
Lindernia spp. *(Uchino and Watanabe, 2002)*

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

◊ Heterozygosity

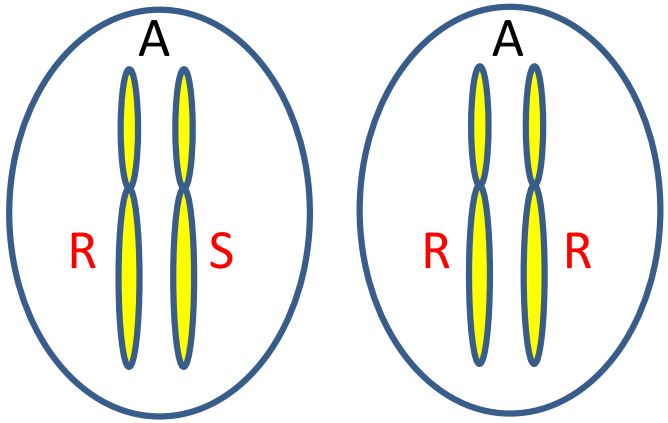
No RR genotype!

Double peak!

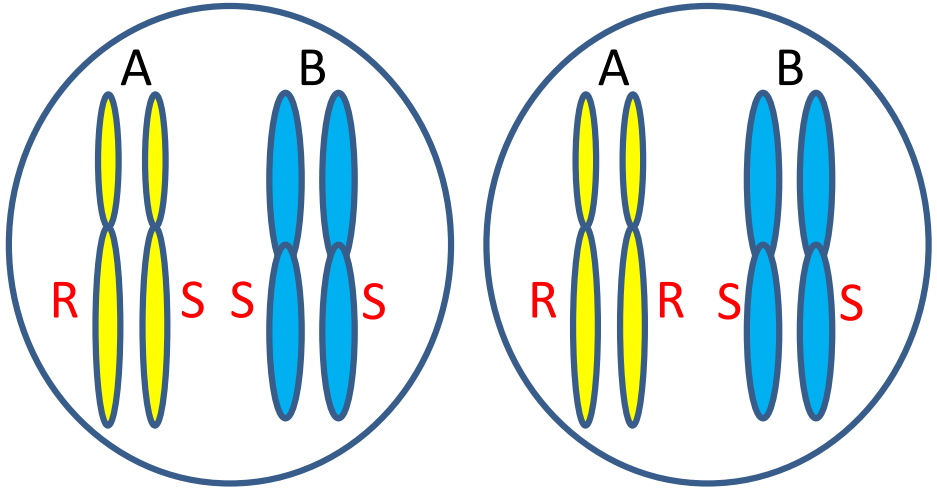


Target-site AHAS resistance mutations: Polyploids

Diploid



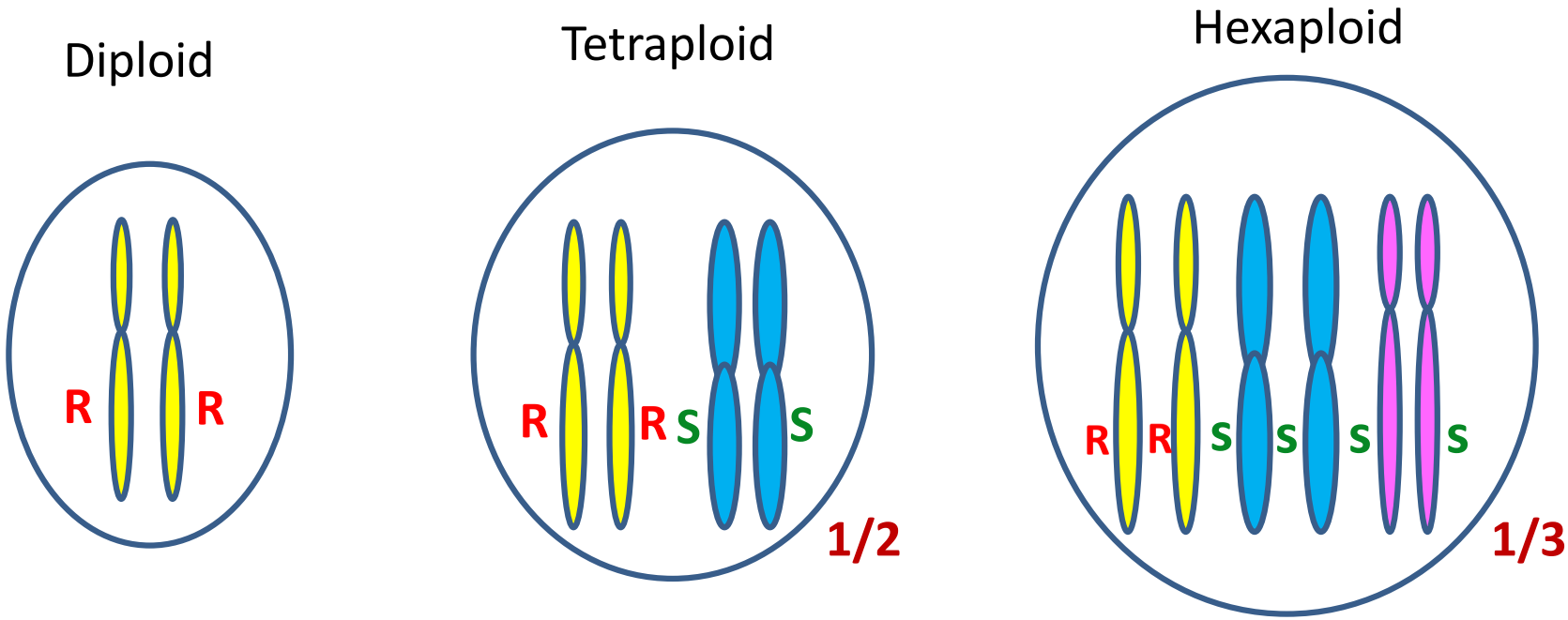
Polyploid



Gene copy-specific PCR-markers!

Target-site AHAS resistance mutations: Polyploids

◊ Dilution effect by multiple S alleles



Assume equal expression, the higher the ploidy, the higher the dilution

(Yu et al 2012, Panozzo et al. 2013)

Target-site AHAS resistance mutations: Polyploids

◊ Expression of multiple gene copies

Pseudogenes (*Ohsako and Tominaga 2007*)

Silenced genes (*Iwakami et al. 2012*)

Differentially expressed genes *(Iwakami et al. 2012*)

Epigenetic regulations (*Scarabel et al. 2010*)

◊ Implications in resistance evolution?

Heredity

Heredity (2012), 1–12

www.nature.com/hdy

ORIGINAL ARTICLE

Herbicide resistance-endowing ACCase gene mutations in hexaploid wild oat (*Avena fatua*): insights into resistance evolution in a hexaploid species

Q Yu[1], MS Ahmad-Hamdani[1,2], H Han[1], MJ Christoffers[3] and SB Powles[1]

Many herbicide-resistant weed species are polyploids, but far too little about the evolution of resistance mutations in polyploids is understood. Hexaploid wild oat (*Avena fatua*) is a global crop weed and many populations have evolved herbicide resistance. We studied plastidic acetyl-coenzyme A carboxylase (ACCase)-inhibiting herbicide resistance in hexaploid wild oat and revealed that resistant individuals can express one, two or three different plastidic ACCase gene resistance mutations (Ile-1781-Leu, Asp-2078-Gly and Cys-2088-Arg). Using ACCase resistance mutations as molecular markers, combined with genetic, molecular and biochemical approaches, we found in individual resistant wild-oat plants that (1) up to three unlinked ACCase gene loci assort independently following Mendelian laws for disomic inheritance, (2) all three of these homoeologous ACCase genes were transcribed, with each able to carry its own mutation and (3) in a hexaploid background, each individual ACCase resistance mutation confers relatively low-level herbicide resistance, in contrast to high-level resistance conferred by the same mutations in unrelated diploid weed species of the Poaceae (grass) family. Low resistance conferred by individual ACCase resistance mutations is likely due to a dilution effect by susceptible ACCase expressed by homoeologs in hexaploid wild oat and/or differential expression of homoeologous ACCase gene copies. Thus, polyploidy in hexaploid wild oat may slow resistance evolution. Evidence of coexisting non-target-site resistance mechanisms among wild-oat populations was also revealed. In all, these results demonstrate that herbicide resistance and its evolution can be more complex in hexaploid wild oat than in unrelated diploid grass weeds. Our data provide a starting point for the daunting task of understanding resistance evolution in polyploids.
Heredity advance online publication, 10 October 2012; doi:10.1038/hdy.2012.69

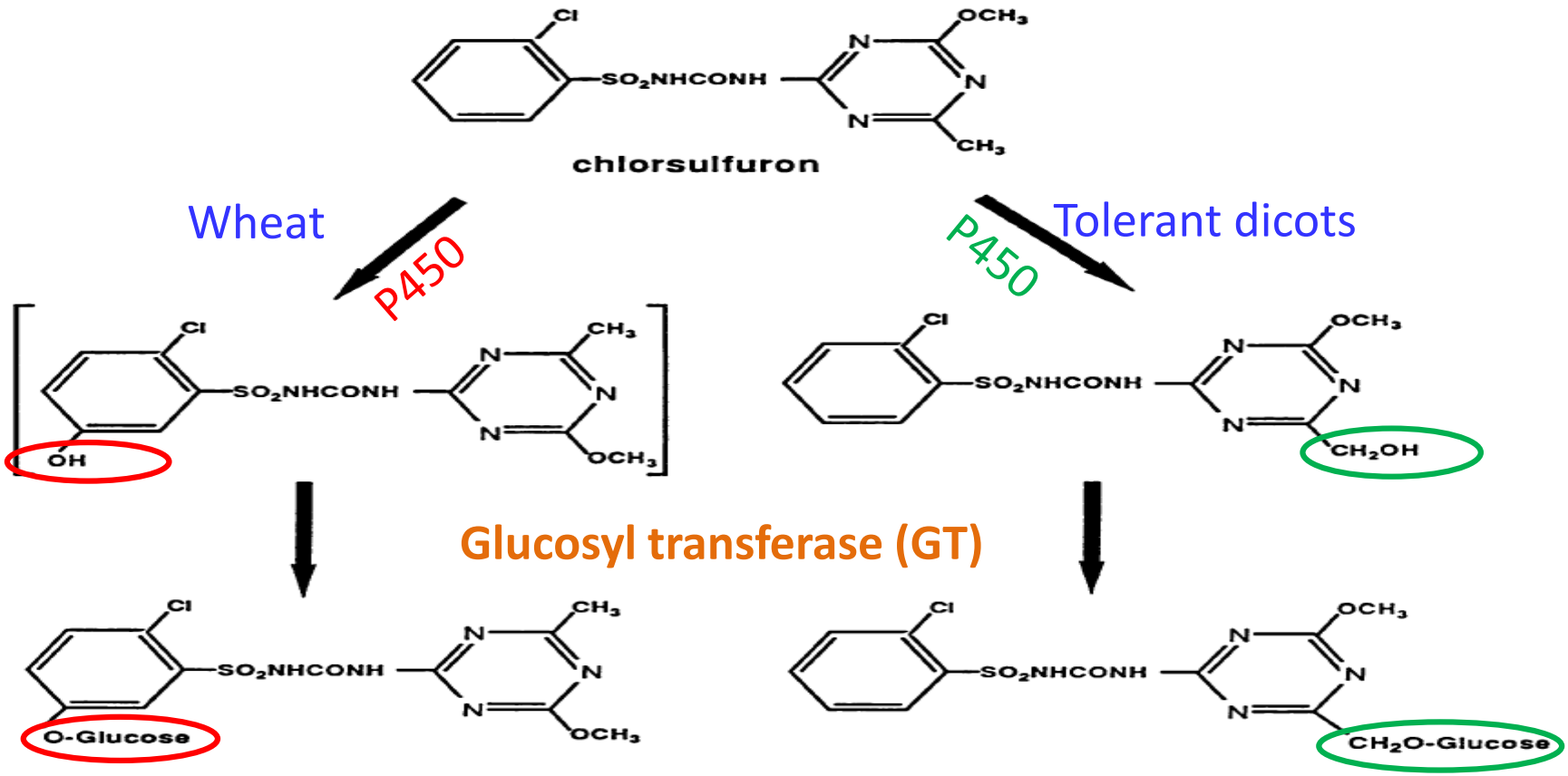
Keywords: ACCase mutation; *Acc1*; herbicide resistance; hexaploid wild oat (*Avena fatua*); polyploidy; resistance evolution

Non-target-site resistance (NTSR)

- Uptake X
- Translocation X
- Metabolism √

  - ✓ Mimic herbicide tolerant crops
  - ✓ Enhanced rates of herbicide metabolism
  - ✓ Involve cytochrome P450s, GTs
  - ✓ Cross resistance to certain herbicide chemistries

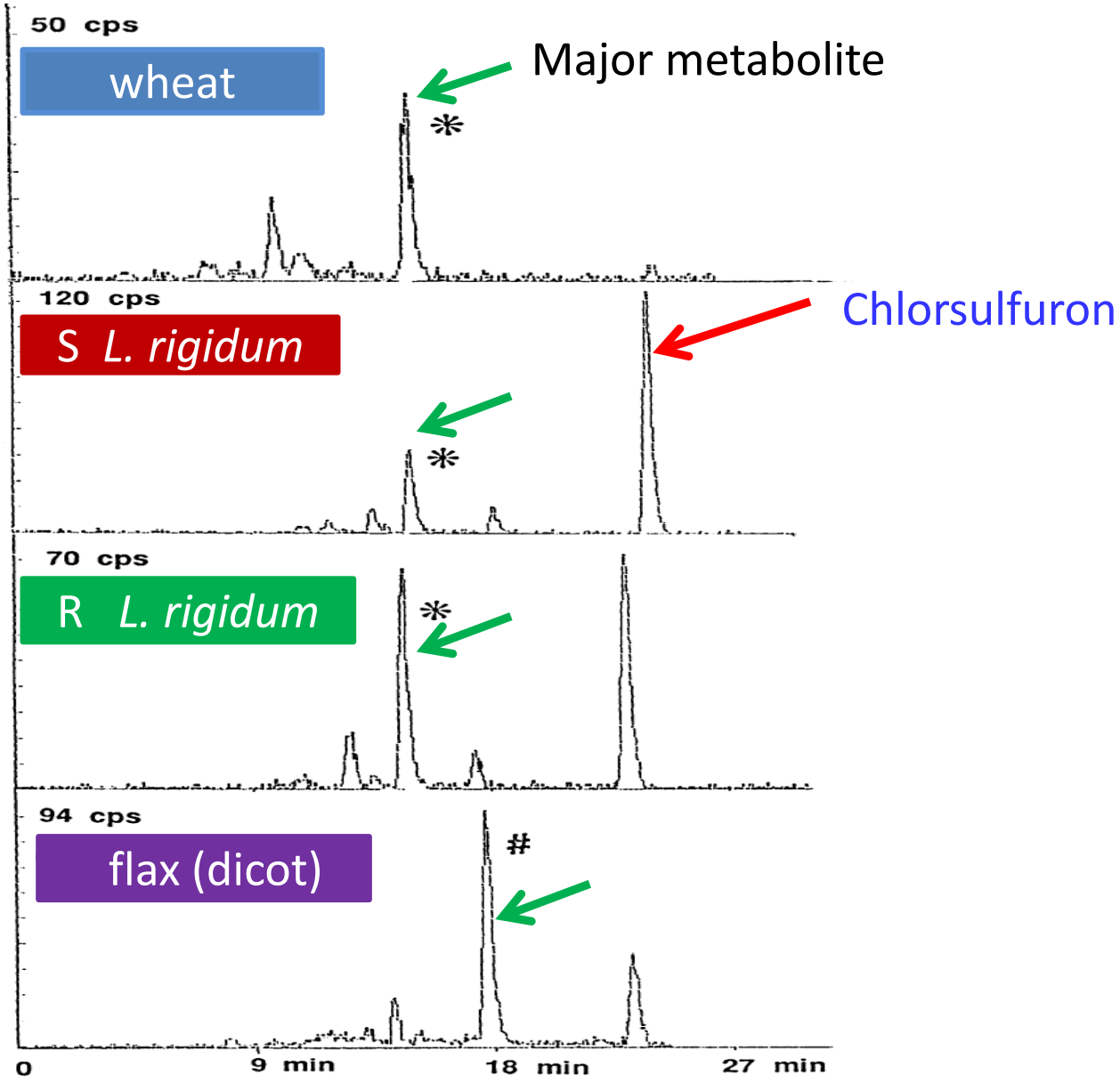
Chlorsulfuron metabolism in tolerant crops



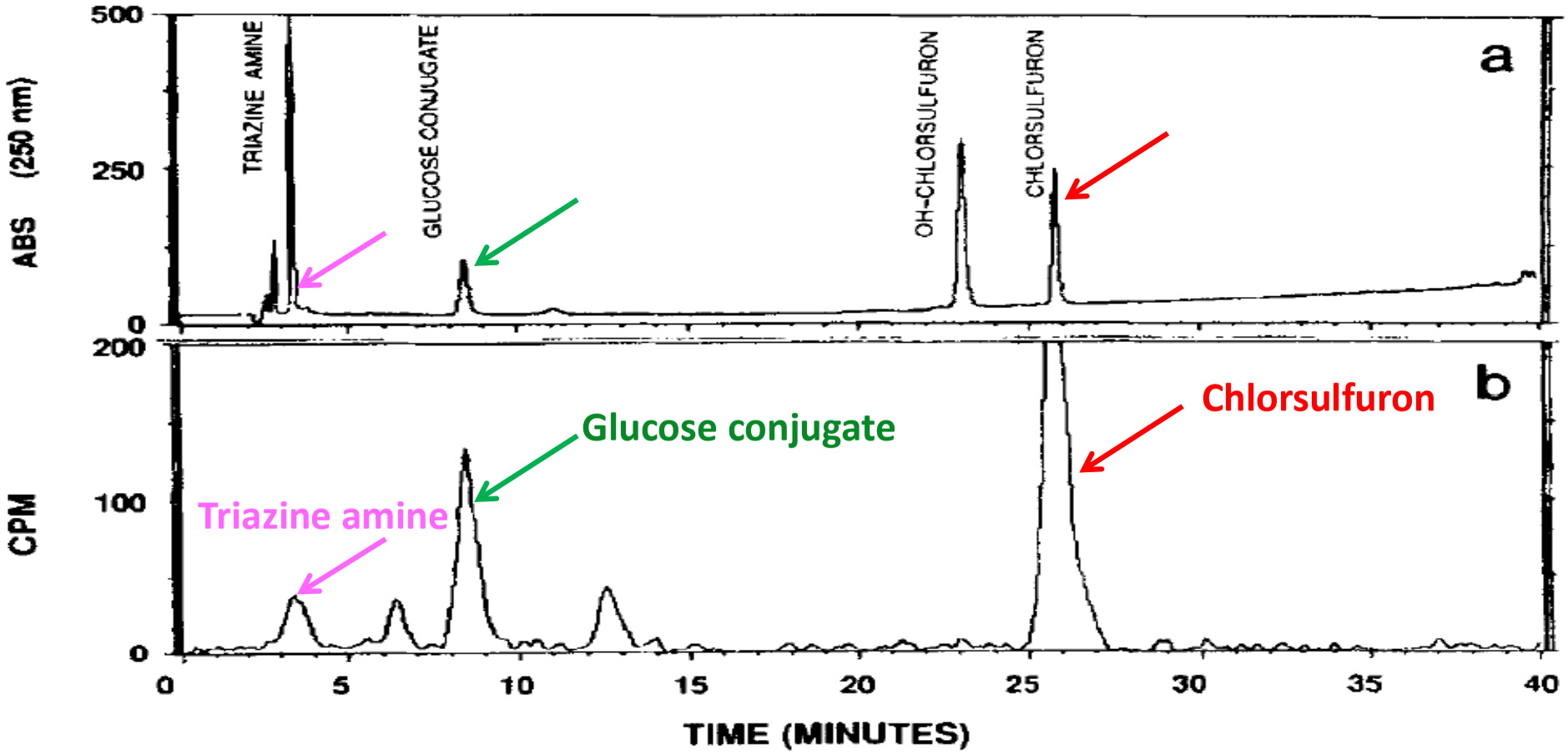
(Modified from Christopher et al. 1991)

Chlorsulfuron metabolism in *Lolium.rigidum*

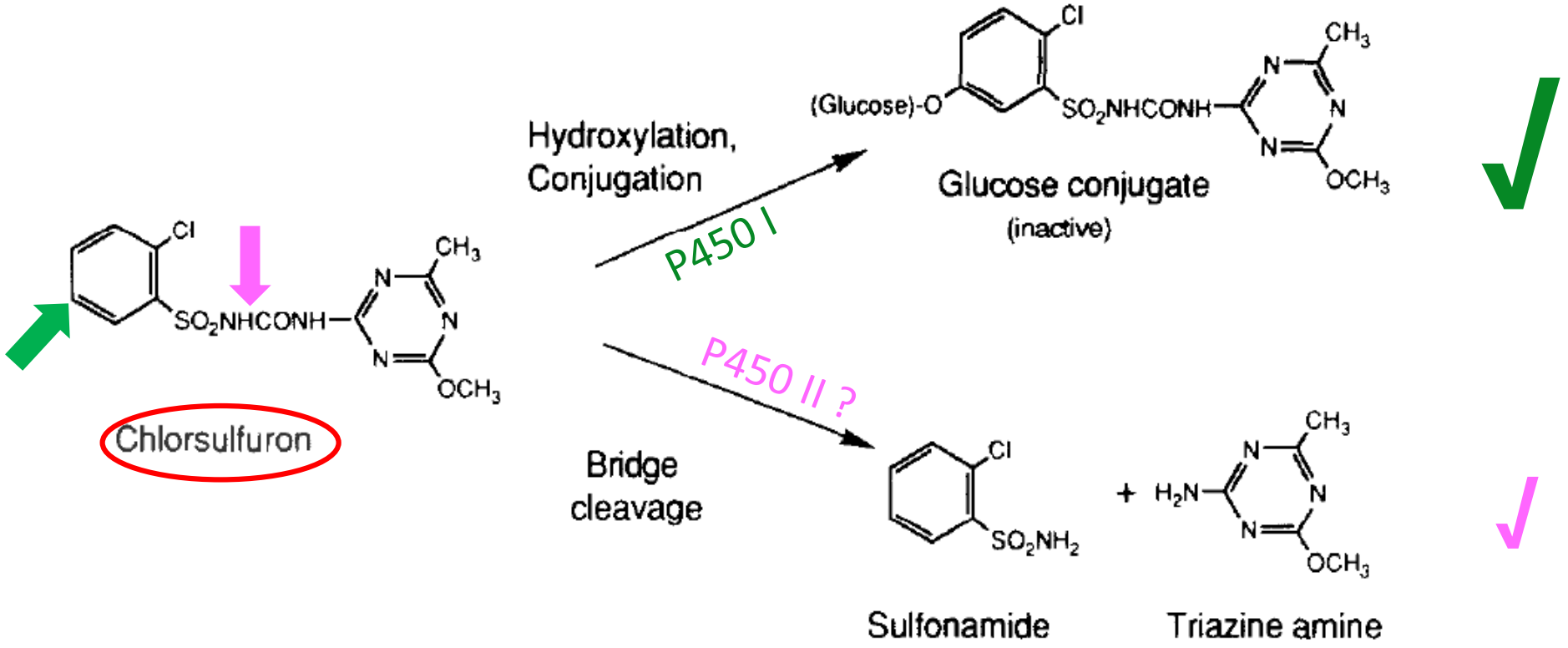
(Christopher et al. 1991)



Chlorsulfuron metabolites in *L. rigidum*



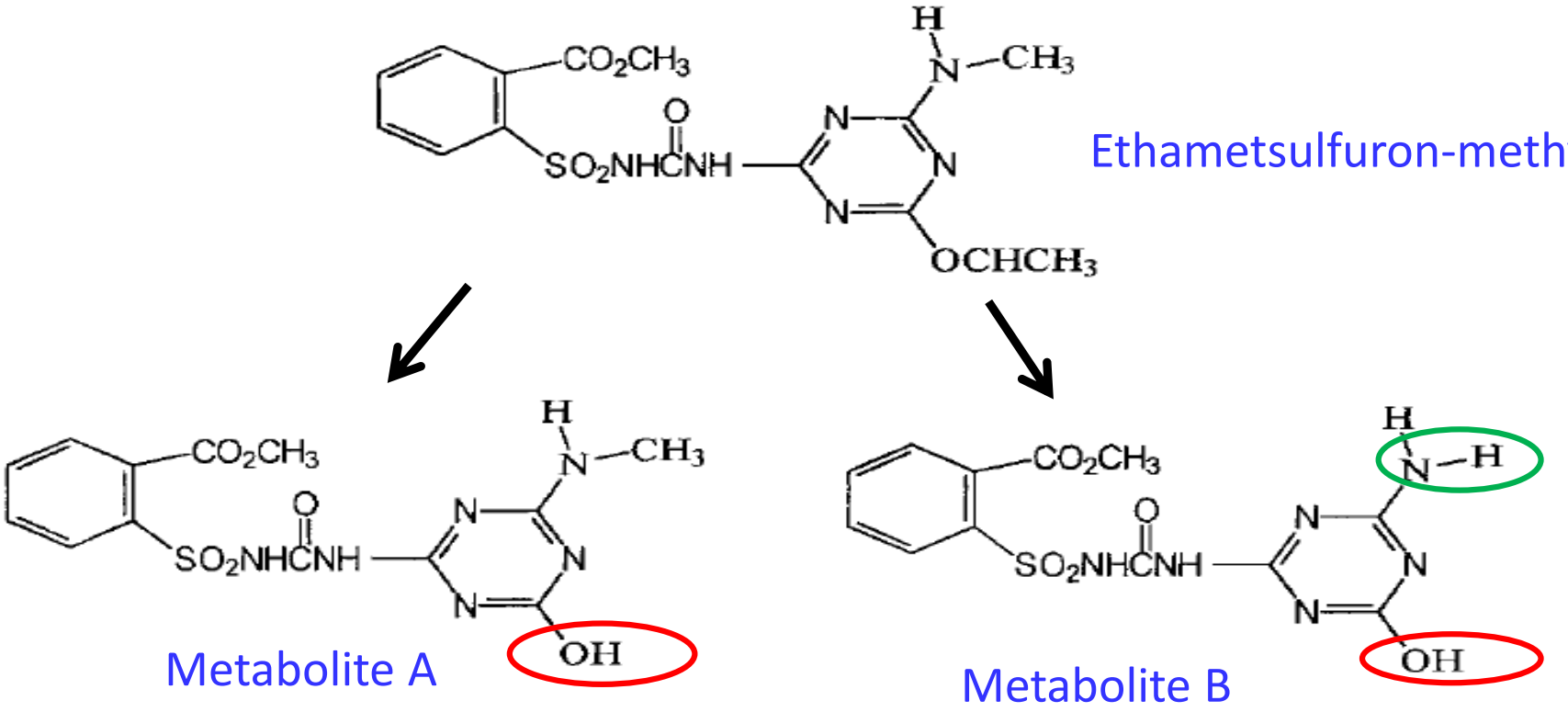
(Cotterman and Saari, 1992)



At least two P450s!

(Cotterman and Saari, 1992)

Ethametsulfuron-methyl metabolism in *Sinapis arvensis* (dicot)



At least two P450s!

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

Propoxycarbzone-sodium (SCT) in *Bromus tectorum*

Penoxsulam (TP) in *Echinochloa phyllopogon*

Enhanced metabolism involving P450s

(Park et al. 2004; Yasuor et al. 2009)

Non-target-site resistance (NTSR)

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

- **Fitness**

  Unknown

- **Genes involved largely unknown!**
  - ◊ Plants have high number P450 genes (200-400)
  - ◊ For a given herbicide, different P450/GT may be involved, in different weed species, in different populations of a given species, or even in different individuals of a given population.

(Preston 2003; Petit et al. 2010; Busi et al 2011, Han et al. 2013 unpub.)

Non-target-site resistance (NTSR)

- **Rapid progress in P450/GT gene discovery is expected!**

  L. rigidum: AHRI-Bayer CropScience, Germany

  A. myosuroides: Delye-Bayer CropScience, Germany

  E. phyllopogon : Japan

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 *(Christopher et al. 1992; Yu et al. 2008)*

- **Implications**

  Risk using low rates and metabolisable herbicides complication in resistance management

Summary

- **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
  - ◊ Metabolic resistance gene discovery
  - ◊ Diagnostic tools
  - ◊ Synergists to overcome metabolic resistance is a possibility (e.g. malathion).

Thank you!