More food needed to feed the world

Ever more People

Less Crop land

FAOSTAT data, 2005
Herbicides a great technological innovation, BUT too much of a good thing = hooked on herbicides.

Large genetically diverse weed populations + persistent herbicide selection = Evolution in action.
Australia has the world’s biggest herbicide resistance problem

WHY?

USA will become #1
Australian sheep then grain belt
*Lolium* seeded & nurtured across half a continent when the sheep was King
Sheep on *Lolium/Medicago* pasture then crops + herbicides + no till

**Area of crops**

**Sheep numbers**

<table>
<thead>
<tr>
<th>Year</th>
<th>Sheep numbers (Million)</th>
<th>Area of crops (ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1965</td>
<td>125</td>
<td>200</td>
</tr>
<tr>
<td>1974</td>
<td>150</td>
<td>175</td>
</tr>
<tr>
<td>1983</td>
<td>175</td>
<td>150</td>
</tr>
<tr>
<td>1992</td>
<td>200</td>
<td>125</td>
</tr>
<tr>
<td>2001</td>
<td>225</td>
<td>100</td>
</tr>
<tr>
<td>2010</td>
<td>250</td>
<td>75</td>
</tr>
</tbody>
</table>
Lolium and resistance in Australia – a unique combination of events

- High densities (1,000 seedlings m\(^{-2}\)) over vast areas (>60 m ha) because planted as pasture

- Genetically diverse & cross-pollinated

- Change from pasture to cropping without diversity, no tillage, universal herbicide reliance

- World’s lowest herbicide rates
12 million hectare Western Australian grainbelt

• In 1970 all *Lolium* susceptible to all herbicides

• In 2008 over 12 million hectares > 70% of crop fields infested with multiple herbicide resistant *Lolium*

• EVOLUTION IN ACTION
When I started back in medieval times (1983)

- 1983. First wheat field in Australia with herbicide (diclofop) resistant *Lolium*, Bordertown, S Aust. (J Heap & R Knight, JAIAS)

- Diclofop mode of action unknown

- I knew nothing about resistance!
**Acetyl coenzyme A carboxylase inhibiting herbicides**

- Inhibit plastidic ACCase, preventing fatty acid biosynthesis.
- Rapid and widespread adoption then resistance evolution across the nation.
- Three chemical classes; Fops, Dims and Dens
ACCase Gene

Big gene (many introns): >15,000 base pairs
Different forms: cytosolic + plastid forms
Plastid ACCase is the herbicide target

prokaryotic multisubunit ACC in plastids

eukaryotic multifunctional ACC in cytoplasm

eukaryotic multifunctional ACC in cytoplasm and plastids of GRASSES

BC  BCCP  CT

β  α
eukaryotic
prokaryotic
ACCase gene–carboxylase transferase domain

7 amino acid substitutions known to endow resistance in R *Lolium* populations
The pattern of target site resistance across ACCase herbicides in a Lolium plant depends on which and how many ACCase gene mutations accumulated AND whether heterozygous/homozygous PLUS the herbicide rate

e.g.
1781 heterozygous = diclofop resistant but clethodim S
1781 homozygous = clethodim R
(at the Aust. clethodim use rate)
Effect of ACCase gene resistance endowing mutations on *Lolium* fitness

- The Ile\textsubscript{178}Leu mutation has no resistance cost (no fitness penalty). It is the most common resistance mutation.
- The Asp\textsubscript{207}Gly mutation has a clear fitness penalty.
Cannot generalise on target site mutations

- Can be BIG, STRONG, DOMINANT mutations (eg Leu-1781-Ile). Even heterozygous individuals unaffected by normal herbicide rate.
- Can be weak mutations (eg Asp-2078-Gly). Heterozygous individuals killed by normal herbicide rate. Homozygous individuals survive but damaged.
- Evolution dictates that ALL possible survival mechanisms (weak or strong) selected.
- Selection intensity (rate) crucial!
- Cross pollination enables accumulation
Acetolactate synthase (ALS) inhibiting herbicides:

- Wheat selective chlorsulfuron from 1982, universally adopted for Lolium and dicot weed control.

- Now, 5 chemical classes:
  - Sulfonylureas, Imidazolinones, Triazolopyrimidines, Pyrimidinyloxybenzoic acids, Sulfonylanminocarbonyl triazolinones
  - Inhibit branched chain amino acid synthesis
Evolved ALS Target Site Mutations in Lolium

**Imidazolinone versus Sulfonylurea:**

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Residue</th>
<th>Substitution</th>
<th>Resistance Pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala</td>
<td>122</td>
<td>Thr, Val</td>
<td>IM only</td>
</tr>
<tr>
<td>Pro</td>
<td>197</td>
<td>Ala, Arg, Gln, Ser, Leu</td>
<td>SU only</td>
</tr>
<tr>
<td>Ala</td>
<td>205</td>
<td>Val</td>
<td>IM + SU</td>
</tr>
<tr>
<td>Asp</td>
<td>376</td>
<td>Glu</td>
<td>IM? + SU</td>
</tr>
<tr>
<td>Trp</td>
<td>574</td>
<td>Leu, Ser</td>
<td>IM + SU</td>
</tr>
<tr>
<td>Ser</td>
<td>653</td>
<td>Asn, Thr</td>
<td>IM only</td>
</tr>
<tr>
<td>Gly</td>
<td>654</td>
<td>Asp</td>
<td>IM + SU</td>
</tr>
</tbody>
</table>

- Switched to the completely new and different chlorsulfuron (Glean) - different chemistry, new unique mode of action (ALS).
- Amazingly, the diclofop resistant population was resistant to chlorsulfuron despite zero use in Australia
Lolium exhibits resistance to dissimilar herbicides, even herbicides not yet discovered! HOW COULD THIS BE?
Non target site resistance

Mechanism(s) minimising herbicide reaching target site enzyme
Herbicide detoxification by metabolism is a widespread and important resistance mechanism in *Lolium*.

Enhanced activity of P450 enzymes that can metabolise many different herbicides.

P450 enzymes active on ACCase, ALS and several other herbicide chemistries.

Little understanding at biochemical or molecular level (VABC ARC collab.).
WLR1 Selection at Low Dose

Gene Frequency

Year

Annual Ryegrass Number

- Pro-Ala 10-10
- Pro-Arg 10-6
- Metabolic 10-3
- Pro-Leu 10-4
- ARG
Glyphosate – world’s most important herbicide

Lolium in Australia was the first global case of evolved glyphosate resistance

Specific inhibitor of EPSP synthase
Glyphosate resistance in Lolium

• Glyphosate resistant *Lolium* populations exhibit one OR two resistance mechanisms:
  • Reduced glyphosate translocation mechanism.
  • Proline 106 EPSPS gene substitutions
Summary

• The evolution of herbicide resistance in *Lolium* in Australia occurs rapidly and can be widespread.
• Genetic diversity and cross pollination in *Lolium* enables enrichment and accumulation of all possible resistance genes.
• Low herbicide use rates and rate cutting in Australia means that all possible resistance genes enriched.
• Although each “paddock” is a distinct evolutionary event there is also some pollen mitigated gene flow site to site.
• Populations & individuals exhibit multiple target site mutations and metabolic resistance.
• Only some mutations endow a fitness penalty.
IS TIME RUNNING OUT?
Adoption of Glyphosate-Resistant Cultivars in the United States

**Soybean**
- 94%

**Cotton**
- 84%

**Maize**
- 43%
N Carolina grower

- Rotation = Corn, Soybean, Cotton
- RR Corn, RR Soybean, RR Cotton
1.3 kg ae glyphosate/ha
A dioecious annual herbaceous weed, growing to 1.5-3.0 m producing approx. 500,000 seed/year

Amaranthus palmeri
What can be done to manage resistance?
Diversity

• # 1 tool for managing herbicide resistant weeds = herbicide diversity!

When on a good thing, don’t stick to it!
  Judicious herbicide rotation/sequences
  Glyphosate-paraquat – sequence/rot’n
  Trifluralin-prosulfocarb rotation

Good agronomy – a vigorous healthy crop is a great weed biol. control agent!

Crop rotation – diversity that makes $ense
Aim to prevent seed production/survival

• In Australia, but not elsewhere in the world, there is widespread acceptance of strategies to minimise weed seed production or survival. Herbicides to minimise weed seed production (pasture topping, crop topping) and non-herbicide tools to kill weed seeds.
Integrated *Lolium* control

Target weaknesses in biology

- Early season emergence “flush”.
- Seed matures the same time as crop AND does not shatter, therefore standing at harvest.
- Seed has a short seed bank life.

Therefore: use effective herbicides, good agronomy to suppress *Lolium* and harvest management.

**KEY= Lolium seed control**
Collect the weed seed containing chaff fraction

- Up to 85% of ryegrass seed can be collected in the chaff fraction
Burn Narrow windrows
Bale everything!!
Harrington harvest seed destructor
“Man Should Not Live by Bread Alone!”

Professor Dyno Keatinge
Director General, AVRDC-
The World Vegetable Centre, Taiwan

11 December 2008
11:00 - 12:00
Molecular & Chemical Sciences
Lecture Theatre (G.33), UWA
(Fairway, Entrance No. 4, Car Park 14 and 21)