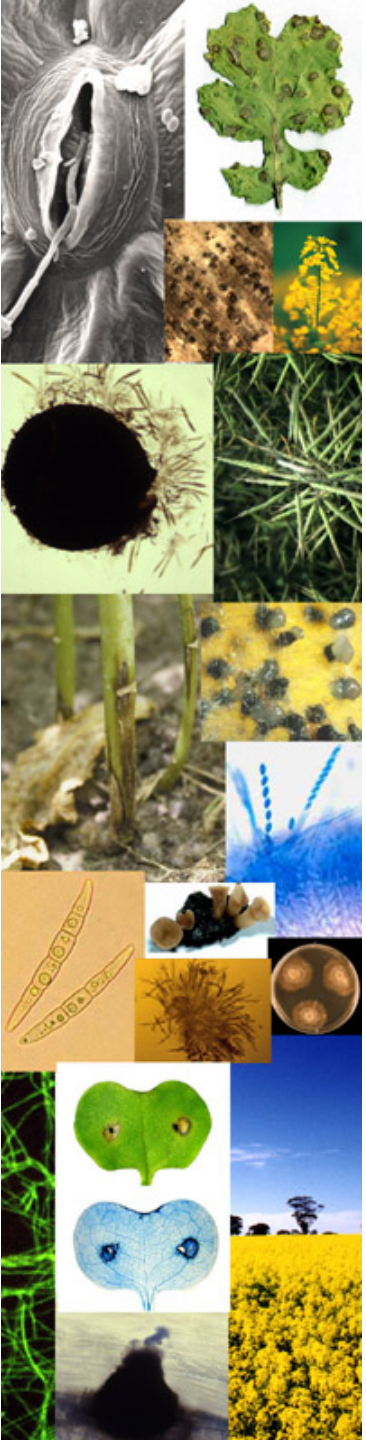

Propensity of the blackleg fungus developing fungicide resistance

Barbara Howlett,
School of Botany,
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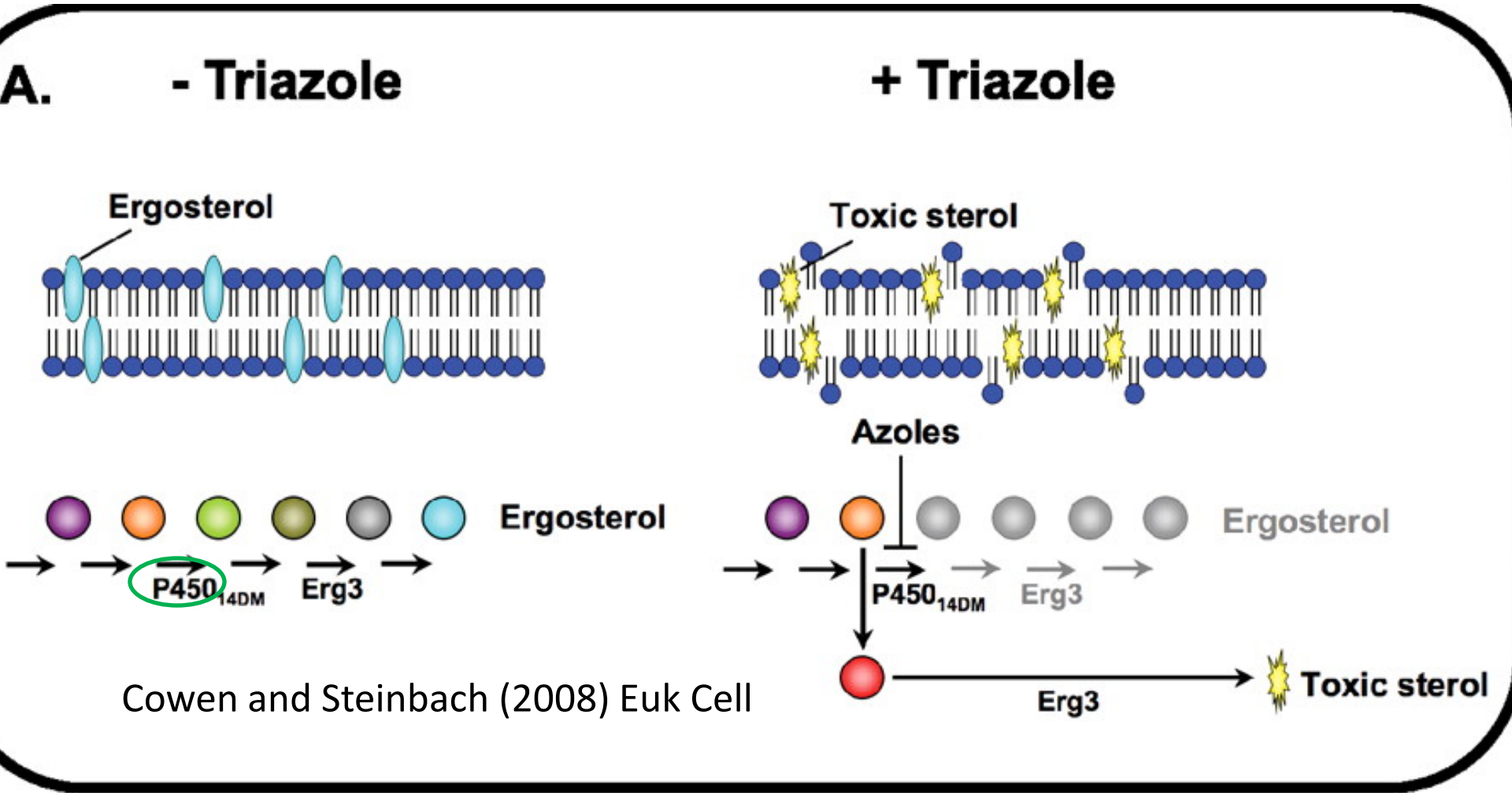
Fungicides for blackleg in Australia

- Metalaxyl (phenylamide) and fludioxononil (phenylpyrrole): Maxim XL: seed
- controls Pythium, not blackleg

- Flutriafol: Impact in Furrow
- Fluquinconazole: Jockey: seed
- Tebuconazole & Prothioconazole: Prosaro: foliar (6 leaf stage) in April 2012?
- All 3 have same mode of action: triazoles/Demethylation inhibitors(DMI)/Ergosterol Biosynthesis Inhibitors (EBI)/class 3

Action of Azole fungicides



Cowen and Steinbach (2008) Euk Cell

Mechanisms of triazole resistance:

mutations in Cyp51 (P450)- V136A, Y137F, I381V; Mutation and /or overexpression of membrane pumps (ABC & MFS transporters)

Mutations and Fungicide Resistance; a number's game

- Fungal populations may have resistant mutant naturally occurring at frequency of 1 in 10^{9} spores
- Selection pressure from repeated fungicide use increases frequency of mutants
- Becomes apparent when frequency of 1 in 10^{2} or frequency of 1 in 10^{1} spores
- Thus resistance can appear suddenly, but may have been building up insidiously
- Have to screen large numbers of isolates to find ones that are resistant

Fungicide Resistance Risk factors: legumes & cereals

Diseases

High risk	Medium risk	Low risk
Wind borne spores sexual & asexual	Rain splash spores History; Monocyclic	Soil or water borne spores; asexual;
Powdery mildew	Ascochytas	Rusts (asexual)
		oomycetes

Fungicides

High risk	Medium risk	Low risk
Strobilurins	DMIs	
Benzimidazoles	SDHI	
Iprodione		

Applications

High risk	Low risk
Repeats of same MOA s	Rotation of MOA groups
Full and repeated doses	Low dose
Foliar?	Seed?

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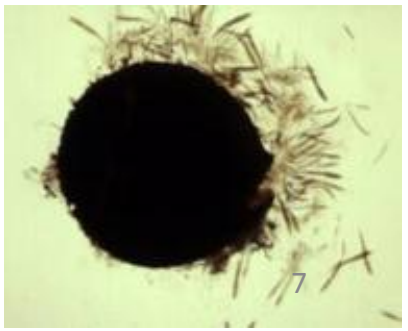
L. maculans - a high risk pathogen for 'overcoming' disease resistance

- Prolific sexual and asexual reproduction
  - Windborne sexual spores (annual sexual cycle);
  - Rainsplash asexual spores (multicyclic)
- Large populations (inoculum) of recombinants
  - Populations evolve very quickly
  - Major gene resistance in canola lines results in strong selection pressure towards virulent isolates
- Major gene resistance overcome





L. maculans - a high risk fungus to develop fungicide resistance I ??

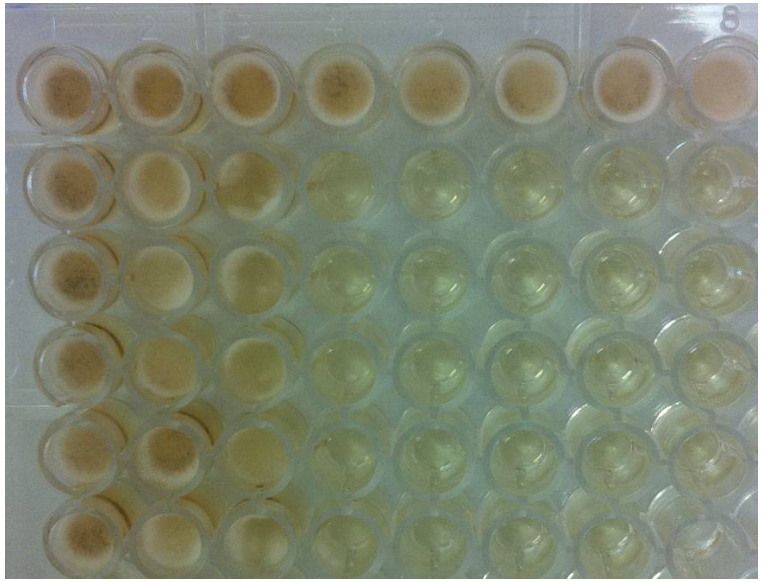
- Haploid fungus: mutations have direct effect on phenotype
- Sexual cycle gives large numbers of recombinants and allows combining of different mutations conferring resistance
- 90% oilseed rape crops in UK have foliar azole spray: no reports of resistance. Climatic conditions in Europe do not favour prolific sexual crossing
- Related fungus *Mycosphaerella graminicola* (Septoria leaf spot) has resistance to triazoles and strobilurin in Europe

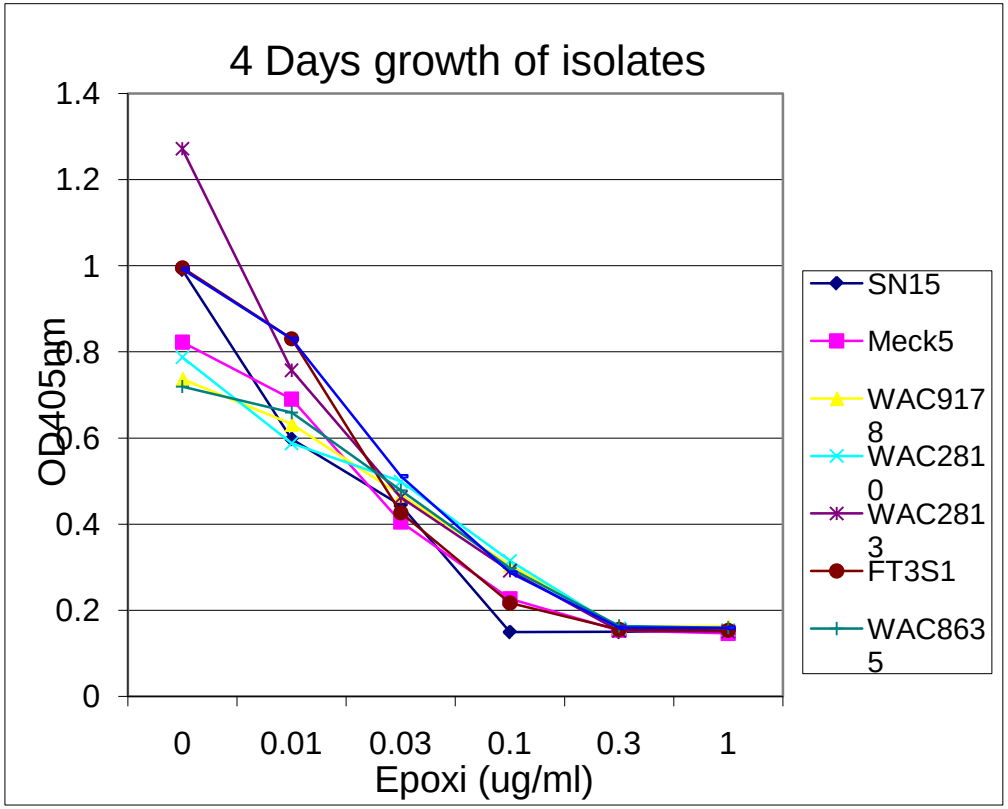
L. maculans - a high risk fungus to develop fungicide resistance II?

- Genome analysis of *L.maculans* shows that:
  - Particular amino acids in Cyp51 are present that when mutated confer to triazole resistance
  - The G143A site in mitochondrial DNA that confers strobilurin resistance is present and can be mutated to resistance
- *L.maculans* has the molecular features that could enable it to develop fungicide resistance under sufficient selection pressure such as repeated use of high levels of same mode of action of fungicides
- Firstly need to determine base-line sensitivities to fungicides

Measuring base-line sensitivity to fungicides *in vitro*

Stagonospora nodorum and Epoxiconazole
Different isolates can have different sensitivities





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Measuring base-line sensitivity to fungicides : glasshouse

Apply fungicides; after 16 h spray fungus on leaves; measure disease

Richard Oliver

Recommendations and Take Home messages

- Determine base-line sensitivities for at risk pathogen/fungicide combinations (Oliver -GRDC grant)
- Evaluate current disease/loss models to ensure fungicides not used unnecessarily
- Introduce new modes of action fungicides
- Maintain adequate break crops – not canola on canola
- Repeated higher doses of fungicides probably will promote resistance (unlike herbicides)
- Keep breeding for disease resistance
- Improve awareness of growers
- Need Australian industry/research/farmer group focussing on fungicide resistance
- Acknowledgments: Richard Oliver, ACNFP, Perth & Derek Hollomon ：www.frac.info/frac/index.htm