THE UNIVERSITY OF MELBOURNE

Off-target effects of herbicides on Blackleg

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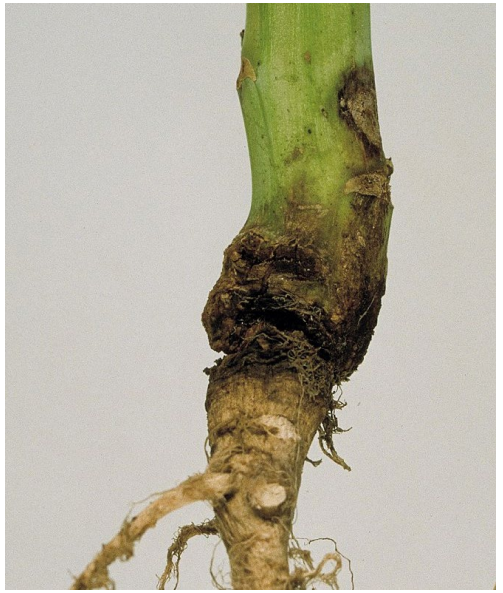
Leptosphaeria maculans

- Fungal pathogen that causes ‘Blackleg disease’ in canola
  - Stem cankers, leaf lesions, upper canopy infection
- Causes an average yield loss of 20-30% in Australia
  - Impacts $6b canola industry



Canola pathogens and herbicides

- Murtza *et al.* 2021 found that herbicides can inhibit various pathogens on canola
  - Timing impacts the disease effect
- *L. maculans* (Blackleg) was not tested, nor were herbicides used on Clearfield® lines

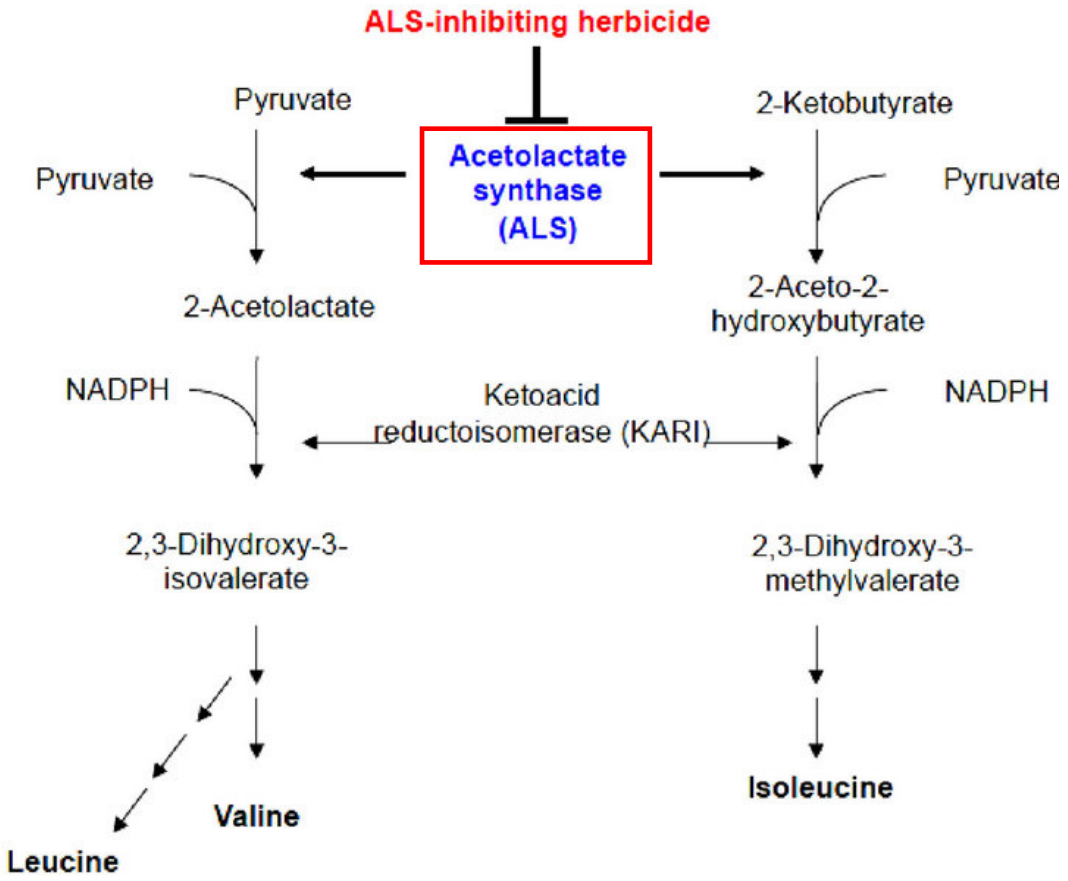
TABLE 2 Symbolic representation of effect of herbicides (atrazine and glyphosate) on each pathogen (*Neopseudocercosporella capsellae*, *Alternaria brassicae*, *A. japonica*, and *Hyaloperonospora brassicae*) for disease parameters %LDI and %LAD with respect to herbicide application pre- and postinoculation with pathogen

		Atrazine		Glyphosate	
Parameter	Pathogen	Preinoculation	Postinoculation	Preinoculation	Postinoculation
%LDI	*N. capsellae*	+	–	+	+
	A. brassicae	+	–	++	–
	A. japonica	+	–	++	–
	H. brassicae	– –	–	– – –	–
%LAD	*N. capsellae*	+	–	+	+
	A. brassicae	+	–	++	–
	A. japonica	+	–	++	–
	H. brassicae	–	–	– – –	–

Note: +, enhanced disease; ++ a stronger effect; +++ an even stronger effect in terms of disease enhancement.
– lower disease; – – a stronger effect; – – – an even stronger effect in terms of disease reduction.

Acetolactate synthase (ALS) inhibitors

- Chemicals which impact the synthesis of essential amino acids
  - Branched chain amino acids (BCAAs)
    - Isoleucine
    - Leucine
    - Valine
- Commonly found in a range of herbicides
  - E.g. Intervix®, Raptor®, Staple®, Atlantis®, Paradigm®

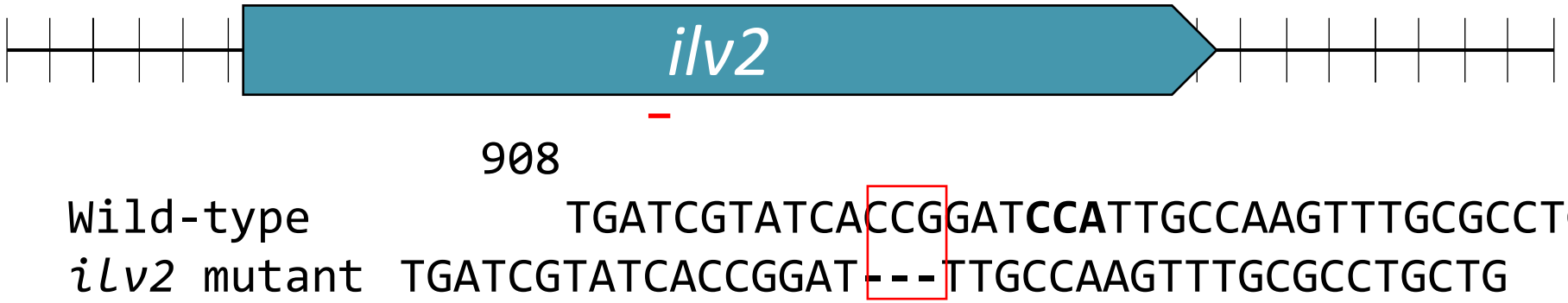


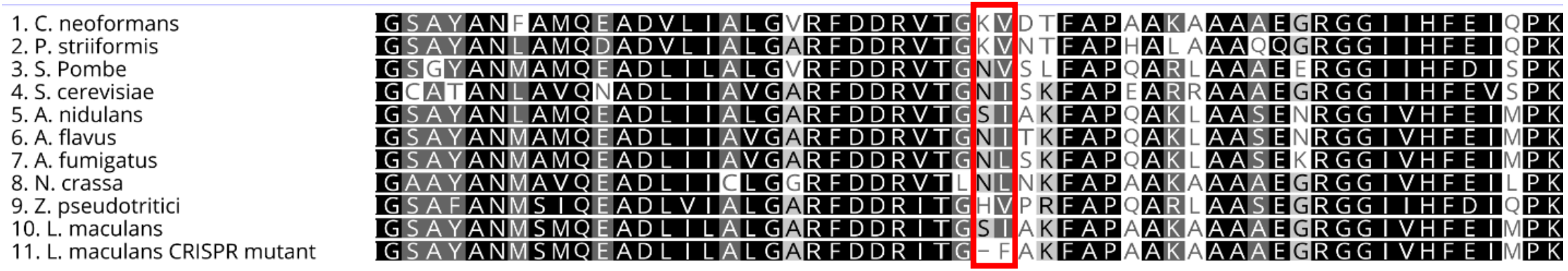
Blackleg and branched chain amino acids (BCAAs)

- *Leptosphaeria maculans* requires BCAAs to remain viable
- Canola plants have very low levels of BCAAs in their leaves
  - *L. maculans* typically makes these amino acids
- The *ilv2* gene encodes acetolactate synthase in *L. maculans*

Testing ALS function in *L. maculans*

Generation of a mutant strain with non-functional *ilv2*





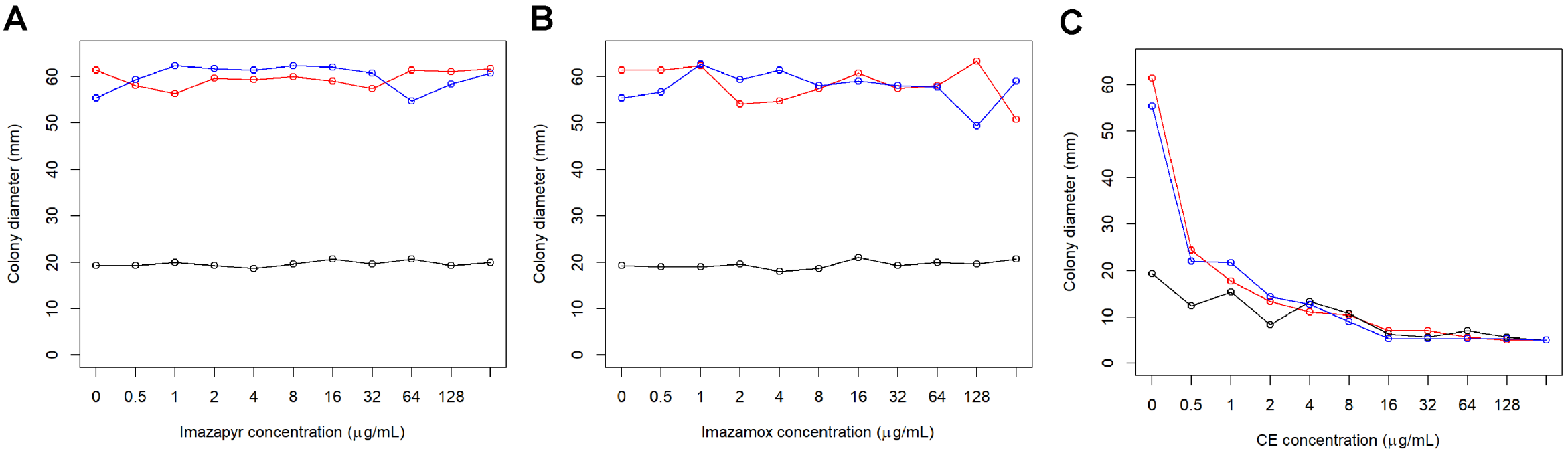
Testing ALS function in *L. maculans*

Strains:

- *ilv2* mutant
  - Non-functional *ilv2*
- *ilv2* complemented strain
  - *ilv2* mutant with a functional *ilv2* copy inserted
- Wild-types

In vitro growth assay with herbicide chemicals

- Imazapyr and Imazamox are active ingredients in Intervix®
- Chlorimuron ethyl (CE) inhibits growth in other fungi



Pathogenicity of ALS-deficient mutant

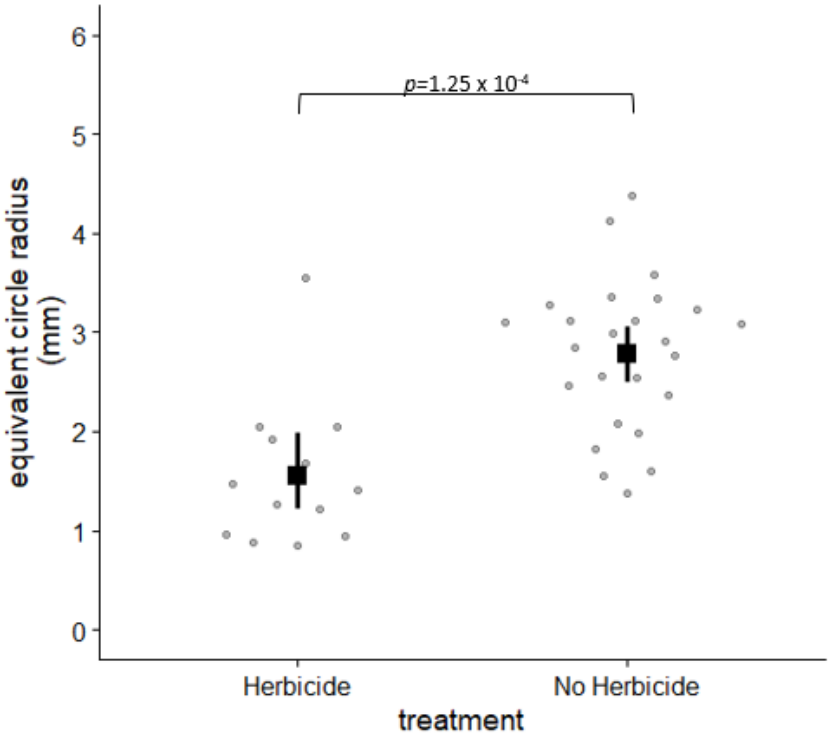
- The *ilv2* mutant is non-pathogenic
  - Pathogenicity is restored through complementation
- *ilv2* is a pathogenicity-related gene

Intervix® inhibits Blackleg

- Application of an ALS inhibitor herbicide results in smaller disease lesions

Herbicide applied

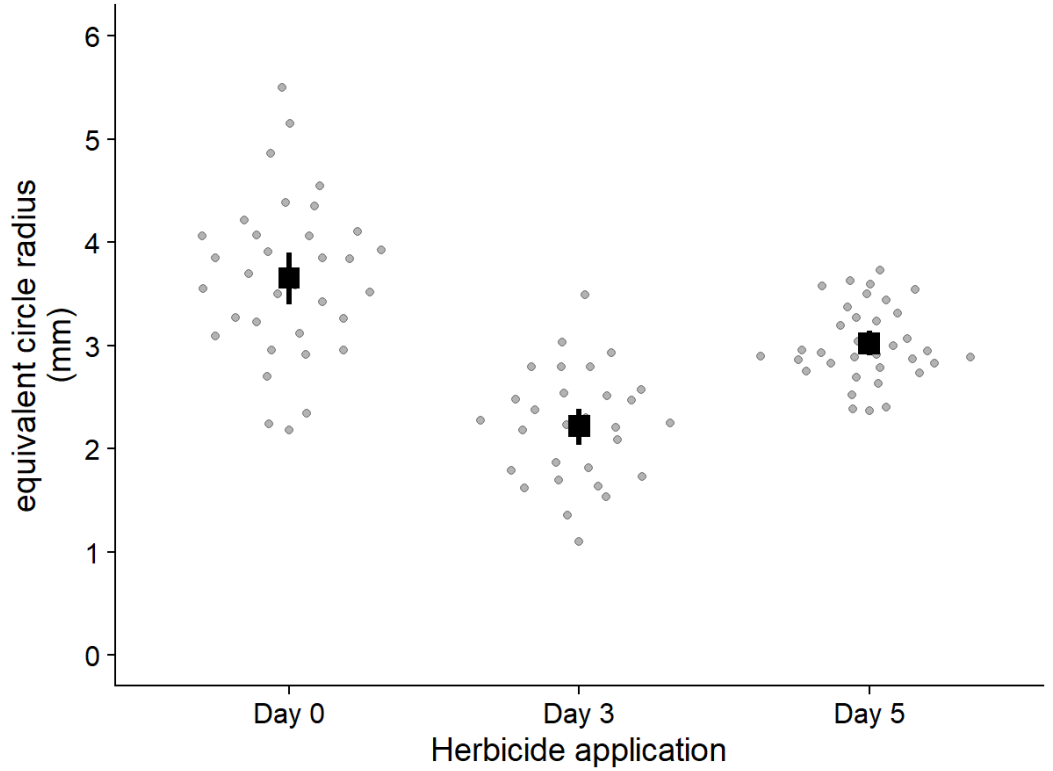
No Herbicide



Timing of Intervix® on Blackleg

- Lesion size is smallest when Intervix® is applied 3 days after disease inoculation
  - As compared to 0 days and 5 days

Water control | No Herbicide | Day 0 Herbicide Application | Day 3 Herbicide Application | Day 5 Herbicide Application



Conclusions

- ALS inhibitor herbicide Intervix® inhibits *L. maculans* on canola plants
  - Smaller disease lesions
  - Not as effective as commercial fungicides
- Timing of herbicide application impacts disease
  - Herbicide applied 3 days after inoculation had smaller lesions
- *ilv2* is a pathogenicity gene in *L. maculans*

Thank you

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Lesion area statistics

- Equivalent circle radius method
  - Calculate the radius of a circle with an area equal to the lesion