



THE UNIVERSITY OF
MELBOURNE

Off-target effects of herbicides on Blackleg

Nick Chong

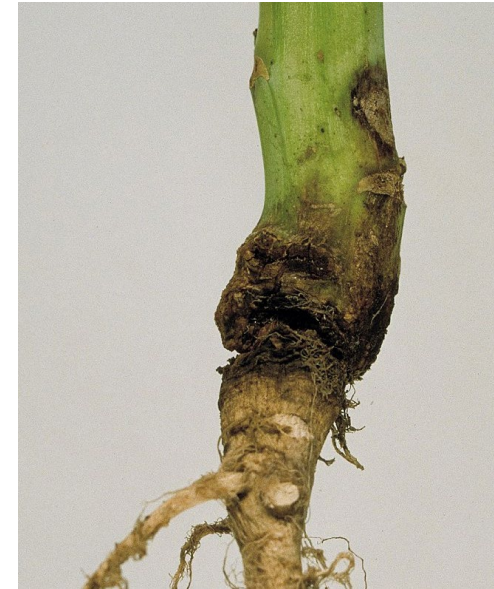
PhD Candidate





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

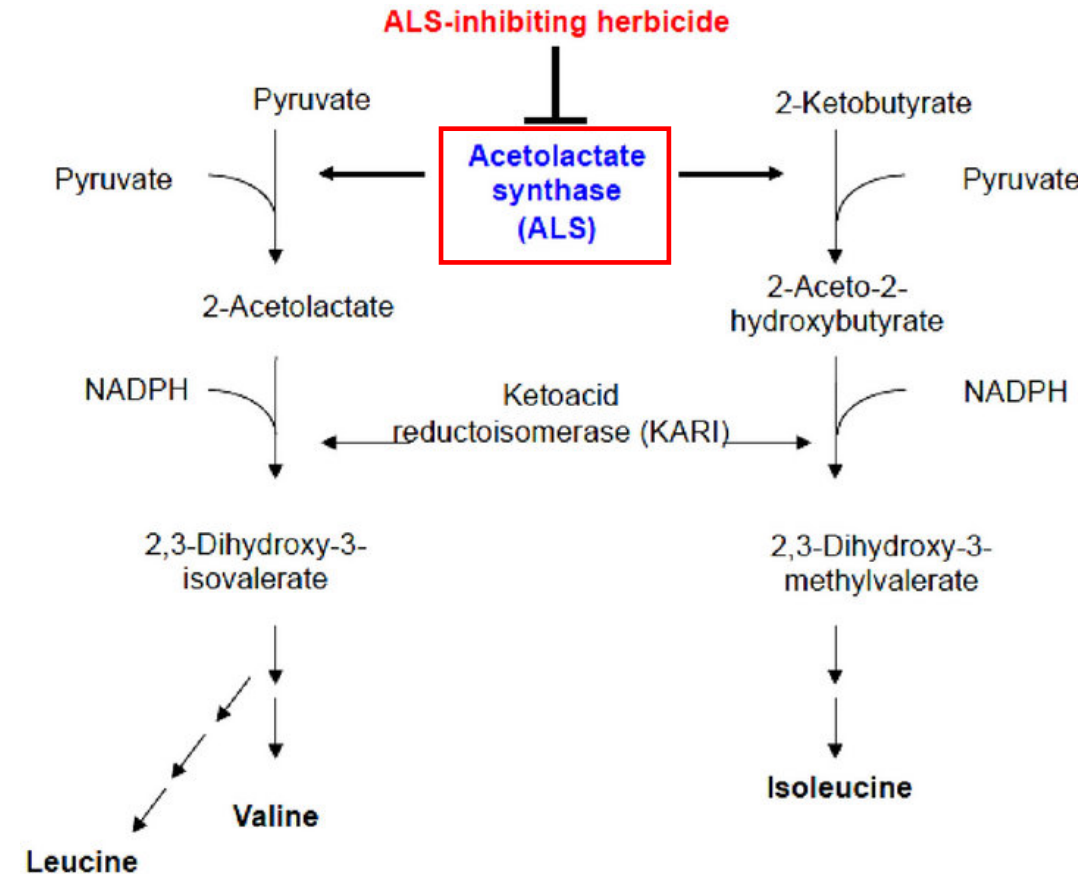
TABLE 2 Symbolic representation of effect of herbicides (atrazine and glyphosate) on each pathogen (*Neopseudocercospora capsellae*, *Alternaria brassicae*, *A. japonica*, and *Hyaloperonospora brassicae*) for disease parameters %LDI and %LAD with respect to herbicide application pre- and postinoculation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

Parameter	Pathogen	Atrazine		Glyphosate	
		Preinoculation	Postinoculation	Preinoculation	Postinoculation
%LDI	<i>N. capsellae</i>	+	-	+	+
	<i>A. brassicae</i>	+	-	++	-
	<i>A. japonica</i>	+	-	++	-
	<i>H. brassicae</i>	--	-	---	-
%LAD	<i>N. capsellae</i>	+	-	+	+
	<i>A. brassicae</i>	+	-	++	-
	<i>A. japonica</i>	+	-	++	-
	<i>H. brassicae</i>	-	-	---	-

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*



908

948

Wild-type

TGATCGTATCACCGGATCCATTGCCAAGTTTGCGCCTGCTG

ilv2 mutant

TGATCGTATCACCGGAT---TTGCCAAGTTTGCGCCTGCTG

1. *C. neoformans*
2. *P. striiformis*
3. *S. Pombe*
4. *S. cerevisiae*
5. *A. nidulans*
6. *A. flavus*
7. *A. fumigatus*
8. *N. crassa*
9. *Z. pseudotritici*
10. *L. maculans*
11. *L. maculans* CRISPR mutant

1. <i>C. neoformans</i>	GSAYANFAMQEADVLIALGVRFDDRVTGKVDTFAPAAKAAAAGRGGIIHFELQPK
2. <i>P. striiformis</i>	GSAYANLAMQDADVLIALGARFDDRVTGKVNTFAPHALAAAQQRGGIIHFELQPK
3. <i>S. Pombe</i>	SGSYANMAMQEADLILALGVRFDDRVTGNVSLFAPQARLAAAEEERGGIIHFDISPK
4. <i>S. cerevisiae</i>	GCATANLAVQNADLIIAVGARFDDRVTGNISKFAPPEARAAAAGRGGIIHFELVSPK
5. <i>A. nidulans</i>	GSAYANLAMQEADLIIALGARFDDRVTGSIKAFAPQAKLAAASENRGGIVHFELMPK
6. <i>A. flavus</i>	GSAYANMAMQEADLIIAVGARFDDRVTGNITKFAPQAKLAAASENRGGIVHFELMPK
7. <i>A. fumigatus</i>	GSAYANMAMQEADLIIAVGARFDDRVTGNLSKFAPQAKLAAASEKRRGGIVHFELMPK
8. <i>N. crassa</i>	GAAYANMAVQEADLIIICLGGRFDDRVTLNLNKFAPAAKAAAAGRGGIVHFELLPK
9. <i>Z. pseudotritici</i>	GSAFANMSIQEADLVIALGARFDDRITGHVPRFAPQARLAAASEGRGGIIHFDIQPK
10. <i>L. maculans</i>	GSAYANMSMQEADLILALGARFDDRITGSIKAFAPAAKAAAAGRGGIVHFELMPK
11. <i>L. maculans</i> CRISPR mutant	GSAYANMSMQEADLILALGARFDDRITG-FAKAFAPAAKAAAAGRGGIVHFELMPK



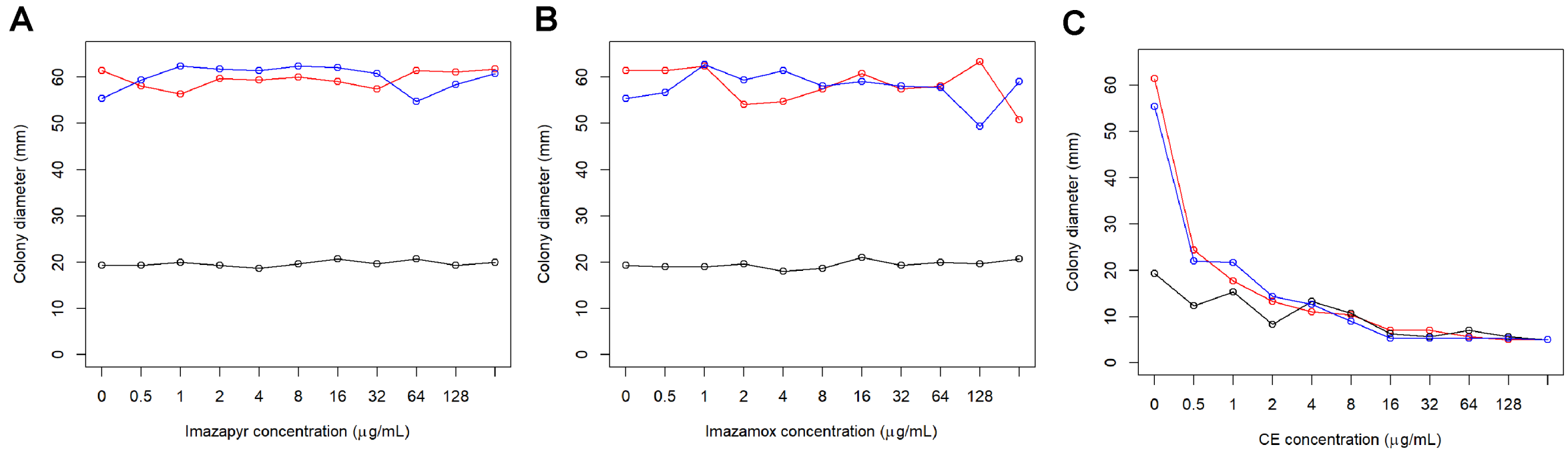
Testing ALS function in *L. maculans*

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



— Wild-type
 — *ilv2* mutant
 — complemented

Pathogenicity of ALS-deficient mutant

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



Water control

Wild-type (D22)

ilv2 mutant

ilv2 comp 6

Intervix® inhibits Blackleg

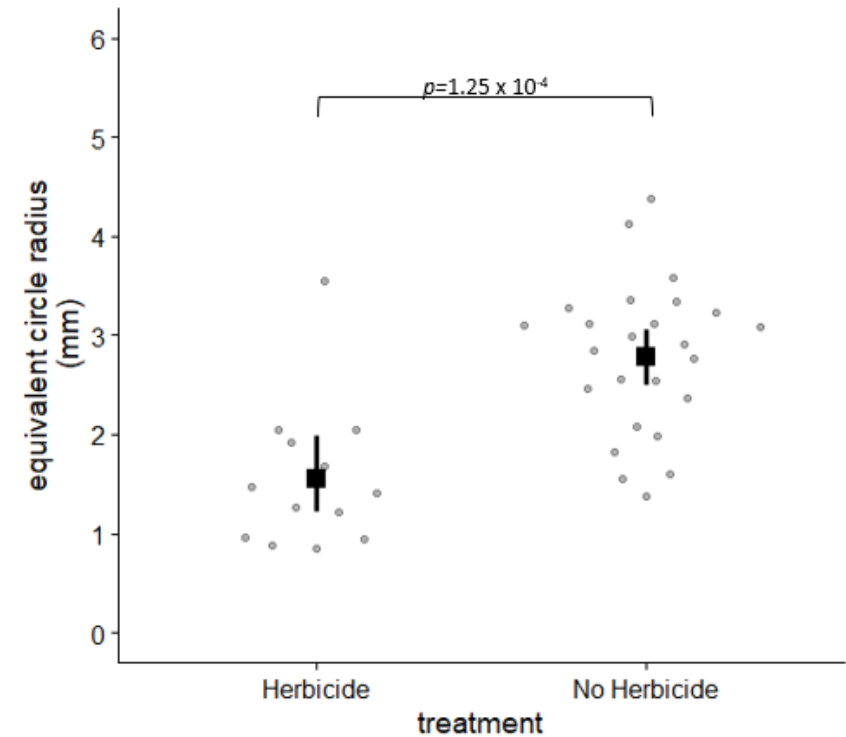
- Application of an ALS inhibitor herbicide results in smaller disease lesions



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



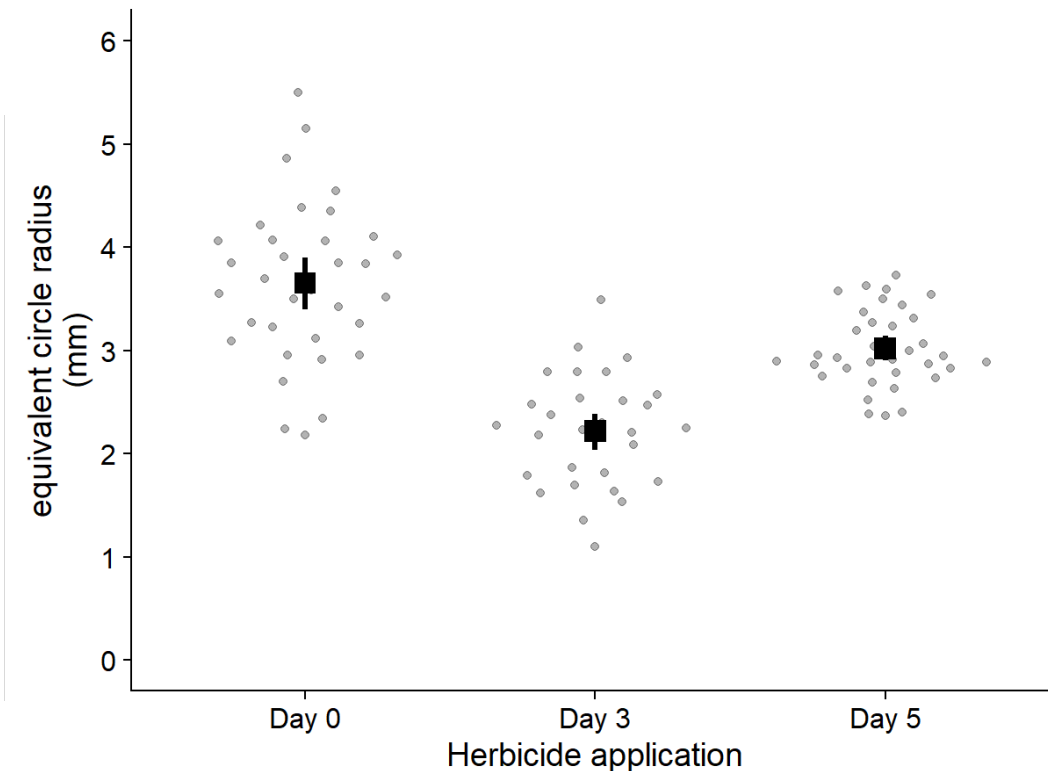
Water control

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*



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Thank you

Nick Chong

PhD Candidate



Lesion area statistics

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

