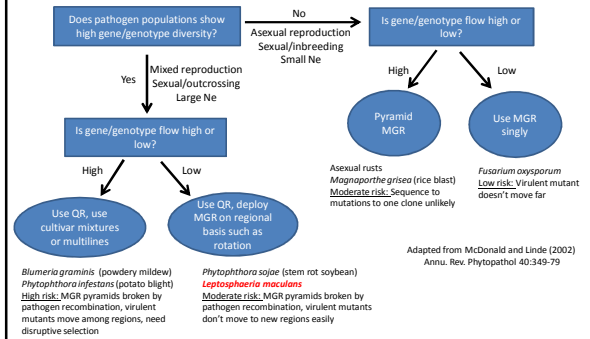


Virulence evolution, rotation and resistance stacking

Angela Van de Wouw
Canola Pathology Meeting 2014

Rotation of resistance genes has been suggested for sexually reproducing pathogens



Evolution of virulence in *L. maculans* populations

- What is the impact of rotation of resistance genes on virulence evolution?
- Are we selecting for isolates that are virulent towards multiple resistance genes?
- What is the impact of stacking of resistance genes on virulence evolution?

Tools:

- We have isolate collections and spore trap populations available to address these questions.
- We have the breakdown of resistance on the Eyre Peninsula involving Rlm1

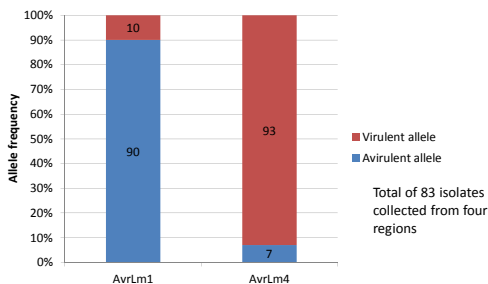
In the beginning – Gen0

- Assume allele frequencies are:

Allele	Nomenclature	Percentage	Frequency
AvrLm1	p1	90%	0.90
avrLm1	q1	10%	0.10
AvrLm4	p2	10%	0.10
avrLm4	q2	90%	0.90

- Hardy-Weinberg principle
- NO FITNESS PENALTY

Allele frequencies of isolates collected between 1997 and 2000



Applying Hardy-Weinberg (HW) principle - Gen0

Allele	Nomenclature	Percentage	Frequency
AvrLm1	p1	90%	0.90
avrLm1	q1	10%	0.10
AvrLm4	p2	10%	0.10
avrLm4	q2	90%	0.90

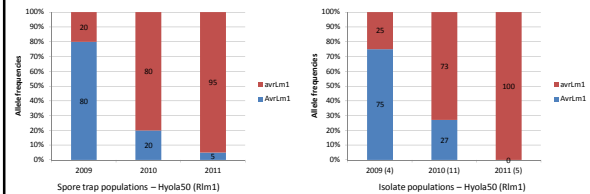
HW	Genotype	Freq	Percentage	HW allele
p1p2	Avr1Avr4	0.09	9	x11
p1q2	Avr1avr4	0.81	81	x12
q1p2	avr1Avr4	0.01	1	x21
q1q2	avr1avr4	0.09	9	x22

Gen1 – Selection with *Rlm1*

- If cultivars grown with *Rlm1*, *AvrLm1* isolates will be selected against.
 - How much selection is acting on the population?
 - How does this impact on allele frequencies?
- Spore trap populations and single isolates can be used to estimate selection pressure.

Population changes over three years

- AvrLm1* allele frequencies change dramatically over three years of selection with *Rlm1*
 - Year 1 = 80% survival of *AvrLm1* isolates
 - Year 2 = 20% survival of *AvrLm1* isolates
 - Year 3 = 5% survival of *AvrLm1* isolates



Changes in allele frequencies in Gen1

- Adjust allele frequencies due to selection

Genotype	AvrLm1	Equation term	Freq	# in 1000 spores	x80% AvrLm1	New freq
p1p2	Avr1Avr4	x11	0.09	90	72	0.085207
p1q2	Avr1avr4	x12	0.81	810	648	0.766864
q1p2	avr1Avr4	x21	0.01	10	12.5	0.014799
q1q2	avr1avr4	x22	0.09	90	112.5	0.133136
				1000	845	1

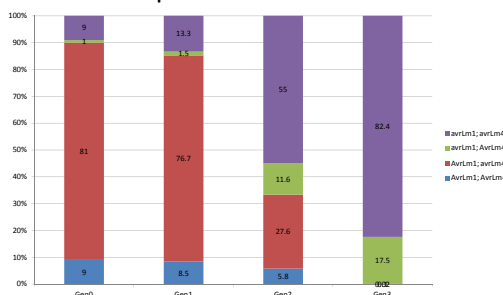
Genotype Parent 1	Genotype Parent 2	Freq	x11	x12	x21	x22	
A1A4	x11	A1A4	x11	0.00726025	1	0	0
A1a4	x12	A1a4	x12	0.06534225	0.5	0.5	0
a1A4	X21	a1A4	X21	0.00136046	0.5	0	0.5
a1a4	X22	a1a4	X22	0.01134414	0.25	0.25	0.25
A1a4	x12	A1a4	x11	0.06534225	0.5	0.5	0
A1a4	x12	A1a4	x12	0.58808025	0	1	0
a1A4	X21	a1A4	X21	0.01134414	0.25	0.25	0.25
a1a4	X22	a1a4	X22	0.10209727	0	0.5	0
a1A4	x21	A1A4	x11	0.00136046	0.25	0.25	0.25
a1A4	x21	A1a4	x12	0.01134414	0.25	0.25	0.25
a1A4	x21	a1A4	X21	0.00021883	0	0	1
a1a4	X22	a1a4	X22	0.00596947	0	0	0.5
a1a4	x22	A1a4	x11	0.01134414	0.25	0.25	0.25
a1a4	x22	A1a4	x12	0.10209727	0	0.5	0
a1a4	x22	a1A4	X21	0.00196947	0	0	0.5
a1a4	x22	a1a4	X22	0.01772522	0	0	1

Frequency of avirulence alleles in Gen1 after selection with *Rlm1*

Allele	Nomenclature	Frequency	Percentage
<i>AvrLm1</i>	¹ p1	0.852	85.2%
<i>avrLm1</i>	¹ q1	0.147	14.7%
<i>AvrLm4</i>	¹ p2	0.099	9.9%
<i>avrLm4</i>	¹ q2	0.901	90.1%

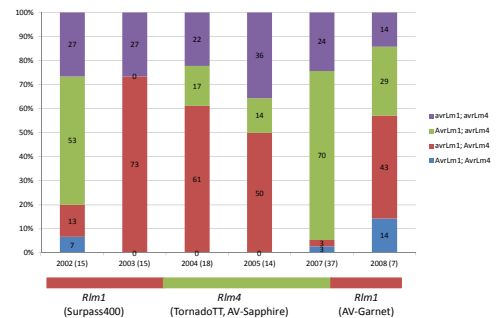
HW	Genotype	Freq	Percentage
p1p2	<i>Avr1Avr4</i>	0.084	8.4%
p1q2	<i>Avr1avr4</i>	0.767	76.7%
q1p2	<i>avr1Avr4</i>	0.014	1.47%
q1q2	<i>avr1avr4</i>	0.133	13.3%

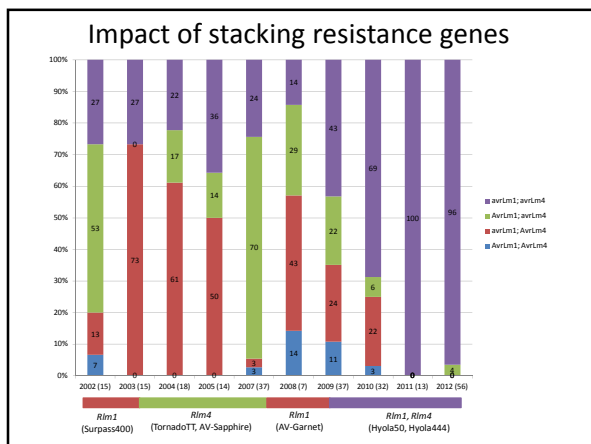
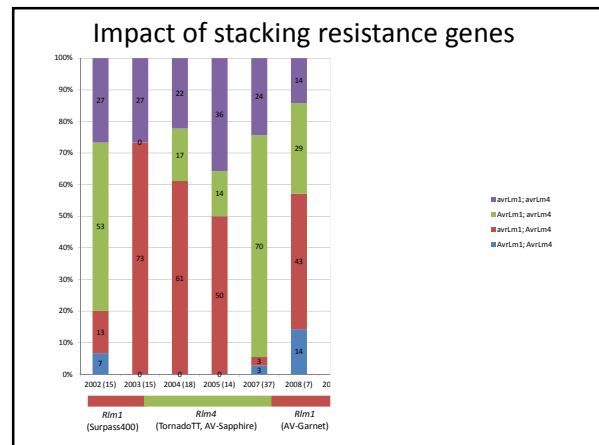
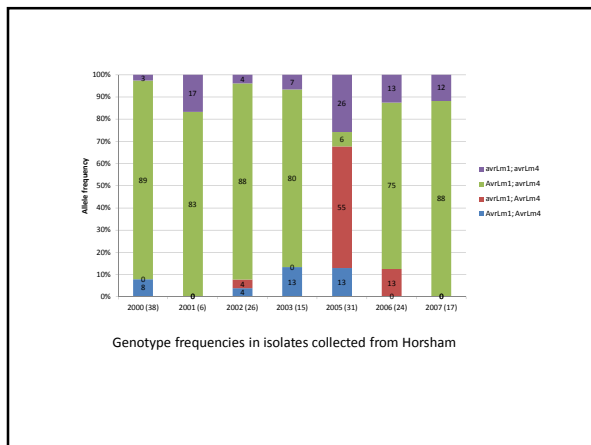
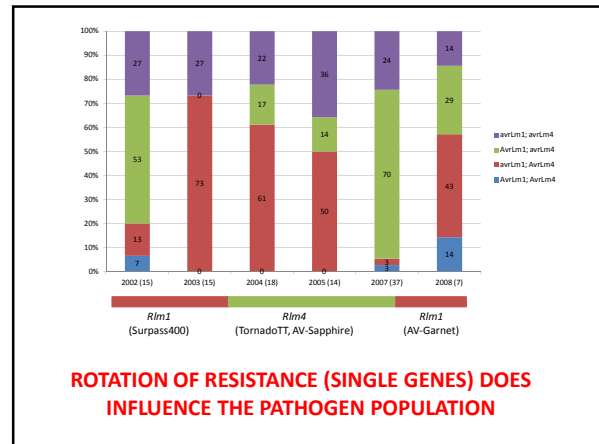
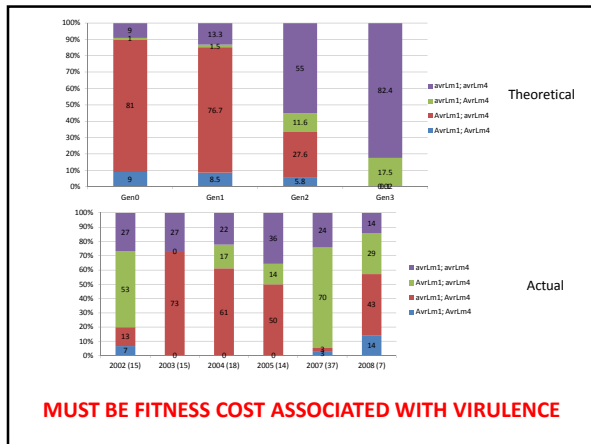
Theoretical changes in genotype frequencies over time



- Majority of isolates are virulent towards both isolates
- Assumes NO FITNESS PENALTY

Actual genotype frequency data





Summary of findings

- Fitness costs must be associated with virulence
- Rotation of single resistance genes can be used to manipulate the blackleg population
- Stacking of resistance gene will select for isolates with virulence to multiple resistance genes