



Using detached leaves and pods to screen for resistance to *Phomopsis* (*Diaporthe toxica*) in *Lupinus albus*

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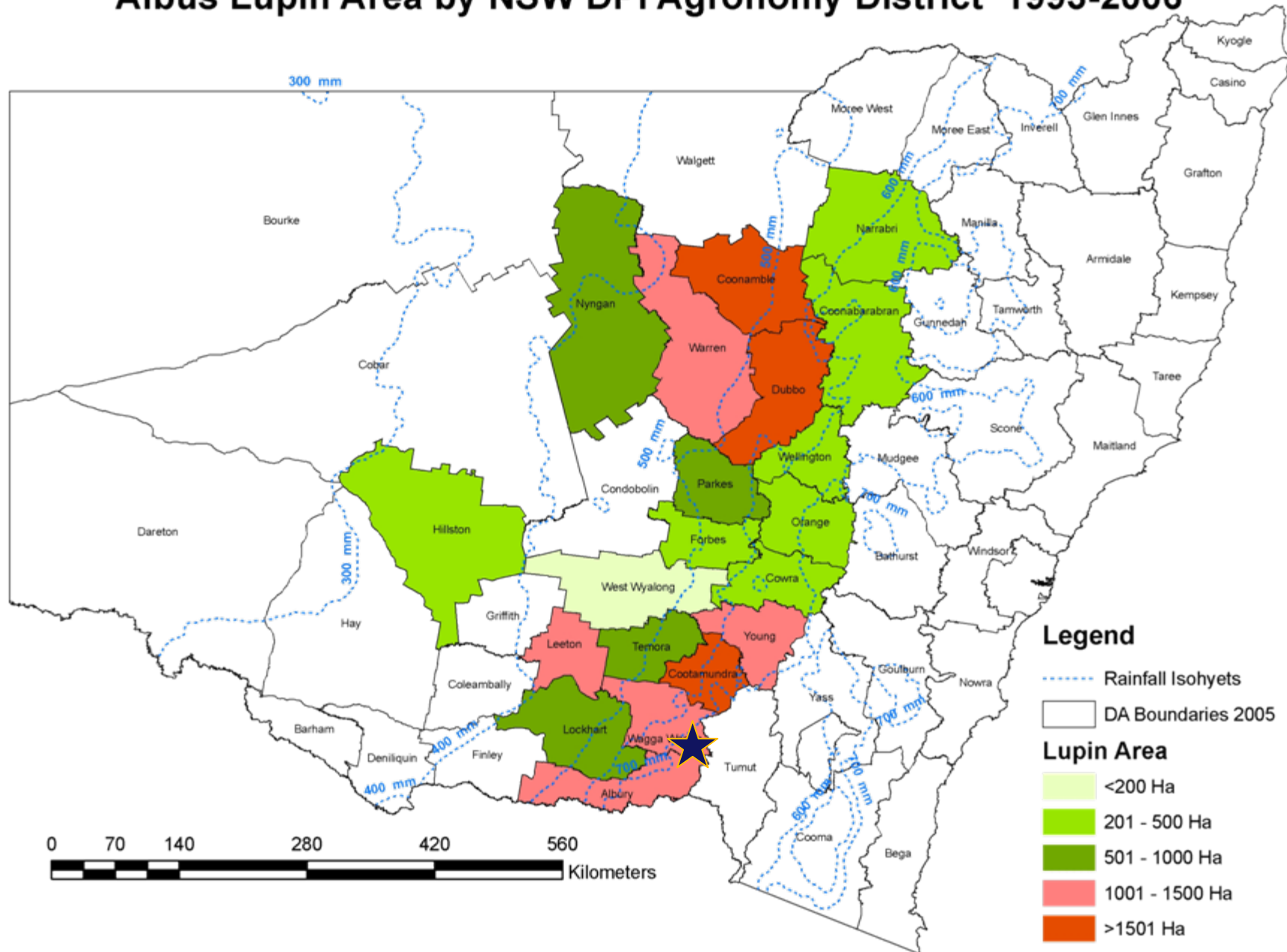
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Introduction



- Albus cropping areas in NSW
- Phomopsis in albus
- Multiphase experiments
- Detached leaf assay
- Detached pod assay
- Correlations

Albus Lupin Area by NSW DPI Agronomy District 1993-2006



Phomopsis in albus in NSW

What happened in Tarcutta in 2004?

- Late season hail damaged crop
- Stubble grazed for 10 days over summer before sick sheep noted and removed from stubble
- Post-mortem testing confirmed lupinosis
- Phomopsis identified from albus stubble and infected seed
- Final stock loss was around 100 ewes and weaners



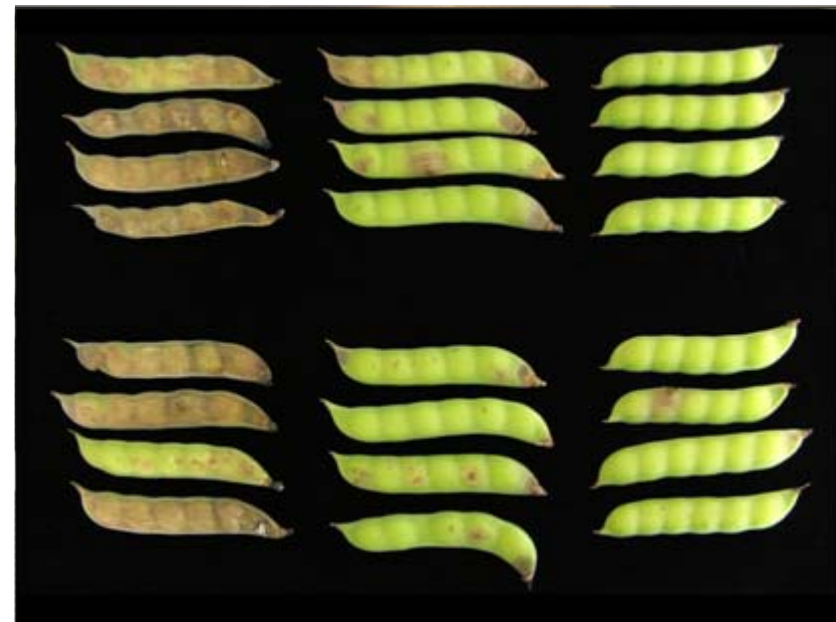
Research questions



- Was the phomopsis infection at Tarcutta due only to the environmental conditions?
- Or, had the fungus developed a strain that was more virulent on albus?
- If so, what is the extent of risk to the industry should this strain become widespread?
- And, can genetic resistance to the ‘virulent’ strain be identified in germplasm and current breeding lines?

Investigating phomopsis in albus

- To date a number of experiments have been conducted
 - Conventional glasshouse screening
 - Field elevation under natural infection
 - Stem infection
 - Seed infection
 - Pod infection
 - Leaf infection



Experimental design

- Consisted of two phases
 1. Field replication
 2. Laboratory procedure
- Problem – limited ability to assess both field and lab errors
- Statisticians have addressed this problem and have come up with a “multi-phase”



Example of inadequate replication in phase 2 of a multiphase experiment

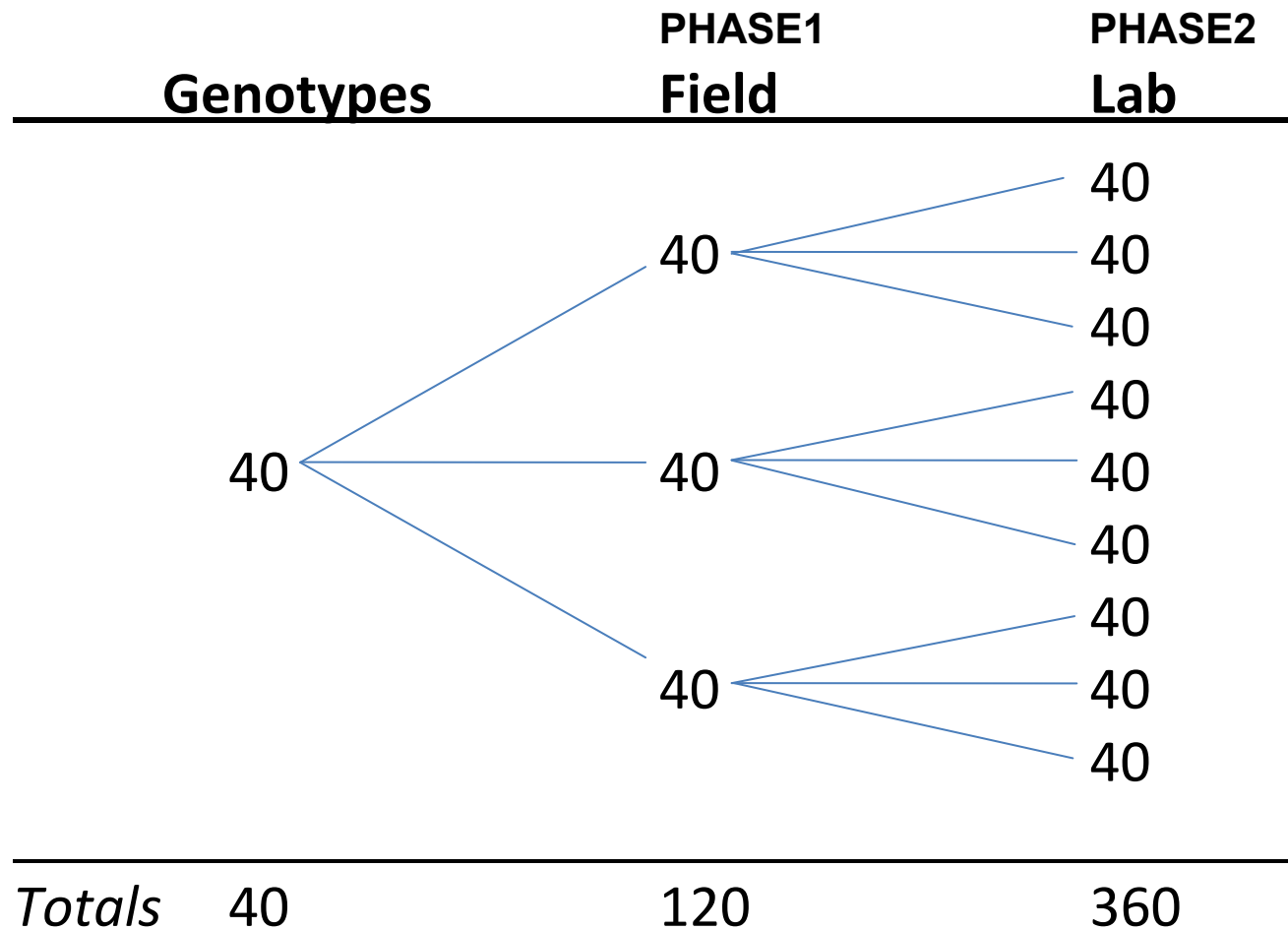
Genotypes	PHASE1 Field	PHASE2 Lab
40	40	40
40	40	40
40	40	40
<i>Totals</i> 40	120	120

The diagram illustrates the flow of genotypes between two phases of an experiment. In Phase 1 (Field), there are 40 genotypes. These 40 genotypes are then replicated 3 times in Phase 2 (Lab), resulting in 120 genotypes. This represents inadequate replication in Phase 2 because each genotype from Phase 1 is only represented by 3 samples in Phase 2, which is insufficient for statistical analysis.

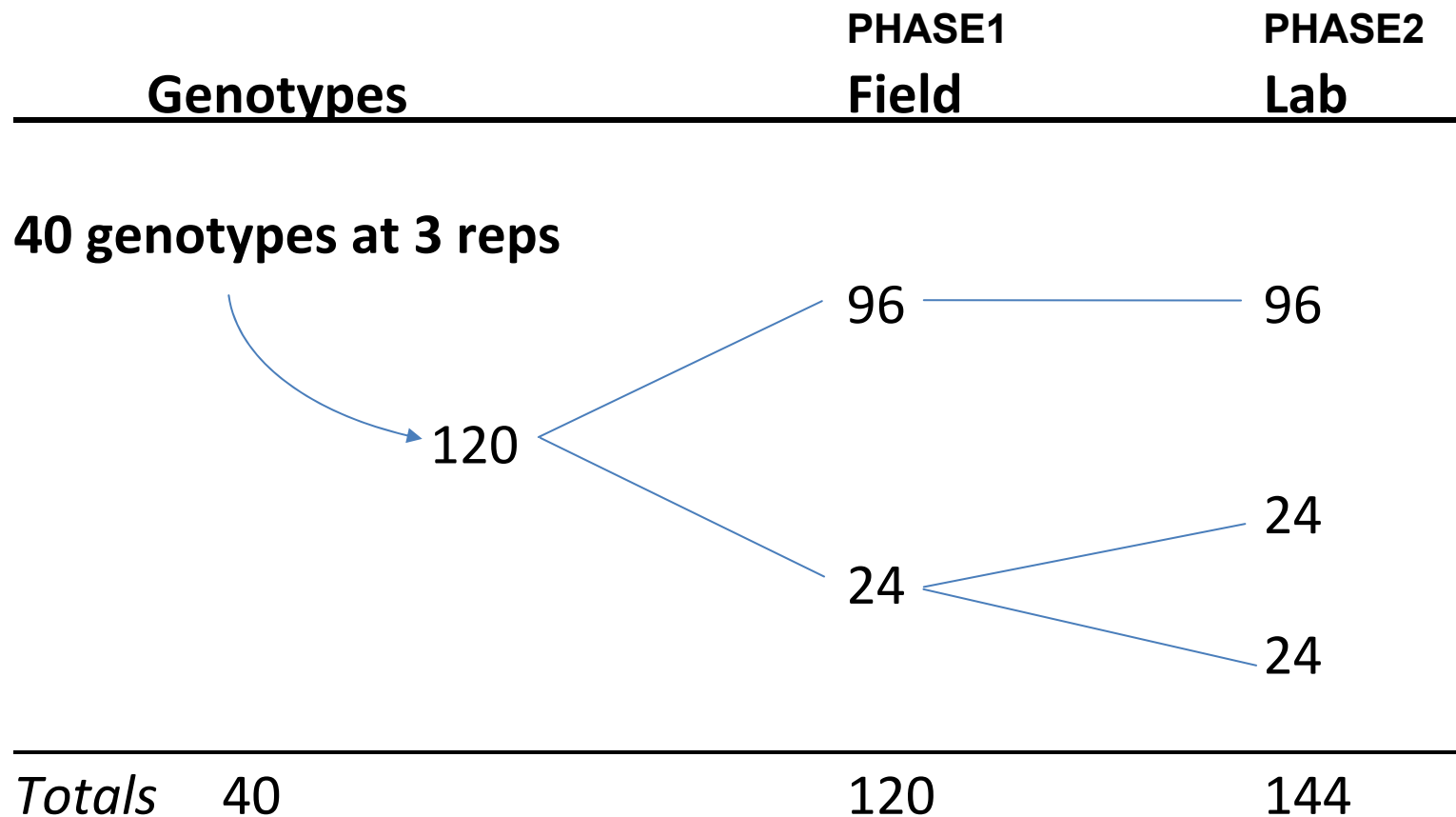
Example of inadequate replication in phase 2 of a multiphase experiment

Genotypes	PHASE1 Field	PHASE2 Lab
	40	40
	40	40
	40	40
+ Lab Controls		10
<i>Totals</i>	40	130

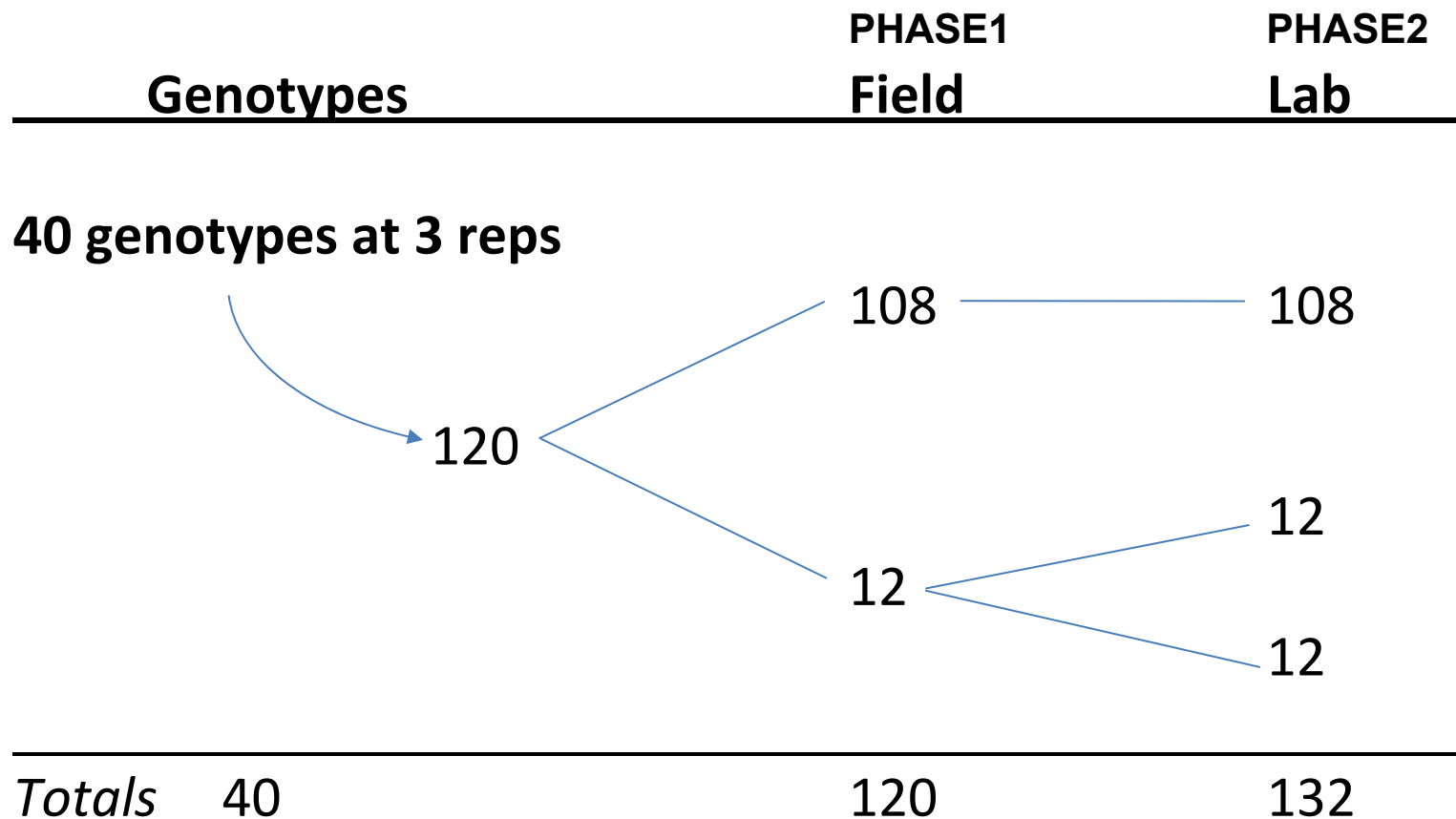
Replication used in detached leaf assay 100% replication in phase 2



Replication used in detached pod assay 33% replication in phase 2



Replication used in detached pod assay 20% replication in phase 2



Example of reduced replication in both phases

Genotypes	PHASE1 Field	PHASE2 Lab
10	10	10
		10
10	10	10
		10
20	20	20
<i>Totals</i>	40	50
		60

Detached Leaf Assay



Phase 1



Phase 2

- 1m rows in a field soil screen house
- 40 genotypes at 3 randomised replicate rows

- 3 leaves removed from each row
- 5×10^6 spores/mL
- Placed onto TAKA media

Technical Agar 10g L⁻¹

Kinetin 10mg L⁻¹

Aueromycin 0.1g L⁻¹

Detached Leaf Assay

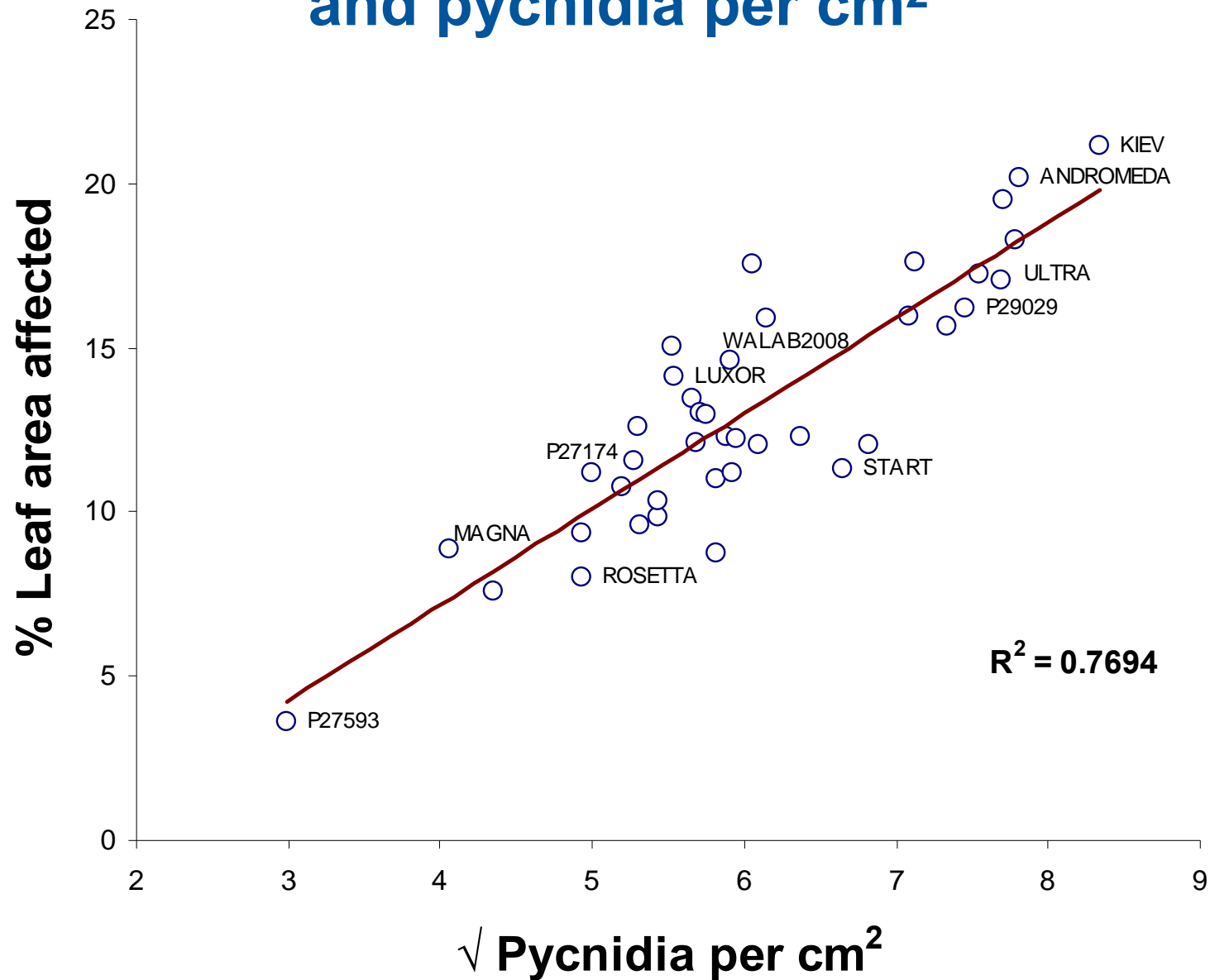
Resistant



Susceptible



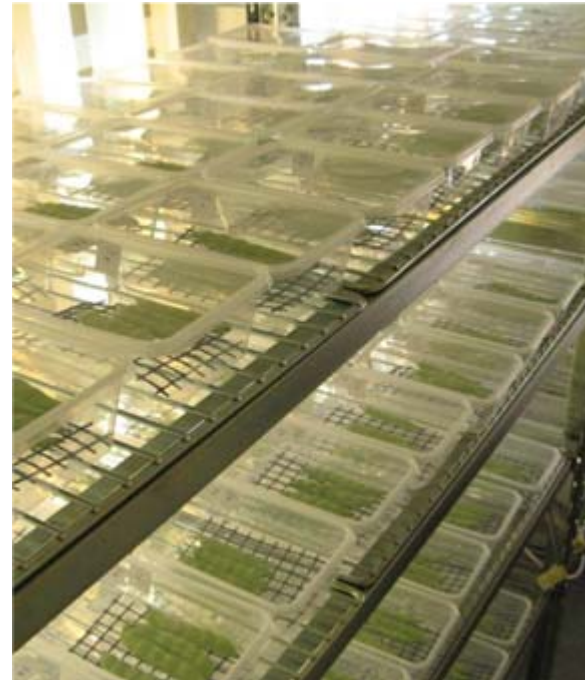
Correlation between %leaf area affected and pycnidia per cm²



Detached Pod Assay



Phase 1



Phase 2

- 4 pods removed from each row
- Immersed in spore solution 5×10^6 spores/mL
- Extra pods taken for rows that were duplicated in phase 2
- Incubated in sealed containers for 17 days
- Lids removed and scored 0, 3, 5 and 7 days later

Detached Pod Assay

Resistant

Susceptible

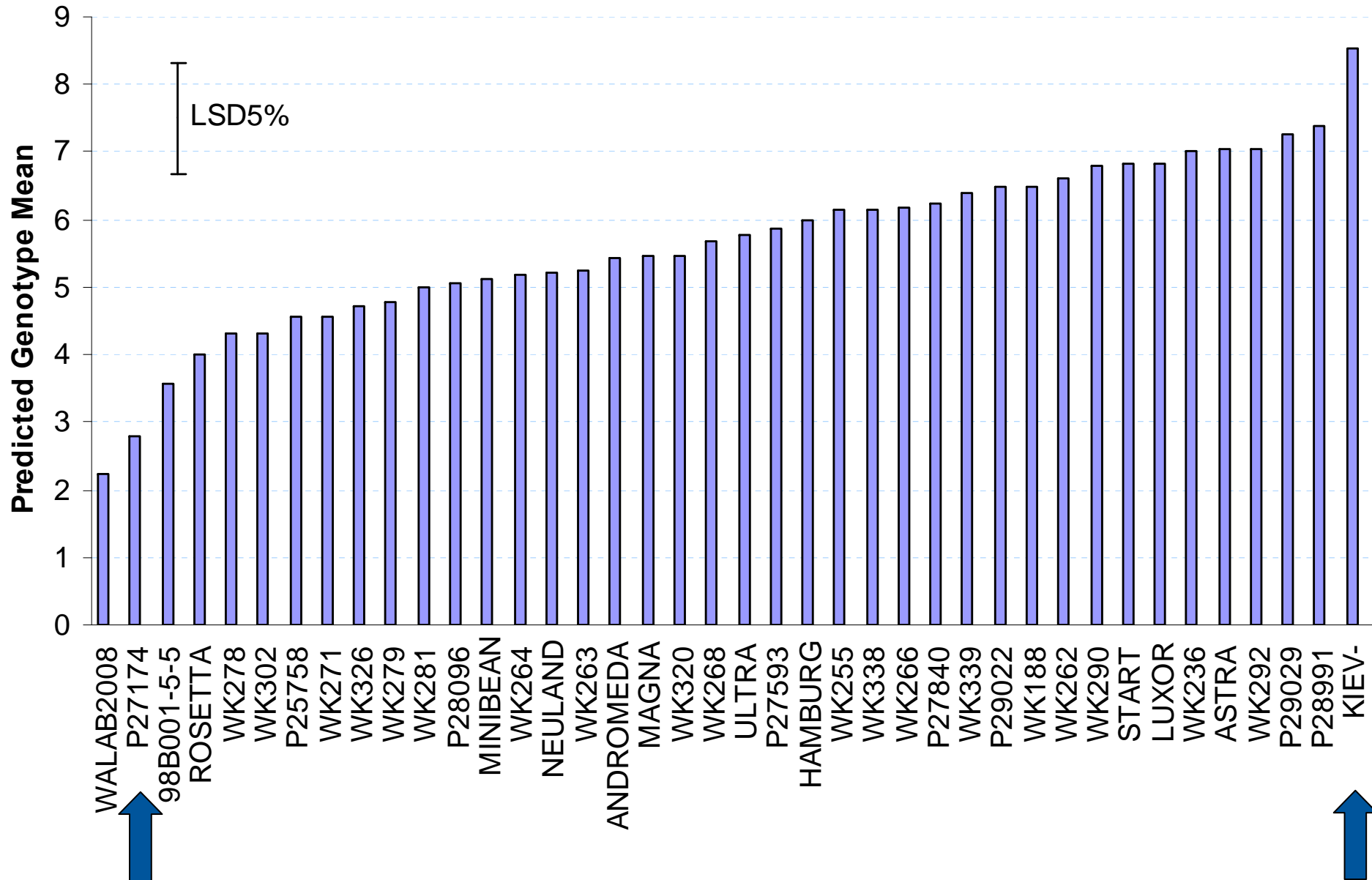


WALAB2008

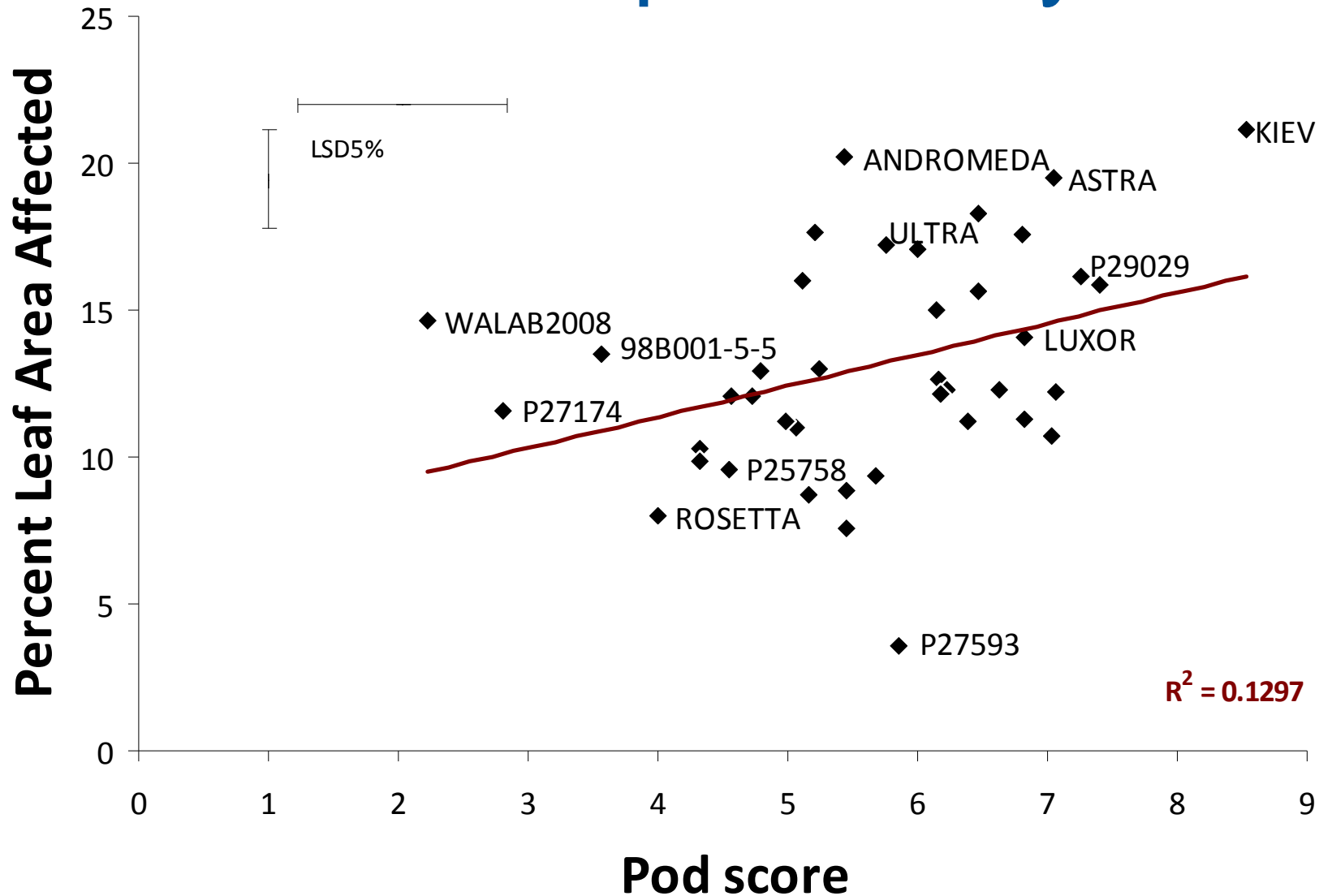
ROSETTA

KIEV-MUTANT

Detached Pod Assay



Correlation between leaf and pod assays



Conclusion

- Correlation between leaf and pod assays was poor
- Suggests resistance to phomopsis in leaves and pods in albus lupins is under independent genetic control
- Work is currently in progress to determine the genetic control of resistance and find markers





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