



Epidemiology of blackleg pathogen *Leptosphaeria maculans*

Bonny Vogelzang, PhD candidate



Supervisors:

Eileen Scott (UA)

Jenny Davidson (SARDI)

Kathy Ophel-Keller (SARDI)

Moin Salam (DAFWA)



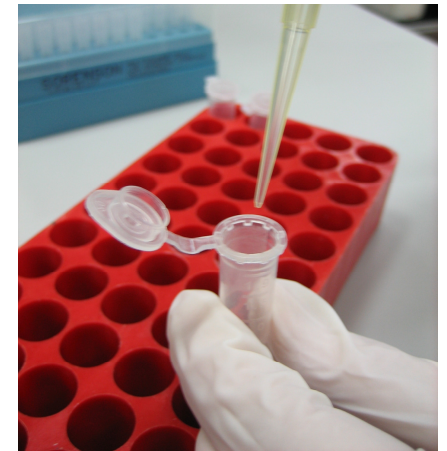
Spore trapping + PCR based diagnostics

Develop as tool for



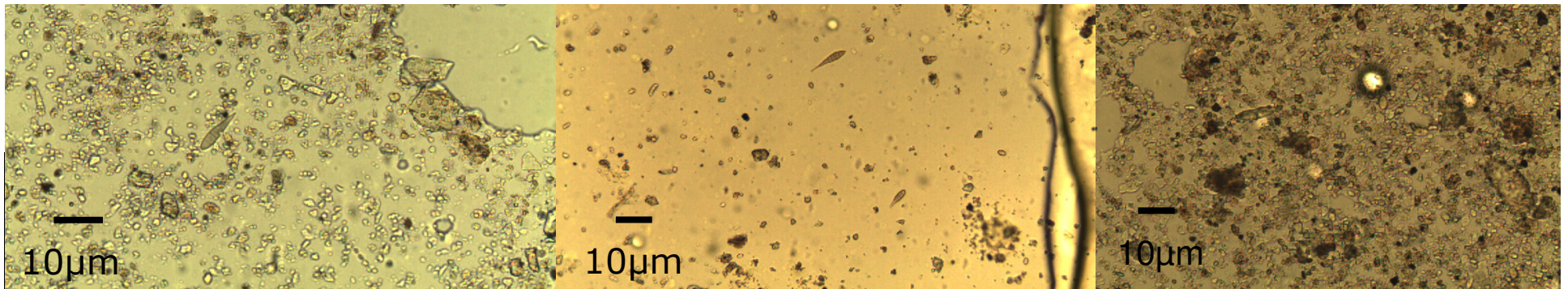
- Biosecurity surveillance
- Epidemiology

- ✓ Fast
- ✓ Reliable
- Sensitive?
- Specific?



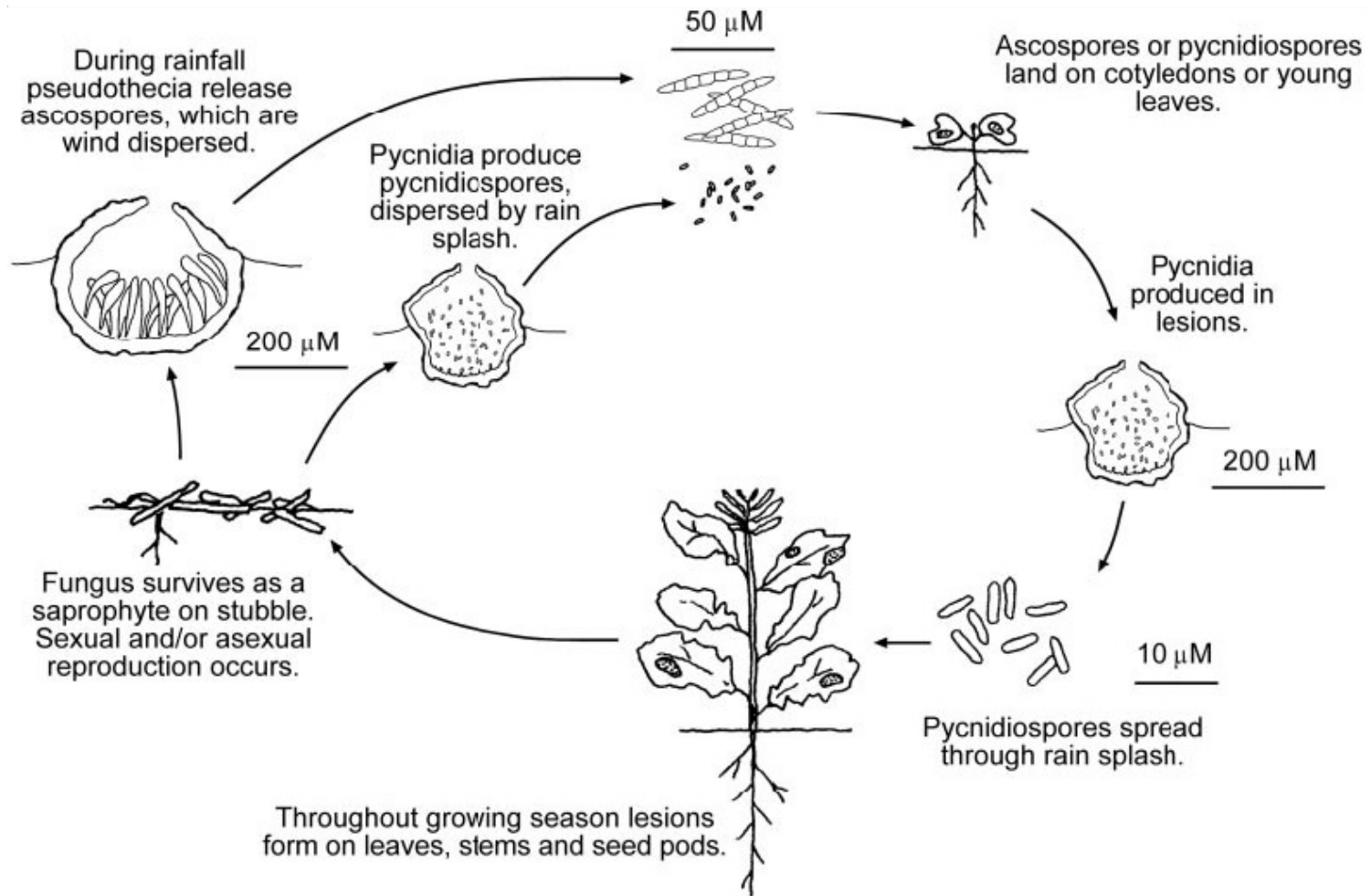
Methods/Results

- Three endemic pathogens as models
 - *Mycosphaerella pinodes* (peas)
 - ***Leptosphaeria maculans* (canola)**
 - *Ascochyta rabiei* (chickpeas)
- Real-time PCR tests available for each
- Confirmation by microscopy, trap plants
- Optimise DNA extraction from spores on tapes
 - ✓ Specificity of PCR tests
 - Sensitivity
 - ✓ Conidia (6 spores)
 - Ascospores – variable DNA yield



Leptosphaeria maculans
Epidemiological questions

1. Verification of epidemiological models
2. Interruption to ascospore production
3. Spore dispersal



Life cycle of *Leptosphaeria maculans* on *Brassica napus*.
(Howlett *et al.*, 2001)

Blackleg Sporacle/ SporacleEzy

Predicts

1. Onset of pseudothecial maturity and
 2. Onset of ascospore showers
- On basis of daily mean temperature and daily mean rainfall

(Salam *et al* 2007; Salam *et al* 2003)

1. Verification of Blackleg Sporacle/SporacleEzy

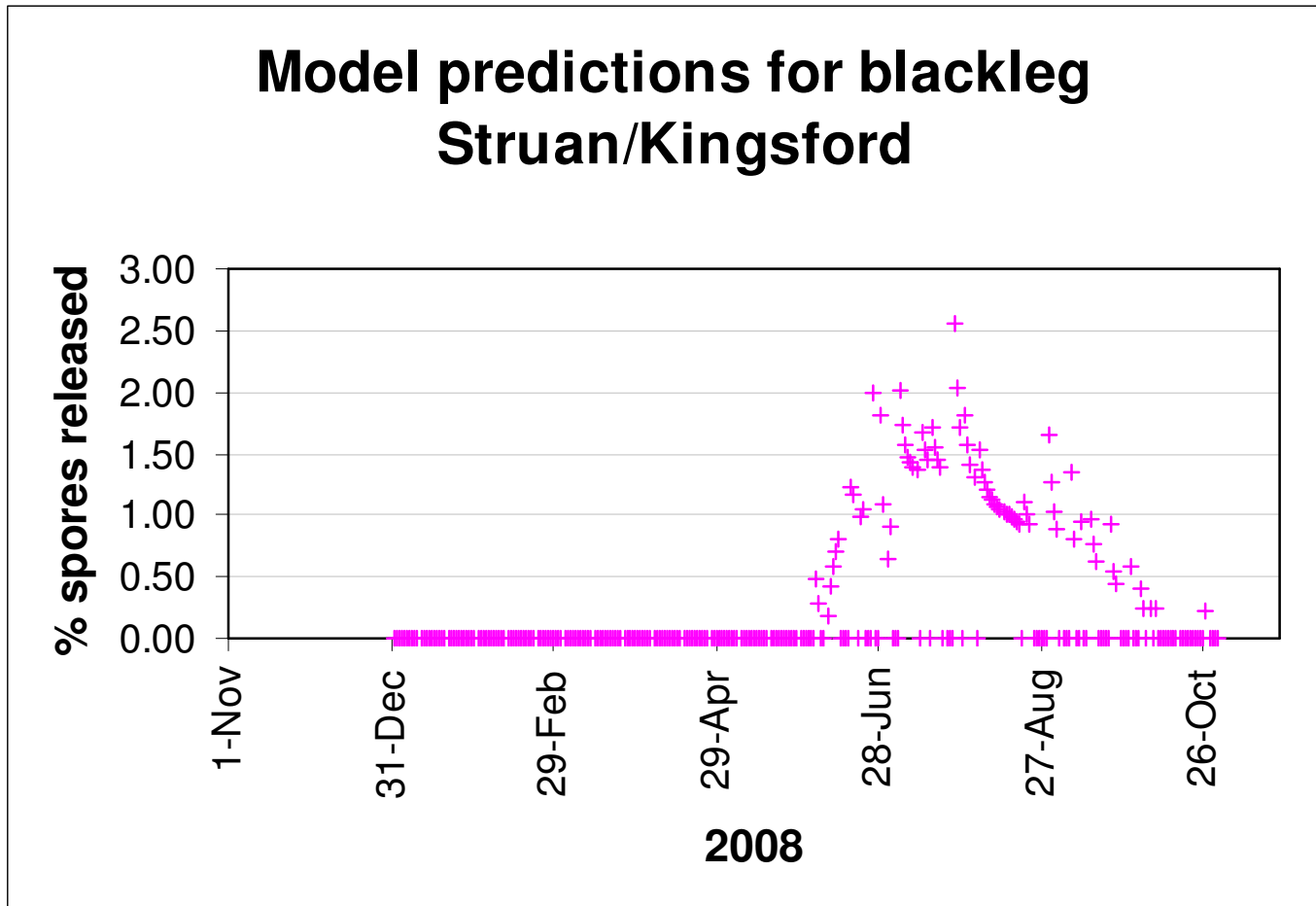
H_0 : That the model correctly predicts days on which spores are released

(a) Yes or no?

(b) How many spores?



Verification of model Spore trapping 2008



Verification of Model

Spore trapping 2009

- Model predicts proportion of spores released by date
 - How does this relate to amount of disease?
- Quantify disease based on prior knowledge of relative disease severity for that region
- Two sets of spore traps / trap plants with different inoculum loads
 - High
 - Medium – low

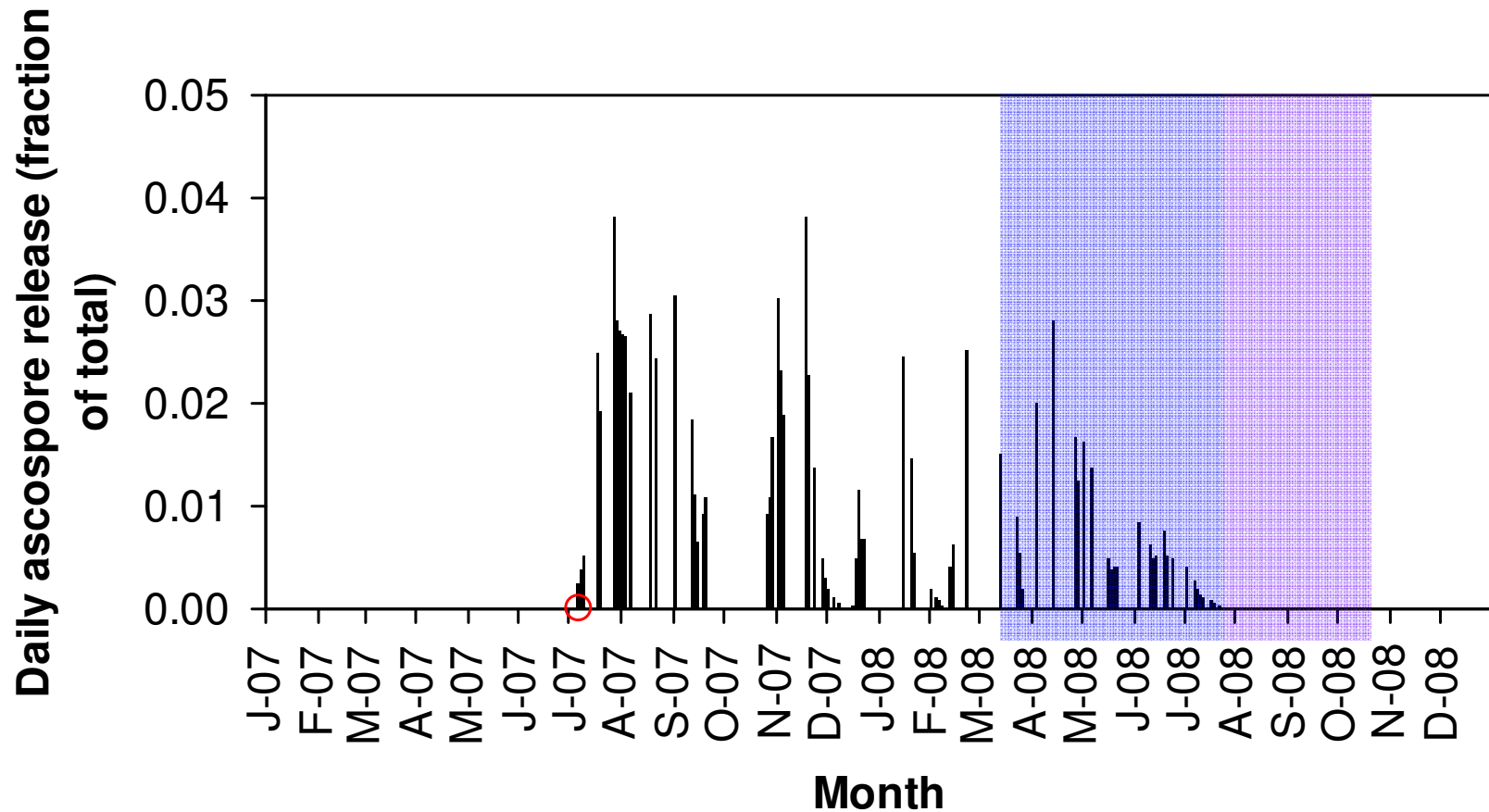
2. Effect of interruption to ascospore production

Background:

- NSW 2006 season started wet, then drought
- April 2007 starting inoculum levels high Why?

Wagga Wagga, New South Wales

Canola blackleg: predicted seasonal pattern of daily ascospore release from infected residues of crop grown in 2006

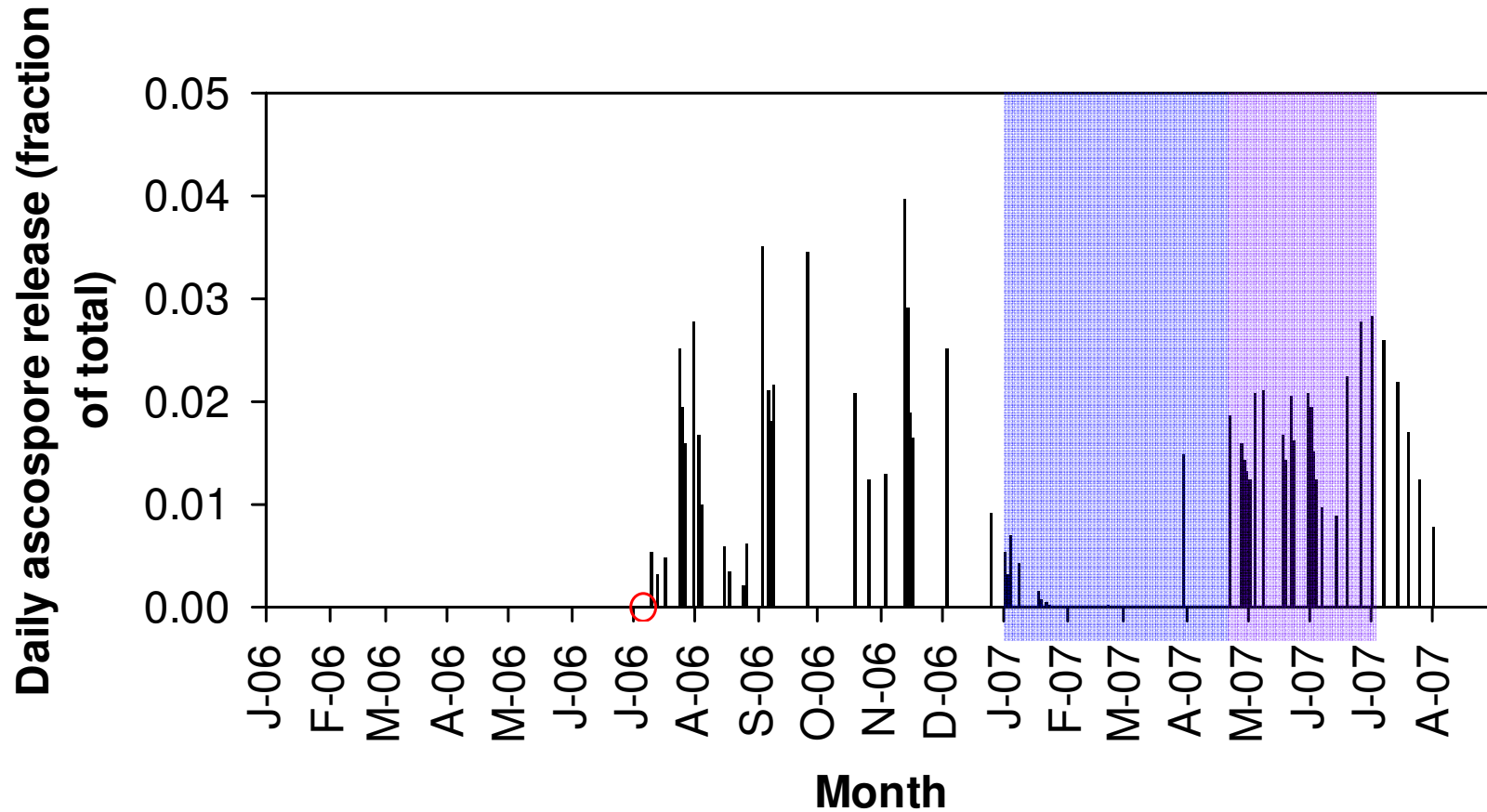


Slide courtesy M. Salam



Wagga Wagga, New South Wales

Canola blackleg: predicted seasonal pattern of daily ascospore release from infected residues of crop grown in 2005



Slide courtesy M. Salam



2. Effect of interruption to ascospore production

Research question:

- Does ascospore release immediately resume following prolonged drought or do pseudothecia dry out and die?

2. Effect of interruption to ascospore production

Methods:

- 2008 stubble collected Struan Feb 09
- Precondition stubble (16°C, 95% RH) => pseudothecial maturation
- Stubble then placed in field but sheltered from rain
- Monthly sampling up to 12 months
 - Ascospores released immediately on wetting?
 - Viability of ascospores?
 - Density of pseudothecia on stubble

3. Spore dispersal study

Background:

- Sub-model on ascospore dispersal based on pea pathogen (*Mycosphaerella pinodes*)
- *L.maculans* spores are larger than *M. pinodes* – likely to drop out earlier

Research question:

- What is the impact of ascospore size on distance of dispersal by wind?

3. Spore dispersal study

Methods:

- Canola stubble infected with *L.maculans*
- Pea stubble infected with *M. pinodes*
- Rotorod air samplers at distances 0, 250, 500, 750, 1500m downwind from stubble
- Stubble wetted 5 minutes per hour
- Air sampling for 3-4 hours
- PCR-based tests, microscopy, trap plants

Expected Outcomes

- Epidemiological models tested using 2008 and 2009 spore trap data for Kingsford, SA
- Model predictions of proportion of spores released related to disease severity under two inoculum load scenarios
- Pseudothecial survival under drought conditions determined
- Data on *L. maculans* ascospore dispersal collected for incorporation into dispersal sub-model

Questions?

References

- Howlett BJ, Idnurm A, Pedras MC (2001) *Leptosphaeria maculans*, the causal agent of blackleg disease of brassicas [Review]. *Fungal Genetics & Biology* 33, 1-14.
- Salam M, Khangura RK, Diggle A, Barbetti MJ (2003) Blackleg Sporacle: A model for predicting onset of pseudothecia maturity and seasonal ascospore showers in relation to blackleg of canola. *Phytopathology* 93, 1073-1081.
- Salam MU, Fitt BDL, et al. (2007) Two weather-based models for predicting the onset of seasonal release of ascospores of *Leptosphaeria maculans* or *L. biglobosa*. *Plant Pathology* 56 412-423.

Experimental system

- Cereal/pulse/oilseed cropping system
- Three model pathogens;
 - ❖ Varying concentrations of ascospores
 - ❖ Morphologically distinct
 - *Mycosphaerella pinodes* (peas)
 - ***Leptosphaeria maculans* (canola)**
 - *Ascochyta rabiei* (chickpeas)