# SPORE TRAPPING COUPLED WITH QUANTITATIVE PCR: POTENTIAL FOR APPLICATION TO BIOSECURITY SURVEILLANCE

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# 1 ABSTRACT

Plant disease surveillance is used in biosecurity to enable early detection of incursions of new pathogens, to monitor disease status during eradication programs, and to demonstrate that an area is free of a particular pathogen for trade purposes. Monitoring for plant pathogens currently relies on detection of symptoms by suitably skilled personnel, but the difficulties of distinguishing diseases based on symptoms, and of timing surveillance to coincide with symptom expression, can result in new pathogens not being detected until they are already widespread. The aims of this research were, firstly, to investigate the efficacy of spore trapping combined with quantitative PCR diagnostic assays for biosecurity surveillance and, secondly, to use the system to test predictions of spore release generated by epidemiological models.

Methodology for detection and quantification of airborne spores using spore traps and quantitative PCR assays was optimised and tested on three model pathogens endemic on pulse and oilseed crops in South Australia:

1. *Leptosphaeria maculans,* cause of blackleg (phoma stem canker) of canola (oilseed rape, *Brassica napus*)
2. *Didymella pinodes,* which causes ascochyta blight (blackspot) of field pea (*Pisum sativum*), and
3. *Ascochyta rabiei*, cause of ascochyta blight of chickpea (*Cicer arietinum*)

The PCR tests were shown to be both specific and sensitive when applied to spore trap samples, and results were borne out by close correlation with microscopic counts of ascospores on tapes of trapped spores. Poor reproducibility of results was largely addressed by replication of PCR assays and use of an exogenous control to allow variation to be taken into account. Implications of the imprecision remaining in the system are discussed in relation to epidemiological research leading to biosecurity surveillance. Storage of spore trap samples at -20$^{o}$C was found to be a suitable system for ascospores of *L. maculans*, with no reduction in the yield of DNA after 6 months. The finding that DNA yield from conidia of *L. maculans* was reduced, however, indicated that further research may be needed before applying these findings to other species and/or types of spore. The potential for reduced yield of DNA from spore trap samples collected during periods of extremely hot weather, and the possibility that dust

may affect yields, were identified as factors to be considered in assessing qPCR results. Nevertheless, the system proved to be generally robust in weather conditions prevailing in southern Australia.

The data from 2 years of monitoring for model pathogens were used to calibrate epidemiological models to the field site and to refine the models, as required. The findings that ascospores of *D. pinodes* were released during rain events in the summer led to incorporation of an additional cycle of ascospore formation and release, commencing during the cropping season, into the G1 Blackspot Manager model (model pathogen 1). Furthermore, the detection of *D. pinodes* DNA in spore trap samples on rainless days led to the incorporation of a relative humidity factor into the model. A minor modification was made to the Blackleg Sporacle model to smooth the model prediction curve (model pathogen 2). These changes, and the calibration of the models to the field site, need to be validated in further seasons. The results of monitoring for *D. rabiei* indicated that few or no ascospores were released from chickpea stubble, suggesting that the second mating type required for sexual reproduction, Mat 1-1, either does not occur in South Australia or is present as only a very small proportion of the population (model pathogen 3).

In investigating questions arising from the above experiments, it was found that 7-month-old canola stubble exposed to prolonged dry conditions continued to release ascospores immediately upon wetting, for up to 5 months (i.e. up until early summer, 12 months after harvest), but did not continue beyond the (Australian) early summer into the mid- and late-summer and autumn of the following year. This suggested that infested canola stubble from the season before the last is unlikely to be a significant source of infection for the current year's canola crop emerging in autumn.

DNA of *D. pinodes* was readily detected in dust generated at harvest but application of the dust to field pea seedlings failed to cause blackspot. The implications of these findings in relation to disease spread and biosecurity surveillance are discussed.

The results obtained using the optimised methodology for spore trapping coupled with PCR diagnostics provided a good match with those obtained using trap plants, and with predictions of epidemiological models. Spore trapping coupled with qPCR proved to be a useful tool for epidemiological studies, which can be applied in biosecurity surveillance.

# 2 DECLARATION

This thesis contains no material which has been accepted for the award of another degree or diploma in any University and, to the best of my knowledge and belief, contains no other material previously published or written by another person, except where due reference is given. I give consent to this thesis being made available for photocopying and loan from the University Library.

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Bernadette Klazina Vogelzang

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# 4 ABBREVIATIONS

| | |
|---|---|
| ¼ PDA | One quarter strength potato dextrose agar |
| AWS | Automatic weather station |
| BOM | Australian Bureau of Meteorology |
| Ct | In quantitative PCR, the cycle threshold at which the increase in fluorescence exceeds the background for the fluorescence-labelled probe |
| DAFWA | Department of Agriculture and Food Western Australia |
| fg | Femtogram ($10^{-15}$ gram) |
| *g* | Earth's gravitational acceleration (measure applied in centrifugation) |
| LCS | Lack of correlation weighted by the standard deviations |
| MSD | Mean squared deviation |
| PCR | Polymerase chain reaction |
| PDA | Potato dextrose agar |
| pg | Picogram ($10^{-12}$ gram) |
| qPCR | Quantitative PCR (real-time PCR) |
| RDTS | Root Disease Testing Service (SARDI) |
| RFLP | Restriction fragment length polymorphism |
| RH | Relative humidity |
| RMSD | Root mean squared deviation |
| RO water | Reverse osmosis water |
| RT-PCR | Reverse-transcriptase PCR |
| SARDI | South Australian Research and Development Institute |
| SB | Squared bias |
| SDSD | Squared difference between standard deviations |
| SE | Standard error of the mean |
| SNP | Single nucleotide polymorphism |
| Syn. | Synonym |
| UK | United Kingdom |
| USA | United States of America |