Epidemiology and management of ascochyta blight of field pea (*Pisum sativum*) in South Australia

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ABSTRACT

Ascochyta blight disease (synonym: blackspot) of field pea has worldwide distribution and regularly causes AUD$25 million loss per annum in Australian field pea (*Pisum sativum*) crops. This study provides new information on the causal pathogens and management strategies to reduce loss from this disease.

Research involving sowing dates, genotypes and fungicide treatments was conducted to identify optimal management strategies. Earlier sowing generally resulted in higher yield except when ascochyta blight was severe. Yield response to fungicide application varied with disease severity, sowing date and genotype. The optimum sowing period was within a week of the first autumn rains in low rainfall regions and 3 weeks after the first autumn rains in medium and medium - high rainfall regions. Earlier flowering genotypes were the highest yielding particularly when sown early and subjected to strategic fungicide applications.

The pathogen, *Phoma koolunga*, was recognised for the first time as a component of the ascochyta blight disease complex in southern Australia. The species was described morphologically. Sequences of the internal transcribed spacer region were distinct from those of the accepted causal pathogens of ascochyta blight of field pea *viz*. *Didymella pinodes*, *Phoma medicaginis* var. *pinodella* and *Ascochyta pisi*. Symptoms on field pea seedlings caused by *P. koolunga* were indistinguishable from those caused by *D. pinodes*, other than a 24 h delay in manifestation of symptoms.

P. koolunga was detected across field pea cropping soils in South Australia but rarely from other Australian states while *D. pinodes* plus *P. medicaginis* var. *pinodella* were widespread. The quantity of DNA of these pathogens detected in soils was positively correlated with ascochyta blight lesions in a pot bioassay. Soil-borne inoculum gradually decreased in the 3 years following a field pea crop. DNA tests and pathogen isolation from naturally infected field pea plants showed *P. koolunga* to be an important component of the

disease complex in South Australia. *P. koolunga* and *D. pinodes* were equally responsible for disease symptoms, while *P. medicaginis* var. *pinodella* had a minor role in the disease complex.

Interaction between *D. pinodes, P. medicaginis* var. *pinodella* and *P. koolunga* was investigated in controlled conditions. Colony diameter of the former was reduced on potato dextrose agar (PDA) amended with filtrate from broth cultures of *P. koolunga*, as was colony diameter of *D. pinodes* on PDA amended with filtrate from *P. medicaginis* var. *pinodella* or *D. pinodes*. This effect was shown to be fungistatic rather than fungicidal. When co-inoculated onto leaves on field pea plants, or onto excised leaf discs, either the quantity of DNA of *D. pinodes* and of *P. medicaginis* var. *pinodella,* or the mean lesion diameter of these pathogens, was significantly reduced when co-inoculated with *P. koolunga*. *P. koolunga* was not influenced by co-inoculation. *D. pinodes* demonstrated self-antagonism.

D. pinodes is considered the principal pathogen of concern in this complex. This study further investigated the relationship between ascospore numbers of *D. pinodes* at sowing and disease at the end of the season. Ascospores released from stubble infested with ascochyta blight were counted periodically in a wind tunnel. A model was developed to predict disease severity in relation to ascospore numbers, distance from infested field pea stubble, and rainfall. The model was validated with an independent dataset. A threshold level of ascospores of *D. pinodes* was identified above which disease did not increase.

The findings from this study have been incorporated into management recommendations for field pea in southern Australia. Growers are encouraged to manipulate sowing dates according to the temporal release of ascospores, and select a cultivar that has the best agronomic yield potential for the sowing date, and to use fungicide strategically. The recommendation also emphasises field selection based on commercial testing for the presence of soil-borne inoculum of *D. pinodes*, *P. medicaginis* var. *pinodella* and *P. koolunga*.

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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STATEMENT OF THE CONTRIBUTIONS TO JOINTLY AUTHORED PAPERS

1. Davidson JA, Hartley D, Priest M, Krysinska-Kaczmarek M, Herdina, McKay A, Scott ES (2009) A new species of *Phoma* causes ascochyta blight symptoms on field peas (*Pisum sativum)* in South Australia. *Mycologia* **101**, 120-128 [published manuscript].

Presented in Chapter 2 Author contributions: JAD designed and conducted the research experiments, analysed the data, and drafted and constructed the manuscript. DH identified genetic sequences and conducted evolutionary analysis and contributed to the manuscript, MP contributed the taxonomic description of *P. koolunga*, MKK provided technical assistance and support of experiments, H and AM conducted DNA analyses and contributed to the manuscript, ESS supervised research, contributed to the research ideas and design, and the editing of the manuscript.

2. McMurray LS, Davidson JA, Lines MD, Leonforte A, Salam MU (2011) Combining management and breeding advances to improve field pea (*Pisum sativum* L.) grain yields under changing climatic conditions in south-eastern Australia. *Euphytica* **180**, 69-88 [published manuscript].

Presented in Chapter 3. Author contributions: LSM and JAD collaborated equally to design and conduct all research experiments, analysed the data, and drafted and constructed the manuscript together. MDL provided technical assistance and support of the field experiments, AL provided field pea germplasm and assisted with editing the manuscript, MUS contributed to the research ideas and design, and the editing of the manuscript.

3. Davidson JA, Krysinska-Kaczmarek M, Wilmshurst CJ, McKay A, Herdina, Scott ES (2011) Distribution and survival of ascochyta blight pathogens in field-pea-cropping soils of Australia. *Plant Disease* **95**, 1217-1223 [published manuscript].

Presented in Chapter 4. Author contributions: JAD designed and conducted all research experiments, analysed the data, and drafted and constructed the manuscript. MKK and CJW provided technical assistance and support of experiments, H and AM conducted DNA analyses and contributed to the manuscript, ESS contributed to the research ideas and design, and the editing of the manuscript.

4. Davidson JA, Krysinska-Kaczmarek M, Herdina, McKay A, Scott ES (2012) Comparison of cultural growth and in planta quantification of *Didymella pinodes*, *Phoma koolunga* and *Phoma medicaginis* var. *pinodella*, causal agents of ascochtya blight of field pea (*Pisum sativum*). *Mycologia* **104**, 93-101 [published manuscript].

Presented in Chapter 5. JAD designed and conducted all research experiments, analysed the data, and drafted and constructed the manuscript. MKK provided technical assistance and support of experiments, H and AM conducted DNA analyses and contributed to the manuscript, ESS contributed to the research ideas and design, and the editing of the manuscript.

5. Davidson JA, Wilmshurst CJ, Scott ES, Salam MU (2012) Relationship between ascochyta blight on field pea (*Pisum sativum*) and spore release patterns of *Didymella pinodes* and other causal agents of ascochyta blight. Plant Pathology [submitted manuscript]

Presented in Chapter 6. JAD designed and conducted all research experiments, analysed the data, and drafted and constructed the manuscript. CJW provided technical assistance and support of experiments, MUS assisted with development of the disease model, contributed to research ideas and design and the editing of the manuscript, ESS contributed to the research ideas and design, and the editing of the manuscript.

Each of these manuscripts is displayed in this thesis in either published or submitted form according to the instructions to author of the specific journal.

This thesis has been prepared according to the University of Adelaide's specifications for 'PhD by publications' format.

The following authors agree that the statement of the contributions of jointly authored papers accurately describes their contribution to research manuscripts 1 to 5 and give consent to their inclusion in this thesis.

Davidson JA ……

Hartley D ……

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ABBREVIATIONS

ANOVA	analysis of variance
AUD	Australian Dollar
AUDPC	area under disease progress curve
AWS	automatic weather station
BLUP	best linear unbiased prediction
CER	controlled environment room
CSIRO	Commonwealth Scientific and Industrial Research Organisation
cv.	cultivar
cvs	cultivars
DAI	days after inoculation
DAS	days after sowing
diam	diameter
DiseaseAugSoil	contribution of ascochyta blight from soil inoculum, measured at the end of August
DNA	deoxyribonucleic acid
DpPmp	*Didymella pinodes* plus *Phoma medicaginis* var. *pinodella*
Early MM	two applications of mancozeb (at 4 nodes and at flowering)
ELISA	enzyme-linked immuno-sorbent assay
G3PD	glyceraldehyde – 3 – phosphate dehydrogenase
GS	growth stage
IAD	internode area diseased
IGS	intergenic spacer
ITS	internal transcribed spacer
LAD	leaf area diseased
LSD	least significant difference
L rainfall	low rainfall
M rainfall	medium rainfall
M fungicide	application of mancozeb at 9 nodes
MH rainfall	medium to high rainfall
MM fungicide	two applications of mancozeb (at 9 nodes and at flowering)
NSW	New South Wales
NUV	near ultraviolet
PCR	polymerase chain reaction
PDA	potato dextrose agar (full strength)
p.i.	post inoculation
QTL	quantitative trait loci
RainAS	rain summed for August and September
RainWinter	rain summed from sowing date to end of July
RAPD	random amplified polymorphic DNA
RDTS	Root Disease Testing Service
Rec	recommended sowing date
Rec-2	sowing date 2 weeks earlier than recommended

Rec-4	sowing date 4 weeks earlier than recommended
REML	residual maximum likelihood
RFLP	restriction fragment length polymorphism
RIE	radiation interception efficiency
RO	reverse osmosis
RUE	radiation use efficiency
SA	South Australia
SARDI	South Australian Research and Development Institute
SD	seed dressing (P-Pickel T®)
%Spores	% of ascospores remaining on stubble estimated by G1 Blackspot Manager
SporesInit	initial number of ascospores
TOS	time of sowing
Vic.	Victoria
WA	Western Australia
WAS	weeks after sowing