Epidemiology and management of cercospora leaf spot (*Cercospora zonata*) of faba beans (*Vicia faba*)

by

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Thesis submitted to the University of Adelaide
for the degree of
Doctor of Philosophy

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June 2011
Chapter 2 Literature review

2.1 Introduction ................................................................. 9
2.2 History of faba beans and the Australian industry ......................... 10
2.3 History and significance of cercospora leaf spot ............................. 14
2.4 The causal agent and the disease .......................................... 15
  2.4.1 Macroscopic description ............................................. 16
  2.4.2 Microscopic description ............................................. 18
  2.4.3 Cultural characteristics ............................................ 18
  2.4.4 Morphological and pathogenic variation ............................ 19
  2.4.5 Host specialisation .................................................. 20
  2.4.6 Production of the phytotoxic metabolite: cercosporin ............... 22
2.5 Epidemiology of cercospora leaf spot .................................... 23
  2.5.1 Effect of temperature and moisture .................................. 23
  2.5.2 Defoliation as an epidemiological component ....................... 25
  2.5.3 Pathogen survival .................................................. 26
  2.5.4 Disease spread .................................................... 27
2.6 Host resistance ............................................................. 29
2.7 Disease management ....................................................... 31
  2.7.1 Cultural practices .................................................. 31
  2.7.2 Chemical control ................................................... 32
2.8 Summary ........................................................................ 34

Chapter 3 General materials and methods ........................................... 35

3.1 Plant growth and maintenance .............................................. 37
  3.1.1 Faba bean cultivars ............................................... 37
  3.1.2 Potting soil ......................................................... 38
  3.1.3 Controlled environment conditions ................................ 38
3.2 Fungi ............................................................................ 39
3.2.1 Collection and storage of *Cercospora zonata* isolates .......................... 39

3.2 Statistical analysis ......................................................................................... 41

Chapter 4  Preliminary studies on *Cercospora zonata* ........................................ 43

4.1 Introduction .................................................................................................... 45
4.2 Materials and methods .................................................................................. 46
  4.2.1 *In vitro* sporulation of *Cercospora zonata* on artificial media .............. 46
  4.2.2 Stubble- and soil-borne inoculum ............................................................ 49
  4.2.3 Infectivity of *C. zonata* in soil fractions ................................................ 53
4.3 Results ........................................................................................................... 54
  4.3.1 *In vitro* sporulation of *Cercospora zonata* on artificial media .......... 54
  4.3.2 Stubble- and soil-borne disease inoculum ............................................. 55
  4.3.3 Infectivity of *C. zonata* in soil fractions ................................................ 59
4.4 Discussion ...................................................................................................... 62

Chapter 5  Host range, prevalence and management of cercospora leaf spot
(*Cercospora zonata*) of faba bean (*Vicia faba*) in southern Australia .................. 65

Chapter 6  Factors affecting infection of faba bean (*Vicia faba* L.) by *Cercospora
zonata* .................................................................................................................. 109

Chapter 7  Temporal and spatial development of cercospora leaf spot of faba bean
influenced by in situ inoculum ........................................................................... 147

Chapter 8  Identification and inheritance of resistance to cercospora leaf spot
(*Cercospora zonata*) in germplasm of faba beans (*Vicia faba*) ....................... 183

Chapter 9  General discussion ............................................................................ 197

Appendices ......................................................................................................... 207

References (Chapters 1 – 4 & 9) ........................................................................ 213
The disease cercospora leaf spot (CLS), caused by the fungus *Cercospora zonata*, has affected faba bean (*Vicia faba*) production regions in southern Australian in recent years. This study provides new information on the prevalence and significance of the disease and the factors that affect severity.

Temperature, wetness period, plant maturity, pathogen variability and inoculum concentration all influenced infection of faba bean by *C. zonata* in a controlled environment. Disease severity was positively correlated ($R^2=0.83$, $P<0.001$) with wet-degree hours ($DH_w$) and premature defoliation (40-50%) of the lower canopy, which was most severe when the pathogen was inoculated at the mid- to late-vegetative crop growth stages. Pathogenicity tests showed that 29 isolates of *C. zonata* collected from 1999 to 2008 varied in aggressiveness; this was not related to geographical origin of isolates or growth rate *in vitro*, but isolates collected from 2005 to 2008 were more aggressive than those collected in the period 1999-2004.

The temporal and spatial dynamics of the disease on susceptible and resistant genotypes of faba bean were examined. A strong association between the incidence and severity of CLS and soil-borne inoculum was established using comparative analyses of disease on plants in soil sown with faba bean every 3 years since 1997 and in adjacent soil with no history of cultivation of faba bean. Spatial patterns of disease development showed that inoculum spread primarily over short distances during the early stages of CLS epidemics, though dispersal of 4 to 16 m from the infested soil was observed. Non-linear regression using a logistic model described disease development over time on susceptible plants in soil with *in situ* inoculum, whereas an exponential model best described disease gradient with distance from the inoculum source and disease development on resistant plants. There was a positive relationship ($R^2=0.93$, $P<0.05$) between disease severity on susceptible plants grown
in soil with infested residue on the surface and the amount of DNA of *C. zonata* detected in the soil. When residues were removed from the soil surface, or depleted rapidly through grazing, the infectivity of soil and the amount of DNA of *C. zonata* detected were significantly less than for soil with residue remaining on the surface. *C. zonata* survived in soil, on infested residue or as fungal propagules in the soil profile, and remained infective for at least 30 months.

The distribution and occurrence, host range and management of CLS of faba bean in southern Australia were studied. *C. zonata* infected narbon bean, lentil and vetch but did not infect pea, chickpea, lathyrus, lupin or canola. A disease survey of 100 commercial faba bean crops in southern Australia showed that CLS was endemic to all districts examined, observed in 87% of crops. Disease severity varied in all districts but was most severe in crops in the south-east of South Australia. Disease incidence and severity were highest in fields planted with faba bean in short rotations (1-4 years) and decreased \( R^2=0.13, P=0.006 \) as the interval between faba bean crops increased. Severity also appeared to be influenced by faba bean residue remaining from the previous year in adjacent fields. CLS manifested as severe lesions on foliage and extensive defoliation, resulting in a 7% reduction in yield in field experiments. Applications of carbendazim, tebuconazole, chlorothalonil and triadimefon significantly reduced CLS severity compared with untreated controls and a single application of either carbendazim or tebuconazole prior to disease onset was identified as an economical application strategy for control of the disease.

A rapid screening technique was developed to identify resistance to *C. zonata* in faba bean genotypes in a controlled environment. All faba bean cultivars commercially available to the Australian industry were susceptible to the disease. The mode of inheritance of resistance to *C. zonata* was determined to be monogenic dominant and this has allowed a relatively simple pathway by which sources of resistance identified in this study can be transferred to adapted faba bean genotypes available to the southern Australian industry.
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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Date ……………………………………

Rohan Benjamin Essex Kimber
STATEMENT OF THE CONTRIBUTIONS TO JOINTLY AUTHORED PAPERS


Presented in Chapter 5. Author contributions: RBEK designed and conducted all research experiments, analysed the data, and drafted/constructed the manuscript. JAD supervised all research. ESS and JGP contributed to the research ideas and design, and the editing of the manuscript.


Presented in Chapter 6. Author contributions: RBEK designed and conducted all research experiments, analysed the data, and drafted/constructed the manuscript. MHR provided technical assistance and support of experiments. JAD, ESS and JGP supervised all research, contributed to the research ideas and design, and the editing of the manuscript.


Presented in Chapter 7. Author contributions: RBEK designed and conducted all research experiments, analysed the data, and drafted/constructed the manuscript. JGP provided technical support in field experiments, research ideas and design. CBD contributed to statistical analyses of data. JAD supervised all research and analyses of data. JAD and ESS contributed to the research ideas and design, and the editing of the manuscript.

Presented in Chapter 8. Author contributions: RBEK developed and conducted all disease screening experiments, analysed the data, and drafted/constructed the manuscript. JGP supervised all research, issued germplasm, designed and analysed genetics of resistance studies, contributed to the research ideas and design, and the editing of the manuscript.

Each of these manuscripts is displayed in this thesis in either submitted or published 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 ‘combination conventional/publication format’.

The following authors agree that the statement of the contributions of jointly authored papers accurately describes their contribution to research manuscripts 1, 2, 3 and 4 and give consent to their inclusion in this thesis.

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ACKNOWLEDGEMENTS

I sincerely thank my supervisors, Prof. Eileen Scott, Dr Jeff Paull and Ms Jenny Davidson for their patient guidance, encouragement and advice. I am particularly grateful for their confidence in my ability and their friendship, mentorship and humour. I also thank them for editorial advice and their accessibility for spontaneous discussion of research ideas.

For financial support, I wish to acknowledge the Grains Research and Development Corporation (GRDC) and the South Australian Research and Development Institute (SARDI).

I would also like to thank;

Dr Kathy Ophel-Keller for her support while balancing study and research duties.

All my SARDI colleagues, especially Drs Mark Sosnowski, Belinda Rawnsley, Liz Drew, Kaye Ferguson and Kristian Peters for their friendship, advice and moral support.

Michelle Russ, Kevin James and Ian Roberts for their invaluable technical assistance and fellow colleagues in the pulse and oilseed pathology group, particularly Marzena Krysinska-Kaczmarek and Chris Wilmshurst, for their encouragement and team-spirit.

The University of Adelaide School of Agriculture, Food & Wine Fungal and Bacterial Plant Pathology laboratory group for their support and scientific discussion forums.

Bruno Carrocci (Arris Pty Ltd) for his assistance in printing of this thesis.

My colleagues in the Australian pulse pathology research community, particularly Kurt Lindbeck, Joop van Leur and Dr Moin Salam, for their expert advice and friendship.

Colleagues among the international pulse pathology research community, especially Prof Dani Shtienberg and Prof Bassam Bayyaa, for their valuable advice and collaboration.

The dedicated community of consultants, agronomists and growers that service the southern Australian faba bean industry, with specific acknowledgement to Wayne Hawthorne who first championed the importance of cercospora leaf spot in faba bean.

John and Trevor Cozens at Orroroo, who were ‘founding fathers’ in forming my deep appreciation of agriculture and the challenges that confront farming communities.

My family: I lovingly thank my beautiful wife Tara, for her patience, encouragement and love and our wonderful daughter Zoe, who was a welcome distraction to writing this thesis. I am also thankful for the generosity and support provided by my mother Kaye, sister Merise, and in-laws Ean, Geoff, Ros, Anna and Matt.

My friends, who share with me the joys and challenges of life - all that is familiar to those who take pleasure in fellowship over fabulous food, wine and coffee.

The Lord God, for what he has given me and, through faith in him, the strength to confront the challenges in life as I walk amidst his wonderful creation.
This thesis is dedicated to the memory of my father,

Barrington Litchfield Kimber

1936 – 1995

So rarely are great and good the same man.

If we are to go forward, we must first go back and rediscover those precious values - that all reality hinges on moral foundations and that all reality has spiritual control.

Dr Martin Luther King Jr.


<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
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<tbody>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>AWS</td>
<td>automatic weather station</td>
</tr>
<tr>
<td>BSA</td>
<td>bean seed agar</td>
</tr>
<tr>
<td>CA</td>
<td>carrot agar</td>
</tr>
<tr>
<td>CER</td>
<td>controlled environment room</td>
</tr>
<tr>
<td>CJA</td>
<td>carrot juice agar</td>
</tr>
<tr>
<td>CLDA</td>
<td>carrot leaf decoction agar</td>
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<tr>
<td>CLPA</td>
<td>carrot leaf pulp agar</td>
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<tr>
<td>CLS</td>
<td>cercospora leaf spot</td>
</tr>
<tr>
<td>CMA</td>
<td>cornmeal agar</td>
</tr>
<tr>
<td>CRB</td>
<td>completely randomised block</td>
</tr>
<tr>
<td>CSIRO</td>
<td>Commonwealth Scientific and Industrial Research Organisation</td>
</tr>
<tr>
<td>cv.</td>
<td>cultivar</td>
</tr>
<tr>
<td>cvs</td>
<td>cultivars</td>
</tr>
<tr>
<td>DAI</td>
<td>days after inoculation</td>
</tr>
<tr>
<td>DAS</td>
<td>days after sowing</td>
</tr>
<tr>
<td>Diam</td>
<td>diameter</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>GS</td>
<td>growth stage</td>
</tr>
<tr>
<td>ITS</td>
<td>internal transcribed spacer</td>
</tr>
<tr>
<td>LAD</td>
<td>leaf area diseased</td>
</tr>
<tr>
<td>LPLA</td>
<td>loss of photosynthetic leaf area</td>
</tr>
<tr>
<td>NSW</td>
<td>New South Wales</td>
</tr>
<tr>
<td>NUV</td>
<td>near ultraviolet</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>PDA</td>
<td>potato dextrose agar (full strength)</td>
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<tr>
<td>RCBD</td>
<td>randomised complete block design</td>
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<tr>
<td>RDTS</td>
<td>Root Disease Testing Service</td>
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<tr>
<td>RO</td>
<td>reverse osmosis</td>
</tr>
<tr>
<td>SA</td>
<td>South Australia</td>
</tr>
<tr>
<td>SARDI</td>
<td>South Australian Research and Development Institute</td>
</tr>
<tr>
<td>V8A</td>
<td>V8 juice agar</td>
</tr>
<tr>
<td>V8B</td>
<td>V8 juice Broth</td>
</tr>
<tr>
<td>Vic.</td>
<td>Victoria</td>
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<td>WA</td>
<td>Western Australia</td>
</tr>
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<td>weeks after sowing</td>
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