

**Aspects of epidemiology of  
*Phoma koolunga* (ascochyta  
blight of field pea)**

**Mohsen Khani**

**Bachelor of Agricultural Engineering, Plant Protection, Shiraz  
University**

**Master of Plant Pathology, Tarbiat Modares University**

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

**School of Agriculture, Food & Wine  
Faculty of Sciences, The University of Adelaide**

**October 2014**



## Table of Contents

<b>Abstract</b> .....	i
<b>Declaration</b> .....	iv
<b>Statement of the contributions to jointly authored paper</b> .....	v
<b>Acknowledgements</b> .....	viii
<b>Chapter 1</b> .....	1
Introduction and literature review.....	1
1.1 Introduction.....	2
1.2 Field pea.....	3
1.3 Ascochyta blight pathogens .....	4
1.4 Epidemiology of ascochyta blight .....	8
1.4.1 Symptoms and disease cycle.....	8
1.4.2 Survival of ascochyta blight fungi .....	11
1.4.3 Transmission of ascochyta blight pathogens via infected seeds .....	16
1.4.4 Effect of ascochyta blight disease on field pea yield .....	19
1.5 Control of ascochyta blight of pea .....	20
1.5.1 Cultural practices .....	20
1.5.2 Chemical control; seed treatment and foliar fungicides.....	25
1.5.3 Resistance of field pea genotypes .....	27
1.6 Summary and aims of research .....	29
1.7 Linking statement .....	30
<b>Chapter 2</b> .....	32
Survival of <i>Phoma koolunga</i> , a causal agent of ascochyta blight, on field pea stubble or by pseudosclerotia in soil.....	32
<b>Chapter 3</b> .....	60

Survival, transmission and control of <i>Phoma koolunga</i> in field pea seed and reaction of field pea genotypes to the pathogen .....	60
<b>Chapter 4</b> .....	93
Appearance of atypical colonies of <i>Phoma koolunga</i> on culture media or artificially inoculated plants in controlled conditions.....	93
<b>Chapter 5</b> .....	108
Investigation of formation of teleomorph of <i>Phoma koolunga</i> in controlled conditions .....	108
5.1 Introduction .....	109
5.2 Materials and Methods .....	111
5.2. 1 Experiments with multiple <i>P. koolunga</i> isolates .....	111
5.2.2 Experiments with <i>P. koolunga</i> isolates representing two mating types .....	112
5.3 Results .....	114
5.4 Discussion .....	114
<b>Chapter 6</b> .....	117
General discussion.....	117
<b>References</b> (Chapter 1, 5-6).....	127

## **Abstract**

Ascochyta blight (blackspot) is a significant disease of field pea (*Pisum sativum*) with worldwide distribution, causing grain production losses of 15 % per annum in Australia. *Phoma koolunga* is a relatively new pathogen of this complex disease in Australian field pea crops. This thesis reports information about aspects of the epidemiology of this fungus in Australian conditions.

The survival of *P. koolunga* on field pea stubble and as pseudosclerotia buried in field soil was examined. The frequency of recovery of this fungus declined over time and it was not recovered from stubble buried in soil or placed on the soil surface in pots outdoors at months 11 and 15, respectively, and later. Pseudosclerotia were produced in Petri dishes containing potato dextrose agar (PDA) amended with fluorocytocin or with sand. The maximum longevity of pseudosclerotia buried in soil in pots outdoors was less than 18 months. Infectivity of inoculum of the fungus decreased over time, as the mean number of lesions on plants inoculated with stubble buried or left on the soil surface for up to 6 and 5 months, respectively, and pseudosclerotia retrieved at 14 months and later from field soil did not differ from the water control in a pot bioassay.

*P. koolunga* was isolated from field pea seed samples harvested from South Australia and Victoria. Disease was transmitted to 98 % of seedlings that emerged from artificially inoculated seeds (AIS) in growth room conditions. Seedling emergence rate from AIS at 8° C soil temperature was lower than at 12, 16 and 20° C and also disease severity on seedlings was greater at the lower temperature. Efficacy of fungicides as seed dressings was examined on AIS. P-Pickel T<sup>®</sup> and Jockey Stayer<sup>®</sup> were the most effective fungicides among six tested for reducing

disease incidence and severity due to *P. koolunga* on seedlings that emerged from AIS sown in soil and on germination paper, respectively.

The reaction of 12 field pea genotypes to one moderately virulent isolate of *P. koolunga* was evaluated by spraying a pycnidiospore suspension on plants in controlled conditions and assessing disease severity at 2-5 day intervals for 21 days. Sturt, Morgan and Parafield showed more severe disease on leaves than the other genotypes at 21 days post-inoculation (dpi), and Kaspera, PBA Twilight, PBA Oura, PBA Wharton and WAPEA2211 were less susceptible. When three isolates of *P. koolunga* which varied in virulence were sprayed on four genotypes of short, semi-leafless type peas, Morgan and WAPEA2211 showed more disease than Kaspera at 21 dpi. Aggressiveness of isolates of *P. koolunga* on these four genotypes differed based on % leaf area diseased up to 14 dpi, but this difference had disappeared by 21 dpi.

Some isolates of *P. koolunga* from seeds showed atypical morphology and reproductive behaviour. These cultures had rhizoid form mycelia on growth media such as PDA. Also, some atypical cultures of *P. koolunga* sectorised from typical colonies on PDA. These sectors and cultures were confirmed as *P. koolunga* by DNA test using *P. koolunga*-specific primers. Mycelium from these sectors produced small lesions on leaves and stems of field pea seedling resembling ascochyta blight symptoms in controlled conditions. Pycnidium-like structures of these atypical cultures did not contain pycnidiospores, but had many round and hyaline fatty guttulae of different sizes, usually smaller than normal spores of *P. koolunga* which never germinated.

Crossing 19 isolates of *P. koolunga* in vitro failed to initiate formation of pseudothecia of *P. koolunga* on pea stem pieces or on several growth media. This

fungus might need specific environmental conditions for production of pseudothecia which still are unknown.

The results of this study provide information about survival of *P. koolunga*, transmission to seedlings via infected seed, control of the fungus in seed and also reaction of field pea genotypes to this pathogen in South Australian conditions. These findings improve the understanding of epidemiology of this disease and consequently can help to improve management of this pathogen in the field.

## **Declaration**

I, Mohsen Khani, certify that 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. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Mohsen Khani.....







## **Acknowledgements**

I thank my principal supervisor, Prof Eileen Scott and co-supervisors, Dr Jenny Davidson and Dr Mark Sosnowski for their invaluable professional guidance and advice, as well as their kind encouragement, throughout this project. I am really grateful to Prof Scott for her support, amazing editorial advice and her passion in giving her time.

I also thank the Iranian Ministry of Sciences, Research and Technology for providing a scholarship to support this study and the School of Agriculture, Food and Wine for operating support.

My gratitude also to:

- Pulse Pathology laboratory members in the South Australian Research and Development Institute (SARDI), particularly Dr Rohan Kimber, for valuable scientific and technical advice throughout this research, Mrs Marzena Krysinska-Kaczmarek for helpful technical advice and Michelle Russ, Jamus Stonor and Dr Suzanne McKay for logistical support
- All members of the Plant Protection group and Plant Pathology group at the University of Adelaide, especially Drs Maarten Ryder and Tijana Petrovic for helpful discussion on many matters throughout this study
- Dr Herdina, SARDI Diagnostic Group, for assistance in DNA testing of samples and Dr Hans de Gruyter of Plant Protection Services, the Netherlands, for his valuable responses to my questions
- Mrs Sabela Munoz Santa and Dr Esmaeil Ebrahimie for their helpful advice in statistics and analysing data
- Dr Kolumbina Mrva for her kind encouragement, support and sharing her experiences during coffee breaks

Finally, I thank my parents, my wonderful wife Negar, my son Rayan and my parents-in-law for their support, patience and love.