Factors affecting short and long distance dispersal of fungal pathogens – chickpea ascochyta blight as a model

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Factors affecting short and long distance dispersal of fungal pathogens – chickpea ascochyta blight as a model

Wind and rain dispersed *Ascochyta rabiei* conidia trapped in the wind tunnel (photo by Steve Coventry)

*All experience is a muddle, until we make a model to explain it. The model can clarify the muddles, but the model is never the muddle itself. "The map is not the territory"; the menu does not taste like the meal.*

*Dr Robert Anton Wilson*
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Abstract

Exotic fungal plant pathogens pose a great threat to Australian agriculture. Some of the most devastating fungal pathogens are transported by rain splash, wind dispersal or a combination of both. Ascochyta rabiei, causal agent of ascochyta blight of chickpea, is a wind and rain borne pathogen already present in Australia. A. rabiei, therefore, provides a suitable pathogen for studying the potential spread of any exotic fungal pathogen having similar dispersion mechanism. In this study, firstly, laboratory and field experiments were conducted to examine the key environmental factors influencing the rain-splash triggered short distance and wind triggered long distance distribution of propagules (conidia) of A. rabiei. Secondly, a weather-based simulation model was developed and implemented for spatio-temporal dissemination of spread of chickpea ascochyta blight in natural environments.

The influence of temperature and relative humidity (RH) was studied on the viability of conidia of A. rabiei to help clarify in what environments the conidia initiate epidemics. Conidia were exposed to conditions of 5 - 45 °C (dry) and 12.5 - 100 % RH. Viability decreased from 100 % after 2 h at all the temperatures tested to 0 % after 144 h of exposure to temperatures exceeding 25 °C. Conidia failed to germinate when incubation period exceeded 8 h at 40 °C. After 4 days of exposure to 30 - 35 °C germination of conidia was 1 - 88 %. Conidia remained viable and able to germinate when given optimum conditions following incubation in RH ranging from 12.5 to 100 % over a period of 96 h at 20 °C. More than 50% of conidia germinated following exposure to the lowest RH (12.5%) at 20 °C.

The effect of wind speed (m s\(^{-1}\)) and rain splash (mL m\(^{-1}\)) on the dispersal of conidia in a purpose-built wind and rain tunnel was investigated. Conidia were trapped on 40 cm tall x
2 mm wide rods placed between 2 and 110 cm along the tunnel; pieces of double-sided sticky tape were applied parallel to the rods at heights of 1 - 6, 11 - 16 and 31 - 36 cm. In the presence of simulated wind and wind-rain, conidia were distributed at least 66 cm, and the distance to which conidia were distributed increased with wind speed. Most conidia, in the order of hundreds to thousands, were trapped close to the inoculum source whereas fewer, in the order of tens to hundreds, were caught further from the source, with rain causing a greater number of conidia to be dispersed. Simulated rain also dispersed conidia to vertical tape positions 31 - 36 cm where none were trapped in the presence of wind alone.

Two field experiments were conducted to investigate the spread of ascochyta blight in natural environments at Kingsford (2007) and Turretfield (2008), South Australia. The disease was assessed following inoculation via infested stubble in plots (11 x 11 m each) for three chickpea cultivars, Howzat (moderately susceptible), Genesis 090 (resistant) and Almaz (moderately resistant). Logistic regression analysis was used to compare the rate of change of disease severity and the distance over which disease occurred. Weather data from a local automatic weather station were compiled and associations between wind direction, wind speed, rainfall, cultivar and disease severity were examined. Specifically, higher rainfall in 2008 was associated with faster rate and further spread of disease. In both years, disease spread was faster and further in Howzat than in Almaz, whereas no disease was observed in Genesis 090. Strong and continual winds in the southern and eastern directions in both years influenced the rate of increase in disease severity and the distance over which disease spread.

A spatiotemporal model was developed, based on Anthracnose Tracer for lupins, to determine the spread of ascochyta blight in natural environments. The model was based
on a published model, written in Mathematica\textsuperscript{TM} and runs on an hourly basis. The model is driven by the hourly weather data, \textit{viz.} air temperature (°C), rainfall (mm h\textsuperscript{-1}), wind speed (m s\textsuperscript{-1}), wind direction (°), and standard deviation of the wind direction (°). The parameters of the model were estimated using the data collected in this study. The model was calibrated using 2007 field data. When validated with 2008 field data, the prediction from the model for the incidence of chickpea ascochyta blight closely matched with observation.

The results from this study have a number of implications. One, the newly developed model can form a basis for studying the likelihood of disease spread for exotic plant pathogens that have similar epidemiology to \textit{A. rabiei}. Two, the model can be used to predict the pathogens, potential to cause damage in the regions where chickpea ascochyta blight has not yet spread. Three, this modelling work can contribute to the formulation of strategies for management of ascochyta blight in chickpea by targeted fungicide application and sowing regimes.
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 to Steven Arthur Coventry and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference is made in the text.

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Steven Arthur Coventry:............................................

Date:.............................................
Statement of the Contributions to Jointly Authored Papers

1. Development of a model to simulate the spread of ascochyta of chickpea in the field. Plant Pathology X:X-X [prepared manuscript]

Presented in chapter 7. Author contributions: SAC designed and conducted all research experiments, analysed the data, and drafted/constructed the manuscript. MUS supervised the model development, calibration and application. ESS, JAD and MUS contributed to the research ideas and design, and the writing of the manuscript.

The manuscript displayed in this thesis is in submission form according to the instructions to authors 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 the research manuscript and give consent to their inclusion in this thesis.

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## List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>BOM</td>
<td>Bureau of Meteorology</td>
</tr>
<tr>
<td>cv., cvs</td>
<td>cultivar, cultivars</td>
</tr>
<tr>
<td>DAFWA</td>
<td>Department of Agriculture and Food Western Australia</td>
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<tr>
<td>E</td>
<td>East</td>
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<tr>
<td>E&lt;sub&gt;k&lt;/sub&gt;</td>
<td>kinetic energy</td>
</tr>
<tr>
<td>E&lt;sub&gt;max&lt;/sub&gt;</td>
<td>average maximum kinetic energy</td>
</tr>
<tr>
<td>E&lt;sub&gt;p&lt;/sub&gt;</td>
<td>potential energy</td>
</tr>
<tr>
<td>FAO</td>
<td>Food and Agriculture Organisation</td>
</tr>
<tr>
<td>g</td>
<td>gravitation acceleration constant</td>
</tr>
<tr>
<td>h</td>
<td>hour</td>
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<tr>
<td>ln</td>
<td>natural logarithm</td>
</tr>
<tr>
<td>MAT</td>
<td>mating type</td>
</tr>
<tr>
<td>MPA</td>
<td>megapascal</td>
</tr>
<tr>
<td>MR</td>
<td>moderately resistant</td>
</tr>
<tr>
<td>MS</td>
<td>moderately susceptible</td>
</tr>
<tr>
<td>N</td>
<td>North</td>
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<tr>
<td>PDA</td>
<td>potato dextrose agar</td>
</tr>
<tr>
<td>R</td>
<td>resistant</td>
</tr>
<tr>
<td>RH</td>
<td>relative humidity</td>
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<tr>
<td>RWA</td>
<td>reverse osmosis water agar</td>
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<td>S</td>
<td>South</td>
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<tr>
<td>SA</td>
<td>South Australia</td>
</tr>
<tr>
<td>SARDI</td>
<td>South Australia Research and Development Institute</td>
</tr>
<tr>
<td>Tunnel</td>
<td>Wind and rain tunnel</td>
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<tr>
<td>W</td>
<td>West</td>
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<td>WA</td>
<td>Western Australia</td>
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| ix           | }
Chapter 1: General introduction

Australian horticultural and agricultural industries are constantly threatened by exotic plant pathogens, which may be introduced through breach of quarantine or by natural mechanisms. Containment and eradication of new incursions of exotic plant pathogens needs a comprehensive knowledge of their epidemiology, which may include modelling the spread of the pathogen by rain, wind and other environmental conditions. However, it is not possible to study the dissemination of exotic plant pathogens by such factors in Australia, so plant pathogens already present in the country may serve as models for exotic plant pathogens.

Threatening exotic plant pathogens which are spread by wind and rain include *Colletotrichum truncatumin*, causing anthracnose on lentils, peas, and *C. lupini* which causes anthracnose of lupins (Plant Health Australia, 2009). A suitable model pathogen to study in place of such *Colletotrichum* spp., and the subject of this thesis, is the fungus *Ascochyta rabiei*, which is already present in Australia, as it is spread in a similar manner over long distances by wind and over short distances by rain splash. *A. rabiei* has both rain-splashed asexually produced spores (conidia) and windborne sexually produced spores (ascospores of the *Didymella* teleomorph), which from this point forward will be termed asexual and sexual spores respectively. Investigation of *A. rabiei* will provide more information on the spread of fungal pathogens in the field and increase readiness for potential future pathogen incursions.

The asexual stage of *A. rabiei* was identified in commercial crops of chickpea (*Cicer arietinum*) crops for the first time in South Australia in 1995 and devastated the South Australian and Victorian chickpea industry in 1998. All strains were identified as mating
type MAT 1 - 1 (subsequently reclassified to MAT 1 – 2, Phan et al. (2003)), through pairing Australian A. rabiei isolates with international standard isolates (Khan et al., 1999a). The ascospores of D. rabiei have been documented in Western Australia at extremely low numbers in laboratory experiments (Galloway & MacLeod, 2003), and further investigation of the presence of the sexual stage in South Australia is needed. D. rabiei was not found in South Australia in these investigations, however, so conidia were investigated as a potential long distance dispersal mechanism as it is not known how far the anamorph can be transported and its role in causing subsequent infections in uninfected regions is not understood.

The aim of this research was to gain greater understanding of the relationship between the environment and spore dispersal with reference to the pathogen A. rabiei. A subsidiary aim was the development, calibration and testing of a simulation model to predict the spread of ascocysta blight in the field.
Chapter 2: Literature review

2.1 Introduction

This review examines the pathogen, *Ascochyta rabiei* of chickpeas, and the role of spore dispersal and infection over short and long distances inherent to cause epidemics. It explores the history, symptoms and effect of ascochyta blight of chickpea on the Australian chickpea industry. It identifies the role of the environment, ultra violet radiation, temperature, relative humidity, wind, rain and wind plus rain in assisting spore dispersal and disease development. It further investigates the use of these factors in various modelling techniques used in plant pathology, with emphasis on simulation modelling to develop a predictive tool for ascochyta blight of chickpeas in Australia as a model for exotic plant pathogens of significance in biosecurity.

2.2 Australian production of chickpeas

The first commercial chickpea crop in Australia was grown in 1978 on the Darling Downs in south-east Queensland (Fletcher, 1994). In 2008 Australia was the world’s 4th largest producer of chickpeas with 378,000 ha and the world’s 2nd largest exporter in 2007, behind India, with 159,584 tonnes exported (FAOSTAT, 2011). Chickpeas are an important food and are consumed by people in India, Pakistan, Bangladesh and Nepal where they serve as an inexpensive high quality protein (Akem, 1999). Chickpeas are also consumed in other parts of the world, including the Middle East, the Mediterranean and Asia (Bestow et al., 1996). In 2007, the total revenue from export of chickpeas from Australia was $94 million (FAOSTAT, 2011).
Chickpeas are an important winter crop in the wheat growing areas of Australia, and are mostly grown in Queensland, New South Wales and Victoria. Nitrogen fixation by chickpeas in association with *Rhizobium* spp. produces residue that boosts nitrogen in the soil for succeeding cereal crops, benefiting and contributing to sustainable cropping (Fletcher, 1994, Pulse Australia, 2006, Akem, 1999).

There are two main types of chickpea, desi and kabuli, which differ in seed size, shape and colour. Desi types have small seeds (less than 26g / 100 seeds) that are irregular in shape and in various colours. Desi plants are shorter, bushier, with smaller leaflets than kabuli, and the flowers are purple with a red pigmentation on the stems and pod. Desi are early to medium maturing and have better tolerance of lower rainfall environments (Bestow *et al.*, 1996). They account for about 85 % of the world total production of chickpeas and are widely grown in Australia (Pande *et al.*, 2005). Kabuli chickpeas have large seeds (greater than 26g / 100 seeds) that are round and pale, flowers are white or cream and there is no pigmentation on the pods or seeds. These mature later and need a longer growing season than desi. Desi and kabuli types both have poor tolerance to frost and water logging.

### 2.3 Ascochyta blight: an Australian epidemic

Ascochyta blight caused by *A. rabiei* is a major constraint to chickpea production in Australia and a devastating disease of chickpeas worldwide. *A. rabiei* was first identified in 1911 in the North / Western frontier of India, which is now part of Pakistan (Pande *et al.*, 2005, Butler, 1918). *A. rabiei* was discovered in commercial crops in South Australia and the Wimmera region of Victoria for the first time in 1995 (Khan *et al.*, 1999a). Severe epidemics developed across the southern region of Australia in 1998. It is likely that heavy rain in south-eastern Australia in 1997 increased seed infection, and the
resulting seeds were kept for sowing next season (Ramsey et al., 1999). Early favourable weather patterns in 1998 caused dispersal of the pathogen, spreading the disease further (Shtienberg et al., 2006). At the time, susceptible cultivars grown in south-eastern Australia were devastated, with production losses of 50 to 100% (Galloway & MacLeod, 2003, Kimber et al., 2007, Shtienberg et al., 2006). In South Australia and Victoria the area sown to chickpeas decreased from 132,000 ha in 1999 to less than 5,500 ha in 2004 (Figure 2.1, ABARE (2010). The total area sown in Australia decreased from 308,000 ha in 1998, just before the onset of disease, to 195,000 ha in 2001 after the disease outbreak. In 2006 the total area of chickpeas sown was 259,000 ha, still considerably less than that before the outbreak of ascochyta blight although some of this reduction was due to long term drought (Slatter et al., 2006). Recently, however, there has been a resurgence in the production of chickpeas with the release of ascochyta blight resistant cultivars by breeding programs; 373,000 ha were grown in 2010 (ABARE, 2010). The majority of production now occurs in northern New South Wales and Queensland (Pulse Australia, 2006) where ascochyta blight epidemics are less severe due to the lower incidence of rainfall during the growing season. South Australia and Victoria presently sow only modest amounts of chickpeas compared to the Australian total, with 14,000 and 21,000 ha, respectively sown in 2010 (ABARE, 2010).
Figure 2.1: Area of Australian chickpea production before and after the Ascochyta blight outbreak in 1998 (ABARE, 2010).

2.4 Symptoms

A. rabiei forms brown lesions with dark haloes and affects all above-ground parts of the plant. Lesions that develop on leaves, stems and seed pods are typically circular, with erumpent, dark-brown pycnidia (Figures 2.2 and 2.3). Symptoms can be visible 4 to 5 days after infection (Kaiser, 1992).

Stem lesions cause stem breakage and death or re-shooting of the plant (Punithalingam & Holliday, 1972) (Figure 2.3). Breakage of stems can result in devastation of chickpea crops (Trapero-Casas et al., 1996). Pod infection often occurs and the developing seed can be infected through the pod wall (Porta-Puglia, 1990).
2.5 Host range

Host range studies have shown that pathogenicity of \textit{A. rabiei} can vary when investigated in natural and controlled conditions. In controlled greenhouse conditions in Syria, \textit{A. rabiei}...
*A. rabiei* was inoculated on common bean and cowpea, and only restricted necrotic lesions without pycnidia were observed (Nene & Reddy, 1987). Khan *et al.* (1999b) inoculated 20 plant spp. with South Australian isolate DAR 71767, maintained them in humidity chambers at 25 °C for 72 h, and found that the isolate was pathogenic to chickpea and four cultivars of common bean (*Phaseolus vulgaris*). Infected plants showed typical ascochyta blight symptoms on leaves and petioles 4 - 5 days after inoculation, and *A. rabiei* was re-isolated.

*A. rabiei* has also been isolated from *Brassica nigra*, *Descurainia sophia*, *Galium aparine*, *Lamium amplexicaule* and *Triticum aestivum* grown in fields where infested chickpea debris of the previous season remained on the soil surface (Pande *et al.*, 2005). Kaiser (1991) documented that alfalfa (*Medicago sativa*) and white sweet clover (*Melilotus alba*) were infected in the field by an isolate of *A. rabiei* from Idaho, U.S.A, and developed necrotic lesions with pycnidia.

Sixteen crop and weed species have recently been reported to have been infected by *D. rabiei* naturally in North Idaho and East Washington. The infected plants, including wheat, clover and pea, were asymptomatic but cultures of surface sterilized stems and leaves showed that *D. rabiei* had invaded the tissues. Isolates of the pathogen from the different hosts were indistinguishable from *D. rabiei* isolated from chickpea, in terms of morphology, and were highly pathogenic to chickpeas (Trapero-Casas & Kaiser, 2009).

### 2.6 The disease cycle

*A. rabiei* has two different spore stages within the disease cycle, the anamorph (asexual stage) and the teleomorph (sexual stage) (Figure 2.4). Primary infection arises from either ascospores or conidia from crop debris or by direct infection from infected seed. Conidia
are dispersed by rain splash (Kaiser, 1997). Pseudothecia form on chickpea crop residues that lie on the soil surface after harvest and ascospores mature in time to infect the new season’s crop. In the northern hemisphere this pseudothecial development occurs in autumn and ascospores are discharged in late winter / early spring. In the southern hemisphere it is presumed that this development also occurs in autumn, when conditions are favourable. The resulting sexual spores are disseminated by wind over long distances (Trapero-Casas & Kaiser, 1987a). Following spore dispersal the fungus penetrates and infects plant tissues resulting in disease. Pycnidia develop within the lesions and the resulting conidia are responsible for secondary spread of the disease through rain splash.

Figure 2.4: Disease cycle of Ascochyta blight of chickpea (Drawing by R.M. Hannan. Source: Kaiser (1997)).
2.6.1 The anamorph

The anamorph, *A. rabiei*, produces conidia, or pycnidiospores, in a fruiting body called a pycnidium. The pycnidia contain straight or slightly curved, hyaline, mainly septate and sometimes unicellular conidia, 10 - 16 x 3.5 μm in size (Punithalingam & Holliday, 1972). On potato dextrose agar (PDA) colonies with pycnidia develop which are creamy to pinkish at first, darkening with time. The wall of the pycnidium has one to two layers of extended pseudoparenchymatous cells with an ostiole 30 - 40 μm wide (Punithalingam & Holliday, 1972). Pycnidia form within 4 - 5 days and appear in concentric rings.

2.6.2 The teleomorph

The teleomorph, *D. rabiei*, produces ascospores in a fruiting body called a pseudothecium, and requires two different mating types MAT 1 - 1 and MAT 1 - 2 (Figure 2.4). This is termed heterothallism (Trapero-Casas & Kaiser, 1992b). Ascospores are hyaline, two-celled with the upper cell broader than the lower, constricted at the septum and 12.5 - 22 x 6 (5-7) μm in size (Kovacevski, 1936).

2.6.3 The teleomorph in Australia

*D. rabiei* was reported in Australia as a result of laboratory experiments using varied wind speeds in wind tunnels to move spores from stubble (Galloway & MacLeod, 2003). Ascospores were trapped on adhesive tape placed on roto-rod air samplers and then viewed microscopically, where up to 1396 ascospores/ g of stubble were found. The presence of ascospores in Western Australia suggests that both mating types are present or that occasional homothallic compatibility exists. Phan et al. (2003) compared the genetic diversity of *A. rabiei* isolates from Western Australia, South Australia and Victoria with isolates from Canada, USA, Tunisia and Syria using Sequence-Tagged Microsatellite Site (STMS) markers. The Australian population was found to have low
genetic diversity compared to the isolates taken from the other countries and only MAT 1 - 2 was detected, thus supporting the idea that occasional homothallic compatibility exists (Leo et al., 2011). The lack of genetic diversity in Australian isolates means that strict quarantine measures are needed to reduce the likelihood of an isolate of MAT 1 - 1 entering the country and devastating the chickpea cvs currently used (Phan et al., 2003).

2.7 Epidemiology

Epidemiology is the study of epidemics, which involves the interaction between the host, pathogen and environment (Blakeman & Williamson, 1994, Jones, 1998). Epidemiology of plant disease provides an understanding of disease progress and the most effective strategies in disease prevention. Detailed investigation of the plant-pathogen interaction of *D. rabiei* has been performed, but there is relatively little information on the environmental factors that affect dispersal of conidia or ascospores. Studies of other rain and wind borne pathogens have provided a foundation for understanding the influence of rain and wind on development and maturation of spores and distances to which they may spread, which may be useful in understanding *D. rabiei*. However, few studies have combined information on the effect of the environment on spore dispersal including survival, and applied it to modelling to understand and predict incursions by exotic plant pathogens not already present in Australia.

2.8 Environmental conditions

Rainfall and high relative humidity (RH) are critical for most epiphytotics, with temperature also playing an important role in development. During the early stages of infection, fungal diseases need favourable environmental conditions such as surface moisture, high RH and suitable temperature, for germination of spores and mycelial growth. Ascochyta blight infection and disease development occur in the temperature
range of 5 - 30 ºC, with an ideal temperature of 20 ºC (Trapero-Casas & Kaiser, 1992b, Trapero-Casas & Kaiser, 2007). Infection occurs at RH of > 95% (Jhorar et al., 1997) and in wetness periods of 10 h or more (Reddy & Singh, 1990, Khan et al., 1999a). A. rabiei, therefore, is infective in areas with cool, cloudy and humid weather (Pande et al., 2005).

A. rabiei can survive for 8 months on infected plant debris at temperatures of 10 - 35°C (Nene & Reddy, 1987) and for 20 months on infected stems at 10 - 35°C (Pande et al., 2005). At suboptimal temperatures, a longer wetness period is required for germination, and germination is rare in hot dry periods (Jhorar et al., 1998). Jhorar et al. (1997) found that no infection of chickpeas occurred at less than 95 % RH in laboratory experiments using humidity chambers. Disease severity increased with increased wetness duration, with 18 h giving the highest disease incidence. Germination of conidia occurred in the first 12 h and increased linearly up to 52 h. Dry periods imposed immediately after inoculation followed by 24 h of leaf wetness lessened severity, and as the dry period increased, disease severity decreased. This was contradictory to Trapero-Casas and Kaiser (1992b, 2007) who found that intermittent dry and wet periods after inoculation increased disease severity. Trapero-Casas and Kaiser (2007) also found that germination on water agar (RWA) occurred within 2 h and that maximum germination was reached at 6 h. High RH (100 - 97 %) produced through adjusting the water potential of the WA also had a profound effect on germination of conidia, with germination declining with decreased water potential, which was dependent on temperature.

Pseudothecia form on chickpea debris and the fungus needs around 2 months to mature and develop ascospores (Trapero-Casas et al., 1996, Dugan et al., 2005). Conditions conducive for development of pseudothecia and release of ascospores are moisture close to saturation point and temperature initially at 20 - 27 ºC (Trapero-Casas et al., 1996,
Shtienberg et al., 2000, Trapero-Casas & Kaiser, 1992a) then cooler periods of 5 - 10 ºC (Trapero-Casas and Kaiser 1992). RH, rather than temperature, is critical for development of this fungus on crop residues (Pande et al., 2005).

2.8.1 Abiotic spread

Fungal spores do not move towards a host without a means of dispersal. Passive distribution of pathogens by wind, water, insects or humans is often responsible for plant disease outbreaks (Agrios, 1997). Spread can be long-distance, by wind, or short distance, by rain splash. Conidia of A. rabiei are splashed by rain and ascospores blown by wind. Kimber et al. (2007) found that in South Australian conditions disease spread occurred over short distances (< 10 m) from individual primary infections and was governed by rain and wind. Disease spread in an outward direction from the primary infection focus and spread down wind was five times greater than spread up wind. This suggested that wind-assisted droplet dispersal contributed to the spread of the disease.

2.8.2 Raindrop splash and spore dispersal

Dissemination of spores by rain splash has often been studied in biological rather than epidemiological terms. Rain often occurs in discrete events, characterised by duration and amount of rain dispersed (mm) (Sache, 2000). Short distance dispersal of spores is often responsible for primary disease outbreaks (Sache, 2000) and spread of spores by means of water is important in short distance disease development, but can also aid in long distance dispersal (Madden, 1997, Wadia et al., 2000, Pande et al., 2005). Rain splash aids in quick dispersal of the pathogen from the plant surface to bordering plants. The distance, speed and subsequent infectivity of these spores are poorly understood, and these factors need clarification.
Rain-splashed conidia are transferred from the primary infected seedlings, which results in scattered foci in the field. Severity is sometimes greatest at the first foci and as the pathogen travels further from the source disease severity decreases in an inverse linear fashion (Kimber et al., 2007). Spores of rain-splashed fungi are generally hyaline and filiform in shape. This shape encourages adhesion of spores to the water surface, because of strong surface tension effects, or because of trapped air within the conidium (Lacey & West, 2006). Some Ascomycetes which lack explosive asci may liberate spores in a mucilage which prevents or limits spread by wind alone (Gregory, 1973). The mucilage dissolves when wet to give a spore suspension in a thin layer of water on the host surface (Lacey & West, 2006), which can then be spread by rain-splash.

During rain, spores can be “scrubbed” from the air when they come in contact with falling raindrops and are subsequently deposited onto a host crop. Raindrops that hit the leaves also transfer energy to spores on the leaves causing them to detach, thus transporting spores to nearby plants on higher and adjacent leaves (Figure 2.5). Spores can be also “washed off” the plant in major rain events, through the leaf being repeatedly hit, which removes spores to the lower leaves or to the soil in a wash off effect. In lighter rain events, however, spores can be transported through splash to adjacent plants and are not scrubbed.
Figure 2.5: Rain dispersal of spores from a wheat plant (Source: Sache, 2000).

Sache (2000) found that thunderstorms removed most wheat rust spores quickly and deposited them on leaves, quickly exhausting the spore supply from the lesions. Light rains, however, were less efficient at spore removal but were better at spreading the pathogen. Intermittent rains with low intensity gave best spore transfer, especially if combined with high wind speeds. Under these conditions spore removal occurred without exhausting the supply, and spores were easily transported by wind (Sache, 2000).

Schoeny et al. (2008) investigated the effect of plant canopy architecture on the splash dispersal of *Mycosphaerella pinodes*, which causes black spot or ascochyta blight of field pea. Experiments were performed in controlled conditions using a rainfall simulator which generated 2 mm rainfall events in still air when applied for 3 minutes. Schoeny et al. (2008) reported that less dense pea canopies, with a leaf area index (LAI) of 0.36, resulted in limited spore dispersal compared with denser canopies with a LAI of 0.48 or greater. The majority of conidia were distributed downwards and very few were transported upwards or across from the foci. Conidia were splash dispersed in total over
very short distances, with half distances (the distance in which the number of spores decreases by half), of 1.6 to 6.5 cm. This study showed that in field conditions, early disease spread is likely to affect the source plant and adjacent plants first due to a less dense canopy. As the canopy becomes denser the pathogen will spread further, until the canopy becomes too dense such that it forms a physical barrier for splash dispersal; any subsequent dispersal is then influenced primarily by wind.

On impact, the surface of an object causes raindrops to divide by transfer of energy. Smaller droplets scatter in different directions through force (Sache, 2000, Geagea et al., 1999). Therefore, after the large drops hit a sporulating lesion, the secondary smaller drops can carry spores to surrounding plants (Sache, 2000, Agrios, 1997). Raindrops of different sizes have different amounts of kinetic energy. Those which reach the ground are 0.2 - 5 mm in diameter, while smaller drops evaporate and larger drops break up when approaching terminal velocity (Fitt et al., 1989). Small drops have reduced kinetic energy and therefore remove smaller numbers of spores whereas larger drops, which occur less often, have high kinetic energy, removing more spores (Sache, 2000). Drops of 2 mm in diameter or greater cause the most amount of splash from leaves but the amount and type of splash is influenced by the leaf surface being dry or wet (Fitt et al., 1989) and by crop structure (Schoeny et al., 2008). This difference in both raindrop separation and distance is important for dispersal of spores and epidemic development.

When a raindrop impacts and mixes with inoculum there are two stages involved in the dispersal process, described by Fitt et al. (1989) as “removal, mixing and splash droplet formation. This includes the raindrop descending from the atmosphere and the drop colliding and mixing with the spore suspension. The drop, when it collides with the suspension, flattens on impact. This causes a radial jet that turns into smaller droplets due
to the action of surface tension, leading to inoculum removal. Consequently, smaller spore carrying droplets are dispersed in various directions, causing adjacent plants to be infected with the pathogen.

Experiments conducted in rain towers in still air (Figure 2.6) have been used to show that one splash may disperse thousands of spores, mostly in the largest droplets (greater than 1000 µm in diameter), while few were dispersed in droplets less than 100 µm in diameter (Fitt & Lysandrou, 1984, Brennan et al., 1984, Fitt et al., 1989). Spore-carrying droplets travelled less than 15 cm with greater than 10,000 spores per droplet and beyond 85 cm with fewer than 100 spores per droplet (Fatemi & Fitt, 1983). This showed that although larger droplets carry larger spores, the distance to which they travel is less than that of smaller droplets carrying fewer spores.

**Figure 2.6:** Inoculum dispersal by rain splash, a schematic diagram resulting from experiments conducted in rain towers at Rothamsted Research Institute (Fitt et al., 1989).
In a study by Gregory et al. (1959), a raindrop of 5 mm in diameter falling on a spore suspension of 0.1 mm deep produced more than 5200 splash droplets, of which 2000 carried one or more spores (Gregory et al., 1959). As distance from the first impact zone increased, droplet deposition decreased. In still air, few droplets travelled further than 70 cm. The experiment also showed that size and velocity of the raindrop and the depth of the spore suspension affect inoculum dispersal. These studies suggest that splash dispersal is therefore greatly determined by velocity of the impacting raindrop. Therefore rain with many large drops having greater terminal velocity will effectively disperse spores of the pathogen further distances from the initial impact than rain with smaller size drops.

Wadia et al. (2000) simulated raindrops using hypodermic needles placed 11 m high, allowing drops to travel at speeds near terminal velocity. The pathogen investigated in this study was Passalora personata, which causes late leaf spot of groundnut. Needles displaced droplets of diameters 4 mm, 3 mm and 2 mm, and larger drops dispersing a greater percentage of conidia over a greater distance.

In a similar study, Geagea et al. (1999) used a 50 mL syringe with different hypodermic needles and a syringe pump to produce constant drops with diameters of 2.5, 3.4, 4.2 and 4.9 mm. Drops fell from 5, 50 and 100 cm and splash droplets collected on glass slides identified the presence of wheat rust spores (Puccinia recondita and P. striiformis) at different distances. When droplet size and height of fall were increased it was found that dispersal of spores in droplets also increased. The amount of spores was also found to increase with drop diameter, with 4.9 mm droplets dispersing four times as many spores as 2.5 mm drops. Fall height also increased spore removal, with 100 cm giving greater removal than 5 cm.
These experiments have been conducted as part of endeavours to explain inoculum dispersal and the spore carrying potential of water droplets in controlled environments.

2.8.3 Wind dispersal

Many fungi reproduce by spores that can become airborne at least once in their life cycles (Lacey & West, 2006). Dispersal by wind is important in many pathosystems. Wind dispersal allows a pathogen to travel much longer distances than can be achieved by rain splash alone, and can result in multiple foci in fields and transportation into a new field or region to start a new epidemic. There are generally four atmospheric levels to which spores can be dispersed by wind; these are the laminar boundary layer, turbulent boundary layer, outer frictional turbulence layer and the convectional layer level, as illustrated in Figure 2.7 (Lacey & West, 2006). The laminar boundary layer is a still layer of air in which little spore dispersal occurs. Spores that disperse from here are usually on the surface of plant residues or soil, such as ascospores of D. rabiei, and are caught by updraft. The variably turbulent boundary layer is where dispersal and mixing are most frequent. Plants with spores release them into the air or they can be blown off plants in strong winds. The turbulent winds then move particles upwards into the next layer, the outer frictional turbulent layer, where horizontal wind currents transport the free spores long distances across into adjacent fields, causing new disease foci. Outdoor air is dynamic, constantly changing with location, weather, season, time of day, and is influenced by three main sets of weather conditions: clear night, cloudy day and sunny day. Clear nights are relatively still with no great movement of spores but, combined with wide temperature fluctuations, result in large scale vertical mixing and thundercloud formation. The cumulus type of cloud system can create an upward draft of wind up to 10 kilometres high, lifting spores to great heights (Petterssen, 1969). During a cloudy day, as
compared to a sunny day or a clear night, there is often an increase of wind velocity, causing great transfer of spores from plants or spores lying on the earth’s surface. Sunny days produce pockets of air that are warmer than the surrounding air; these rise upwards as thermals resulting in removal of spores from the surface of the earth.

**Figure 2.7:** The effect of weather and atmosphere level on spore dissemination (Gregory, 1973).

Spores are transferred from host surfaces or captured by wind when released from the pathogen. Many Ascomycetes produce fruiting bodies which contain asci filled with sap in which the ascospores are formed. Through a fall in RH, pressure inside the ascus becomes high and the ascus is triggered to open, scattering spore-laden sap into the atmosphere. Ascospores are discharged from pseudothecia of *D. rabiei* during periods of
rain and may spread over 100 m from the source (Kaiser, 1992, Traper-Casas & Kaiser, 1987b). This phenomenon is common in many wind dispersed pathogens. Depending on air turbulence and velocity, the spores can travel downwind or horizontally in the same way as particles contained in smoke (Agrios, 1997). Figure 2.8 illustrates passive spore release from an infected leaf, which would occur at the turbulent boundary layer. Gusts of wind create turbulence near the leaf, which detaches spores from the surface. The spores are then disseminated up and through the air, to be deposited on a new leaf surface.

**NOTE:**
This figure is included on page 21 of the print copy of the thesis held in the University of Adelaide Library.

**Figure 2.8:** Wind dispersal of spores from a wheat plant (Source: Sache, 2000).

At low velocity, wind can remove spores from leaves, and spore removal increases with increased turbulence (Sache, 2000). However, the number of spores caught with trap plants decreases quickly with distance from the source, so wind dispersal may depend on quantity of spores and wind characters (Sache, 2000). Vertical spore dispersal followed
by horizontal wind capturing the spores contributes to rapid contamination of newly emerged leaves. Spores are transported above the canopy level and are blown across the field, infecting more plants if conditions are conducive.

In Spain and America, ascospores of *D. rabiei* are spread over long distances and give rise to new infection foci in fields kilometres from the source (Kaiser & Küsmenoglu, 1997). *D. rabiei* can survive in a free state in the soil surface and also on crop residues (Kaiser, 1997, Pande et al., 2005), where surface wind turbulence can pick up the free spores or crop residues containing spores at the laminar boundary level, transporting them kilometres.

The airborne ascospores were the primary source of inoculum infecting chickpea fields located 10 to 15 kilometres away from the nearest infected field in the Pacific Northwest of America (Trapero-Casas & Kaiser, 1992a). In a 5-year trial in Spain, Tapero-Casas et al (1996) found ascospores to be the major source of inoculum for epidemics due to their ability for long distance spread.

### 2.8.4 Wind and rain

Wind acts on a larger scale than rain in transporting spores but the two factors interact, so their combined influence on spore removal, transport and deposition should be considered. Rain provides the mechanisms to remove spores from plant surfaces and disperse them over short distances while wind combined with turbulence can transport these spores upwards and across a field. Rain also works in the reverse, collecting fungal spores transported in the air and washing them onto plants below, as was found by Rowell and Romig (1966) where high elevation spores of wheat rusts were very quickly brought to the crop surface by falling raindrops.
Fatemi and Fitt (1983) found that small droplets, < 100 µm in diameter, travel further than larger droplets in wind but larger droplets travel further in still air as the force of the larger droplet is greater after initial dispersal. The distance over which spores will travel in droplets is small unless combined with wind. Fitt et al. (1989) conducted experiments with plants and pathogens in a rain tower and wind tunnel, and showed that airborne spores released by rain splash and transported by wind are likely to be important in long distance spread of pathogens. Kaiser (1992) found that wet and windy conditions are critical in the spread of *A. rabiei*. Likewise, Zachos et al (1963) reported that, in the presence of wind driven rain, *A. rabiei* was spread in the direction of the prevailing winds.

### 2.9 Viability of disseminated spores

During dispersal, spores are exposed to a wide range of environmental factors that may affect their viability, including temperature, rainfall, RH and solar radiation. Viability means in general "capacity to live and grow successfully" (Oxford concise dictionary; accessed online 27 September 2010) and is specifically used in this case to understand if germination is possible after long distance dispersal. This is important in Australian conditions, since differences in rainfall patterns, temperature and cultural practices may play an important role in the pathogen’s spread and survival.

It is unknown for how long spores remain viable during dispersal (Fitt *et al.*, 1989). Long distance dispersal is a complex process and viability is influenced by the physical environment. It was suggested by Nagarajan and Singh (1990) that for spores to be able to travel long distances they need to withstand dry weather, low temperatures and have thick walls and pigmentation to protect against ultraviolet light.
Nagarajan and Singh (1990) suggest that the probability of a viable spore reaching a target and causing infection after long distance dispersal is very low, but a real possibility. Long distance dispersal allows gene flow and the pathogen to adapt to regional environmental changes. In 1978, sugar cane rust, caused by *Puccinia melanocephala*, was transported thousands of kilometres by wind from South Africa to Australia. Upper air circulation patterns validate that urediospores may have been transported above 6 - 10 kilometres, where dispersal across the inter-tropical convergence zone is possible but where radiation and subzero temperature are likely to affect viability (Watson & de Sousa, 1983, Nagarajan & Singh, 1990). Even though viability of spores was reduced, a few still managed to cause disease after such a long migration. Duration of dispersal will also play a role in viability as spores are exposed to extremes of temperature, radiation and RH over a longer time. In the case of urediospores, transport and deposition needs to happen within 120 h or very high loss of viability occurs (Nagarajan & Joshi, 1985).

### 2.10 Disease gradients

Understanding the nature and scope of dispersal of pathogen propagules such as spores is fundamental to the understanding of epidemic development. Modelling of both dispersal of plant pathogen propagules and spread of plant disease has been a cornerstone of plant disease epidemiology (Jones, 1998).

When infective spores are deposited on susceptible hosts in favourable environmental conditions, a spore deposition gradient will lead to a disease gradient. A disease gradient is the point in time or space where disease decreases because of a drop in spore numbers brought about by an increase in distance from the original infection (Trigiano et al., 2004). In ideal conditions, disease spreads from areas of severe infection to those of slight infection (Lacey & West, 2006). The epidemic is the result of many different spore
production and release events over many days, each event influenced by wind speed and direction, and rainfall (Jones, 1998). Disease gradients can occur across fields when spores from nearby inoculum arrive mainly at one side of the field. Individual disease foci may arise from distant sources kilometres away and give rise to scattered individual patches of disease previously unseen within a field.

2.11 Modelling

Modelling used in epidemiology must give the investigator the ability to infer what is happening to the pathogen of interest in the system being studied. This gives the investigator the ability to understand the effect of variables such as wind, rain and cultivar on the spread of the pathogen and make sound judgments on possible control or eradication measures.

Often statistical models used to predict epidemics are empirical, meaning that they describe a relationship between factors through statistical calculations (Stapper, 1986). These models allow for correlations to be made between the pathogen spread and the factors affecting it. These models produce outputs but with no real reference to underlying physical and biological variables and have been termed “input/output” and “black box” models (Jones et al., 2010). These models are commonly used in science due to the limited statistical knowledge required to perform them and the many programs available to help with the statistical analysis. Such a modelling technique is often applied and gives valuable information, as reported by Zhang et al. (2004) where linear regression was used to describe the spatial and temporal distribution of Mycosphaerella pinodes in the field. Zhang et al. (2004) showed that the distance of pathogen spread from the foci was influenced by wind directions and cultivar, and also the rate of increase in severity over time was greater in the direction of the wind in susceptible cultivars. This
form of spatio-temporal modelling provides valuable information on the spread over space and time, allowing a correlation between wind direction and cultivars to be shown, however, such a model cannot predict the spread of the pathogen. With the increase in computational power, however, more complex models can be produced to perform such a task (Jones et al., 2010). One such model is the predictive simulation model. The predictive model is based on parameters taken from field and laboratory data which are used to validate and calibrate the model. If no field or laboratory data are available, published and unpublished data can be used to calibrate the model. After calibration, validation and parameter sensitivity testing, a model is produced that can be used in various agroecological zones and which can run with local weather data only. This form of modelling and analysis helps to increase knowledge of disease gradients, disease severity, and distance of disease spread as influenced by pathogen biology, temperature, cultivar and wind and rain, and can predict the epidemic spread over time and space. This makes predictive models an ideal tool to use in predicting a pathogen’s spread so measures can be put in place to reduce its impact within the system.

In Western Australia, a simulation model has been developed to predict the onset of pseudothecium maturity and seasonal ascospore showers for blackleg disease in canola, caused by the fungus Leptosphaeria maculans (Salam et al., 2003). The model uses daily mean temperature and daily total rainfall, the factors that drive the progress of maturity of pseudothecia on the infested canola stubble (Salam et al., 2003). The model has satisfactorily predicted the timing of the onset of pseudothecium maturity. This model has two main parts; prediction of onset of pseudothecium maturity, and consequent ascospore showers, at a number of locations within Australia, over several seasons.
Salam et al. (2006) have also developed a detailed spatio-temporal model to assess the effect of rain, temperature and wind on maturation, spore release and spore dispersal of *Mycosphaerella pinodes*, a pathogen involved in the blackspot complex of field peas. This model predicts the onset, and progression of ascospore maturity and spread of spores from the source of infection. The model maps the risk of blackspot in neighbouring fields, based on previous field pea history (Salam et al., 2006). This helps farmers to select the best fields for peas in the coming seasons and to plan crop rotation for the lowest risk of disease for several years in advance. The model also allows growers to make tactical decisions on when to sow the next year’s crop (Salam et al., 2006). Using up to date weather data and forward projecting with historical weather data for a location, the model predicts the likely “ascospore load” at given times of sowing for field peas in Western Australia. Previously, the management of blackspot has relied on delaying sowing between 2 - 4 weeks past the opening rains to avoid the ascospore showers, and to avoid planting adjacent to infested stubble.

Diggle et al. (2002) produced a simulation model focused on the short distance rain-splashed dispersal of anthracnose of lupins from infected seeds. The model (“Anthracnose tracer”, Figure 2.9) is able to simulate the loss of healthy growing points of lupins, which are the terminal and lateral shoot apices, branches and inflorescences. Initially one growing point is formed from each seed and this growth increases over time. In the model, the growing point development is calculated via temperature, meaning that when certain temperature requirements are met the model will simulate the growing point development. Local weather data are used in the model to allow prediction of growth. The key elements to the model are the simulation of the growing points, the simulation of the spread of the pathogen via rainfall, the deposition of the pathogen on plants in the field and the cultivar resistance rating, which influences the likelihood of a plant becoming
infected. Much of the information used as parameters in this model has come from published and unpublished data and from small scale experiments. The final product was a predictive tool to estimate crop damage due to anthracnose of lupins. It was tested a few years after anthracnose of lupins was first discovered in Western Australia in 1996. Questions were raised if anthracnose would cause more serious crop loss in a higher rainfall environment than in the lower rainfall environments; the model was able to give quantitative information on the different disease levels that might occur in each rainfall zone and it was found that the disease was more likely to damage widely planted Lupin crops in northern Western Australia where higher rainfall zones and warmer temperatures occur. These results assisted farmers by indicating that in high rainfall areas susceptible cultivars should not be planted if seed infection is above 0.01 %.

To date, no predictive simulation models have been developed, to my knowledge, to simulate the spread of ascochyta blight of chickpeas and are needed to help in disease management. Models that have been developed for ascochyta blight of chickpea thus far are empirical. Examples of such models, which have been valuable in understanding the spread and dispersal of ascochyta blight of chickpea, are Kimber et al. (2007) logistic model that describes the development of A. rabiei of chickpea influenced by wind and rain in the field and Shtienberg et al. (2005) model, which describes the influence of temperature and wetness period on pseudothecial maturity of D. rabiei on chickpea debris and thus timing of chemical applications.
Figure 2.9: Schematic diagram of the “AnthracnoseTracer” model showing development and dissemination as characterised by the model. Parallelograms show the external drivers influencing the system and rectangles show the plant infection process (Diggle et al., 2002).

2.12 Current disease management

2.12.1 Infected seed

Seed infection is a major means of introducing ascochyta blight into a new area. The success of seed infection as a source of inoculum is dependent on various characters, including the percentage of seed infected, rate of seed to seedling transmission, the rate of development of an epidemic from seedling foci, and the comparative influence of alternative sources of inoculum (Davidson & Kimber, 2007).
Seed infection rates in the Pacific Northwest of the USA in commercial chickpea seed lots varied from 0.5 to 31% (Kaiser, 1992), while Maden (1975) found up to 70% of seeds tested to be infected with *A. rabiei* in Turkey. Seed to seedling transmission is important in disease development as diseased seeds develop into diseased seedlings causing new foci and subsequent epidemics, where the pathogen is then spread by wind and rain. Seed infection rates as low as 0.25% are enough to cause epidemics when weather conditions are favourable (Kaiser, 1992). The use of disease free seed is an important strategy against ascochyta blight and has been employed to reduce primary inoculum.

### 2.12.2 Cultural practice

Infested residues are an important means of the pathogen survival between chickpea seasons, therefore it is important that residues break down or are removed before sowing the next chickpea crop. Burial of residue in soil has been shown to reduce pathogen survival (Navas-Cortes *et al.*, 1995), by hastening residue and pathogen decomposition. Buried *A. rabiei* inoculum is no longer viable after 2 - 5 months, but stubble borne inoculum has remained viable on the soil surface for 2 years (Chongo & Gossen, 2001). Burial of residue prevents dispersal of conidia by rain splash and ascospores by wind. Burial also reduces ascospore production, as it inhibits the formation and maturation of the teleomorph on the infested residues (Navas-Cortes *et al.*, 1995). A rotation of 3 - 6 years is suitable for a disease break in most regions, but in warm, moist regions, such as New South Wales and Queensland, 1 - 2 years with a non-host crop is considered a long enough rotation due to rapid residue breakdown (Davidson & Kimber, 2007).

Delayed sowing in late winter in the northern hemisphere is a strategy used to reduce severe outbreaks of ascochyta blight of chickpeas. Ascospores are released at certain
times of the year depending on a favourable environmental setting. Conditions are less favourable for disease development in late winter, but late sowing can reduce yield (Singh & Reddy, 1996). In Australia, pea crops are sown 2 - 3 weeks after what would be the optimum time to avoid the peak period of ascospore release of *M. pinodes*, but again yield may be reduced due to short growing seasons (Bretag et al., 2000). Burning stubble can be used to control *A. rabiei*, but this action can cause losses of organic matter and nutrients for the soil (Pande et al., 2005). The increasing amount of plant debris left on the soil surface with minimum tillage is a potential hazard for increasing the severity of epidemics. Proximity to infested stubble is also important since wind-borne *D. rabiei* ascospores may travel long distances or infected debris may blow into bordering fields, hence site selection is an important part of disease control.

Intercropping (the practice of growing two or more crops in close proximity) has also been shown to be effective in reducing the spread and severity of *A. rabiei*. Gaur and Singh (1944) showed the combination of either mustard, barley or wheat sown with chickpea gave minimum disease severity compared to sowing chickpea alone. The combination of barley and chickpea gave the maximum seed yield and maximum reduction in disease severity.

### 2.12.3 Disease resistance

Breeding for ascochyta resistance in chickpea began in India in the 1930s, with the first resistant cultivar developed and released in the 1940s (Luthra et al., 1941). Breeding has been further developed by groups such as the International Centre for Agricultural Research in Dry Areas (ICARDA) in Syria where new cultivars of both desi and kabuli chickpea are produced. After success in breeding for yield and *A. rabiei* resistance, many cultivars have become susceptible to new pathotypes of *A. rabiei*. Resistance breeding is
a major objective in the chickpea industry in many countries, including Australia. Commercial chickpeas cultivars with moderate and high resistance to ascochyta blight have recently been released to the Australian industry. These include lines such as Almaz and Nafice with moderate resistance, and Genesis 508 and Genesis 090 with high resistance (McMurray & Hobson, 2011).

Plant age appears to have an effect on resistance to ascochyta blight of chickpeas. Chongo and Gossen (2001) investigated the association of plant age and growth stages with regards to disease resistance in field studies and controlled conditions. Four cultivars of varying resistance were assessed, kabuli types cvs UC27 (susceptible), Stanford and B90 (moderately resistant), and one Desi type cultivar, Myles (moderately resistant). It was concluded that resistant cultivars (Stanford, B90 and Myles) showed decreasing resistance with the increasing age of the plant, notably at the flowering stage. Disease was also found to be slight on seedlings but severe on podding plants. Susceptible cultivars UC27 however succumbed to disease at all stages of growth and showed no plant age-related resistance.

2.12.4 Chemical control

Fungicides have been approved for the control of ascochyta blight in several countries, but the need for repeated applications on susceptible cultivars makes this uneconomical in areas of low yield (Pande et al., 2005). In Australia, susceptible chickpea cultivars may be grown by using strategic applications of foliar fungicides, such as chlorothalonil and mancozeb, applied several times during the growing season (Shtienberg et al., 2006). The economics of this practice are determined by the severity of the epidemic. Fungicide sprays are applied prophylactically, before disease is evident and ahead of rain fronts to prevent new infections. Seed treatments (e.g. chlorothalonil, benomyl, carbendazim,
thiabendazole, thiram and mixtures) are also an integral part of disease control but these only reduce, not prevent, transfer of the pathogen from seed to seedlings (Kimber & Ramsey, 2001).

2.13 Summary

Factors affecting spread of spores, such as wind and rain, are important in the dispersal of fungal spores and understanding their role is crucial to modelling fungal epidemiology. Ascochyta blight of chickpeas is a devastating disease only recently seen in Australia, with much to understand about its epidemiology. Because of the similarities of this disease to other diseases that threaten Australia’s plant biosecurity, such as anthracnose of lentils, lupins and peas caused by Colletotrichum truncatum and C. lupini, A. rabiei is suitable for use as a local organism to model spore dispersal and epidemic development. Such a model will be the first of its kind for Ascochyta blight of chickpea and it can be used to predict the potential threat of exotic plant pathogens with similar epidemiological behaviour and identify agroecological zones most at risk.
Chapter 3 : General materials and methods

3.1 Fungi

3.1.1 Source of A. rabiei isolates

Single spore derived isolates of A. rabiei, stored in the pathogen collection in Pulse and
Oilseed Pathology (POP), South Australian Research and Development Industry
(SARDI), were made available for this study. These isolates had originated from infected
chickpea plants collected from commercial crops and research trials during disease
surveys across South Australia and Victoria from 1998 to 2009. Isolates were stored as 3
mm diameter plugs, from sporulating single spore cultures, in 1.5 mL Nunc® cryotubes
(Nalge Nunc International, Denmark) containing 1 mL of sterile Reverse Osmosis (RO)
water. The tubes were labelled with isolate number, location and date/year of storage and
stored at 5 °C. The isolate used in the majority of this study was isolate 141/99, collected
from Kingsford, SA in 1999, unless specified otherwise.

Working cultures were routinely maintained on Streptomycin Potato Dextrose Agar
(SPDA) (Appendix A) under black light and fluorescent light (one florescent black light
NEC 20w/T10 and two Philips cool-white 18W/850 daylight tubes, PJ White and Co.,
Adelaide) for 12 h day/night at 22 ºC.

3.2 Preparation of inoculum

To prepare inoculum, isolates were subcultured onto SPDA plates and incubated for 7 -
14 days as described above. The cultures were flooded with 5 mL of sterile RO water and
the surface of the culture gently rubbed with a sterile glass rod to dislodge conidia. The
conidial suspension was poured into a sterile beaker and subsequently filtered through
four layers of sterile muslin to remove mycelial fragments. Conidia were quantified with the aid of a haemocytometer and adjusted to the desired concentration.

3.3 Field studies

3.3.1 Chickpea material

Seeds of chickpea cultivars and breeding lines used in these studies (Table 3.1) were obtained from SARDI from diseased-free plants stored at 12 °C soon after harvest. Chickpea seed were inoculated with rhizobium in moist peat, NODULAID™ 100, group N Chickpea inoculant (Becker Underwood Pty Ltd, New South Wales, Australia) according to the manufacturer’s instructions prior to sowing.

Table 3.1: Cultivar description, geographical location, year of collection and resistance rating of chickpea material used in this study

<table>
<thead>
<tr>
<th>Chickpea cultivar and description</th>
<th>Seed source ¹</th>
<th>Year it was sourced</th>
<th>Resistance rating ²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Howzat - Desi type, medium to tall height, early flowering, medium size, light brown seed</td>
<td>Balaklava, SA</td>
<td>2005</td>
<td>MS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MS</td>
</tr>
<tr>
<td></td>
<td>Turretfield, SA</td>
<td>2007</td>
<td>MS</td>
</tr>
<tr>
<td>Almaz - Kabuli type, medium height, late flowering, large cream seed</td>
<td>Riverton, SA</td>
<td>2005</td>
<td>MR</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MR</td>
</tr>
<tr>
<td></td>
<td>Turretfield, SA</td>
<td>2007</td>
<td>MR</td>
</tr>
<tr>
<td>Genesis 090 - Kabuli type, medium height, mid flowering, medium to small cream seeds</td>
<td>Riverton, SA</td>
<td>2005</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>Turretfield, SA</td>
<td>2007</td>
<td>R</td>
</tr>
</tbody>
</table>

1. Seeds were sourced from chickpea breeding trials and commercial trials in South Australia (SA)
2. MS = moderately susceptible, MR = moderately resistant, R = resistant
3.3.2 Field sites

Experiments on epidemiology were undertaken at Kingsford Research Station, 53 km north-east of Adelaide, South Australia (SA) in 2006 (S 34.54748, E 138.78052) and 2007 (S 34.54521, E 138.78117), and at Turretfield Research Station, approximately 60 km north-east of Adelaide in 2008 (S 34.54760, E 138.82225). The distance between the 2006 and 2007 field trials was approximately 0.26 km and approximately 3.7 km separated these trials from the 2008 field trial. The nearest commercial crops of chickpeas at the Kingsford property had been planted in 1999 to the north of the 2006 field trial site and in 2003 south of the 2006 field trial site at the Turretfield site. The 2008 trial was the first chickpea planting ever undertaken in that field.

A trial in 2006 and 2007 was conducted at Waite Campus, Urrbrae (S 34°57'56.60, E138°38'1.52), South Australia in a bird proof enclosure (70 by 18 m of plantable space), with irrigation used to facilitate initiation of disease (Appendix B).

Meteorological data (listed below) were recorded at hourly intervals by an automated weather observation system (AWOS) (Telvent Australia Pty Ltd, Melbourne, Victoria owned by the Australian Government Bureau of Meteorology) at Roseworthy Research Station (ID: 023122, Latitude: -34.51, Longitude: 138.68, Height: 65.0 m) situated approximately 13 km from the 2008 chickpea trial site and 10 km from the 2006 and 2007 chickpea trial sites. The data were accessed from the Climate & Consultative Services Bureau of Meteorology (BOM), Adelaide, SA and downloaded via the Internet. The data collected from the Roseworthy weather station included the date and time, air temperature, percentage relative humidity, average wind direction, wind speed in m s$^{-1}$ and rainfall in mm.
3.3.3 Field site management

Plots were sown to dimensions 12 x 12 m at Kingsford (2007), however, at Turretfield (2008) were sown to 11 m x 9.5 m. All plots had 1.75 m gaps between them. Plots were sown by the Clare crop improvement group, SARDI. For analytical purpose and uniformity all sites were analysed and will be described in subsequent chapters as 9 x 9 m² plots. Germination (viability) of the chickpea seeds was tested at the POP SARDI laboratory and the appropriate adjustments to the sowing rate was performed to ensure approximately 40 germinated plants per m². Seeding rate was 45 seeds per m² for Howzat, Almaz and Genesis 090. Plots at Waite Campus (Howzat only) were sown with the help of the faba bean breeding team to 15 m x 60 m (totalling 900 m²) and the seeding rate was 45 seeds per m².

3.3.4 Fertiliser and chemical applications

Various treatments were applied prior to sowing. The fertiliser Diammonium phosphate™ (DAP) (Incitec Pivot Ltd, Southbank, Vic) was applied at a rate of 80 kg ha⁻¹. Herbicides were applied as follows: Treflan™ (Trifluralin 480 g L⁻¹, Dow Agrosciences, Frenchs Forest, NSW) at 1.2 L ha⁻¹, Balance™ (Bayer Cropscience, East Hawthorn, Vic) at 100 g ha⁻¹, Flowable Simazine™ (Nufarm Australia Ltd, Laverton North, Vic) at 800 mL ha⁻¹. The insecticide Endosulfan™ (Nufarm Australia Ltd, Laverton North, Vic) was applied at 500 mL ha⁻¹. The fertiliser Monoammonium phosphate™ (MAP) (Incitec Pivot Ltd, Southbank, Vic) at 40 kg ha⁻¹ was applied at seeding. This was applied by the Clare crop improvement group, SARDI at Kingsford and Turretfield and assistance was given by the faba bean breeding team at the Waite Campus.
3.3.5 Stubble and seed collection

Ascochyta blight affected chickpea stubble was collected, after crop maturity, from Kingsford and Turretfield trials (2006 - 2008) to be used as an inoculum source for field and laboratory experiments in the following year. The stubble was placed into large hessian bags labelled with the date of collection, cultivar and year of trial, and was stored in a dry environment at room temperature in the Plant Research Centre at Waite Campus. The spore load per bag of chickpea stubble was not quantified before or after storage.

At the end of the 2007 and 2008 growing seasons, each plot was harvested in E and W sections (Appendix B). The seed was subsequently threshed to remove the seed coat at the University of Adelaide, Plant Research Centre using a Kingaroy thresher. The seed collected from each section (E and W) of each plot was weighed and stored in hessian bags.

3.4 Wind and rain tunnel

3.4.1 Wind tunnel development

A rain tower/ wind tunnel, hereafter designated “the tunnel”, was designed to study dispersal of spores of *A. rabiei* by rain splash and/or wind. The design was based on equipment used by M. Keller (*pers. com.*, The University of Adelaide, December 2006), and Fitt *et al.* (1986) and was constructed by INSKIP Dust and Fume Extractions, Playford, Salisbury North, SA. The wind tunnel was 5.5 m long by 1.5 m tall with a 240 v single-phase fan delivering air at 1.0 to 4.7 m s\(^{-1}\) (Figure 3.1A). The air was recycled and filtered via a filter made of cotton and a filter made of straw situated one at each end of the wind tunnel. Wind speed was measured via a hand held telescopic anemometer (Series 471, Digital Thermo anemometer, Dwyer Instruments, Inc., Michigan City,
Indiana, USA) which projected through a hole in the top of the tunnel and was inserted through a rubber bung. The main duct of the tunnel was made of galvanized metal; the 1.3 m long by 0.5 m wide by 0.5 m tall viewing chamber had a clear, movable PVC door reinforced with stainless steel (Figure 3B). Flexible black polyethylene pipe (19 mm) ran the width (0.5 m) of the wind tunnel at the beginning of the observation chamber. Four green 4 mm variable flow drippers (Pope Drip Watering Solution, Beverly, South Australia) were used to calibrate drops of water and to simulate rain droplets; drippers were set at a constant rate of 25 mL min$^{-1}$ per dripper, calibrated with multiple measuring cylinders. The dripper system was plumbed into the mains water at the University of Adelaide via copper piping and pressure measured via a gauge (P/N W300, LWG pressure gauge, L.W. Gemmell and Associates, Vic). The water flow was turned on and off via a valve situated on the side of the tunnel.

### 3.5 Statistical analysis and modelling software

Experiments were analysed using Genstat edition 10.1 (Service pack) for PC/Windows 2007 (Rothamsted Experimental Station, UK). Spatial modelling was performed using the program Mathematica 5.2 for Microsoft Windows© (1988-2005 Wolfram Research, Inc.).
Figure 3.1: Development of the wind and rain tunnel (“the tunnel”) (A) at Inskip Dust and Fume Extractors, Playford, Salisbury North, S.A. The “tunnel” recycles air via a cotton filter located at the end (right) of the tunnel and the air is filtered and becomes laminar via a straw filter at the other end (left) of the tunnel, the direction of air flow is indicated by the blue lines on the tunnel. The wind speeds (1 - 4.7 m s⁻¹) are produced via a 240 v single phase fan located bottom centre of the tunnel and baffles shown at the end (left) of the tunnel allowing for varying control of the wind speed. An irrigation line is situated at the start of the experimental chamber with variable flow drippers allowing control of water flow. The 1.3 m long by 0.5 m tall by 0.5 m wide experimental chamber (B) allows for plant and pathogen material to be exposed to the various wind speeds. Wind speed is measured via a telescopic anemometer which projected through a hole in the top of the tunnel and was inserted through a rubber bung.
Chapter 4: The effect of temperature and relative humidity on the viability of *A. rabiei* conidia

4.1 Introduction

The influence of wetness and temperature on germination of conidia of *A. rabiei* was studied predominantly in the 1970s and 1990’s (Trapero-Casas & Kaiser, 1992b, Kaiser, 1973). While some aspects of this pathogen have been well studied (Trapero-Casas & Kaiser, 2007, Akem, 1999, Nene & Reddy, 1987, Kaiser, 1992), others have received less attention, especially in regard to viability and how environmental conditions affect the pathogen during dispersal. Kaiser (1973) found that *A. rabiei* survived in naturally infected chickpea tissue for a period of 2 years when exposed to various conditions of temperature, relative humidity (RH), and ultra violet light (UV). The impact of burying infested stubble on survival of pycnidia and ascospores has also been studied in both field and laboratory conditions (Gossen & Miller, 2004, Navas-Cortes *et al.*, 1995), with the conclusion that this practice reduces the viability of the inoculum. However, there is little in the literature describing the viability of conidia over time in different environments. Conidia are associated predominantly with short distance dispersal but it is conceivable that they may have a role in dissemination over longer distances. Understanding the effect of temperature and RH on the viability of conidia of *A. rabiei* will help to clarify the role of conidia as long and short distance dispersal structures which can initiate disease epidemics. The process of transportation and deposition is often costly to conidia as they must survive extremes of temperature, RH and UV and may have only a small chance of landing on an appropriate host (West & Fitt, 2005).
Conidia of *A. rabiei* were studied because in Australia only one mating type has been detected, and ascospores of *D. rabiei* have been detected in Australia only at extremely low numbers in laboratory experiments (Galloway & MacLeod, 2003). The aims of the experiments reported in this chapter were to expose conidia of *A. rabiei* to a range of environmental conditions that they may experience during dissemination and assess the effect of these conditions on conidial viability.

### 4.2 Material and methods

#### 4.2.1 Isolate preparation

An isolate of *A. rabiei* (isolate 141/99, Kingsford, South Australia) was used as inoculum for all the viability experiments. This had been stored as a single conidium-derived isolate in sterile water at 4 ºC since 1999. Conidial suspensions were prepared from 5 - 7 day old cultures on potato dextrose agar (PDA), as described in section 3.2. The suspensions were adjusted with sterile distilled water to a concentration of $5 \times 10^5$ conidia per mL with the aid of a haemocytometer.

#### 4.2.2 The effect of incubation temperature on subsequent germination of conidia at room temperature

To determine the effect of temperature on the viability of conidia of *A. rabiei*, aliquots (10 mL) of conidial suspension were vacuum-filtered onto each of 123 sterile Millipore filter membranes (pore size 0.45 µm, 47-mm diameter, mixed cellulose esters; Millipore, Billerica, MA, USA) as described by Leong *et al.* (2006). Each membrane was placed into a separate 9-cm plastic Petri dish and dried at room temperature in a laminar flow cabinet for approximately 60 - 120 minutes. Following drying, the lids were replaced and the Petri dishes were wrapped in aluminium foil to provide darkness, and then placed in a
set of eight incubators, with 15 Petri dishes per incubator, for up to 24 h. Each incubator was set at 5, 10, 15, 20, 25, 30, 35 or 40 °C and a tgu-4500 tinytag data logger (Hastings data loggers, Port Macquarie, New South Wales) was placed in each incubator to confirm the set temperature before beginning the experiment. Three membranes from each temperature were destructively sampled at each of the following time intervals; 2, 4, 6, 8 and 24 h and processed as described below. The control (0 h) was sampled directly after the conidial suspension was dry.

The experiment was repeated as above except that 93 Petri dishes containing membranes were incubated at 25, 30, 35, 40 or 45 °C and three membranes were destructively sampled at 48 h intervals for a period of 12 days, i.e.; 0 (control), 48, 96, 144, 192, 240 and 288 h.

This experiment was conducted a third time with 130 membranes exposed to temperatures of 20, 25, 30, 35 or 40 °C, and time periods 0 (control), 8, 24, 48, 72 or 96 h. Five membranes from each temperature treatment were destructively sampled at each of the above time periods.

Immediately after removal from the incubator, or following drying of membranes for the controls, each membrane was wetted with 5 mL of sterile RO water, removed from the Petri dish with sterile forceps and placed in a McCartney bottle containing 5 mL of sterile RO water. The 5 mL of RO water remaining in the Petri dish was pipetted aseptically into the appropriate McCartney bottle and the bottle was vortexed for 30 seconds to dislodge spores from the membrane into the 10 mL of RO water. Aliquots of 250 µL of each spore suspension were spread evenly over water agar (RWA) - coated slides (Naseri et al., 2008). The slides were placed into sterile Petri dishes, one per dish, and incubated at
room temperature (approximately 22 °C) under blacklight and fluorescent light for approximately 48 h. Slides were examined using the compound microscope at 400 x magnification, without a coverslip. The percentage spore germination was determined by observing 60 conidia selected at random on each RWA slide. Conidia were considered to have germinated if the germ tube was at least one-half the longitudinal axis of the spore (Trapero-Casas & Kaiser, 2007).

4.2.3 The effect of relative humidity on the viability of conidia of *A. rabiei*

Membrane filters bearing conidia of *A. rabiei* isolate 141/99 were prepared as described in section 4.2.2.

Specific relative humidities were generated by using saturated salt solutions, as shown in Table 4.1 (Winston & Bates, 1960, Dhingra & Sinclair, 1995). Volumes of 100 mL of each of the salt solutions were poured into plastic Bonson Homeal containers (Mick Savill Packaging Pty Ltd, Research Road, Pooraka, SA) and these were closed and allowed to equilibrate over 24 h at 20 °C. Four replicate Petri dishes containing 0.45 µm filter membranes bearing conidia of *A. rabiei* were uncovered and placed in an offset formation (Figure 4.1A) floating on top of the saturated salt solutions. This design allowed the airspace above each membrane to be contiguous with the air above the saturated salt solution. The plates were incubated at 20 °C in darkness.
Figure 4.1: (A) Petri dishes placed in a stacked formation inside a Bonson Homeal container. Each Petri dish contained a 0.45 µm filter bearing conidia of A. rabiei, and was floated on top of the saturated salt solution. (B) Bonson Homeal containers with each salt solution and membranes in the 20 °C incubator.

Four membranes from each relative humidity treatment (Table 4.1) were sampled at 8, 24, 48, 72 and 94 h intervals, and the conidia were harvested from the membranes and germination assessed as described in section 4.2.2. A total of 120 membranes were assessed.
Table 4.1: Saturated salt solutions and the resulting relative humidity generated in closed containers at 20 ºC.

<table>
<thead>
<tr>
<th>Desired relative humidity (%) at 20 ºC</th>
<th>Saturated salt solution</th>
<th>Amount of salt added to 100 mL of water to achieve saturated solution at 20 ºC</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 (control)</td>
<td>Water (H₂O)</td>
<td>NA</td>
</tr>
<tr>
<td>90 b</td>
<td>Magnesium sulphate</td>
<td>25 g</td>
</tr>
<tr>
<td></td>
<td>(MgSO₄)</td>
<td></td>
</tr>
<tr>
<td>80.5 a</td>
<td>Ammonium sulphate</td>
<td>74 g</td>
</tr>
<tr>
<td></td>
<td>(( NH₄)₂SO₄)</td>
<td></td>
</tr>
<tr>
<td>50 b</td>
<td>Glucose (C₆H₁₂O₆)</td>
<td>91 g</td>
</tr>
<tr>
<td>44 b</td>
<td>Potassium carbonate</td>
<td>156 g</td>
</tr>
<tr>
<td></td>
<td>(K₂CO₃)</td>
<td></td>
</tr>
<tr>
<td>12.5 b</td>
<td>Lithium chloride</td>
<td>55 g</td>
</tr>
<tr>
<td></td>
<td>(LiCl)</td>
<td></td>
</tr>
</tbody>
</table>

a Calculation based on Winston and Bates (1960)
b Calculation based on Dhingra and Sinclair (1995)

4.2.4 Statistical analysis

Statistical software, Genstat 10th edition (Rothamsted Experimental Station, UK), was used to analyse the influence of temperature and relative humidity on spore germination. Due to time restrictions each individual experiment was only conducted once. Hence the data were analysed using Friedman’s test, a non-parametric equivalent of a two-way ANOVA (Dytham, 2003). A ranking table was compiled to compare the differences between the treatments. All individual experiments were designed as randomised blocks with three to five replicates of Petri dishes assigned to a given temperature or RH.
Assumptions were then made by comparing the germination percentage and the mean rank, with lower mean rankings indicating that fewer conidia germinated.

4.3 Results

4.3.1 The effect of incubation temperature on germination of conidia of A. rabiei

The effect of incubation temperature in dry conditions over a period of 24 h on subsequent germination of conidia of *A. rabiei* is shown in Table 4.2. For the first 6 h, at 5 to 40 ºC, temperature had no apparent effect on germination, which remained at 100 % (rank 21.33). Germination was only reduced after 8 h of exposure to 40 ºC, decreasing to 45 % (rank 8.5), and after 24 h at 40 ºC another significant reduction in germination occurred, down to 3 % (rank 1).

The effect of incubation at 25, 30, 35, 40 and 45 ºC in dry conditions on conidial viability assessed at 0, 48, 96, 144, 192, 240 and 288 h is shown in Table 4.3. Friedman’s non-parametric ANOVA shows that viability was significantly affected by both temperature and time (P = 0.000). No germination 0 % (rank 11.67) occurred when conidia were incubated at 45 ºC or 40 ºC for any time period tested, compared to 100 % (rank 31) germination in the control. Similarly, conidia incubated at 35 ºC and 30 ºC had low germination, less than 5 % after 96 h of dry incubation and 0 % (rank 11.67) after 192 h. At 25 ºC viability was not greatly affected until after 240 h of dry incubation when germination decreased to 0 %.

The effect on germination of conidia after dry incubation at 20, 25, 30, 35 and 40 ºC for an 8 h time period and then subsequent 24 h periods up to 96 h is shown in Table 4.4. The Friedman’s non-parametric ANOVA showed that germination was significantly affected by temperature over time (P = 0.000).
Incubation at 40 ºC reduced germination to 32 % (rank 5) in the shortest time assessed, being 8 h. Germination of conidia incubated at 30 and 35 ºC was recorded as 75 % when assessed at the 24 h, after which viability of conidia incubated at 35 ºC decreased significantly (P < 0.05) over time compared to 30 ºC. Germination of conidia incubated at 20 and 25 ºC was similar, between 96 % (rank 22.1) and 90 % (rank 15.7), over the 96 h period and germination was similar to the control that yielded 100 % (rank 25.8) when spores were spread on agar at 0 h.
Table 4.2: Viability (% germination) of *A. rabiei* conidia exposed to a range of temperatures (5, 10, 15, 20, 25, 30, 35 and 40 °C) over time (0, 2, 4, 6, 8 and 24 h) on 0.45 μm Millipore membranes. Viability was assessed as the percentage of conidia that germinated on RWA at 22 °C. Mean ranks calculated in Friedman’s two-way test are presented in parenthesis. The control (0 h) is presented as a footnote with its mean ranking. The Chi squared approximations were significant (*P* = 0.000) for temperature and time.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>35</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>8</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>45</td>
</tr>
<tr>
<td>24</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>3</td>
</tr>
</tbody>
</table>

Control assessed at 0 h with 100 % germination and mean rank (21.33)
Table 4.3: Viability (% germination) of conidia of *A. rabiei* exposed to a range of temperatures (25, 30, 35, 40 and 45 °C) over time (0, 48, 96, 144, 192, 240 and 288 h) on 0.45 μm Millipore membranes. Viability was assessed as the percentage of conidia that germinated on RWA at 25 °C. Mean ranks calculated in Friedman’s test are presented in parenthesis. The control (0 h) is presented as a footnote with its mean ranking. The Chi squared approximations were significant (P = 0.000) for temperature and time.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Temperature °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25</td>
</tr>
<tr>
<td>48</td>
<td>41 (28.67)</td>
</tr>
<tr>
<td>96</td>
<td>23 (26.67)</td>
</tr>
<tr>
<td>144</td>
<td>41 (29.33)</td>
</tr>
<tr>
<td>192</td>
<td>28 (28)</td>
</tr>
<tr>
<td>240</td>
<td>0 (11.67)</td>
</tr>
<tr>
<td>288</td>
<td>0 (11.67)</td>
</tr>
</tbody>
</table>

Control was assessed at zero hours with a 100 % germination and mean rank (31)
Table 4.4: The viability of *A. rabiei* conidia exposed to a range of temperatures (25, 30, 35 and 40 °C) over time (0, 8, 24, 48, 72 and 96 h) on 0.45 µm Millipore membranes. Viability was assessed as the percentage of conidia that germinated on WA at 22 °C. Mean ranks calculated in Friedman’s test are presented in parenthesis. The control (0 h) is presented as a footnote with its mean ranking. The Chi squared approximations were significant (P = 0.000) for temperature and time.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Temperature °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20</td>
</tr>
<tr>
<td>8</td>
<td>91 (15.9)</td>
</tr>
<tr>
<td>24</td>
<td>91 (18.2)</td>
</tr>
<tr>
<td>48</td>
<td>95 (21)</td>
</tr>
<tr>
<td>72</td>
<td>90 (15.7)</td>
</tr>
<tr>
<td>96</td>
<td>94 (19.4)</td>
</tr>
</tbody>
</table>

Control was assessed at zero hours with a 100 % germination and mean rank (25.8)

4.3.2 The effect of relative humidity on the viability of conidia of *A. rabiei*

The effect of relative humidity of 100 (control), 90, 80.5, 55, 44 and 12.5 % at 20 °C over a period of 8 to 96 h on subsequent germination of conidia is shown in Table 4.5. The Friedman’s non-parametric ANOVA showed that germination was significantly affected by relative humidity over time (P = 0.000). Germination of conidia incubated in 80.5 to
100 % RH was generally similar for all the periods tested, with a small reduction in germination after 72 and 96 h.

**Table 4.5:** The viability of conidia of *A. rabiei* exposed to relative humidity of 100, 90, 80.5, 55, 44 and 12.5 % at 20 °C over 8 to 96 hours on 0.45 µm Millipore membranes. Viability was assessed as the percentage of conidia that germinated on RWA at 22 °C. Mean ranks calculated in Friedman’s test are presented in parenthesis. The Chi squared approximations were significant (P 0.000) for relative humidity and time.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>100 (control)</th>
<th>90</th>
<th>80.5</th>
<th>55</th>
<th>44</th>
<th>12.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>95 (22.12)</td>
<td>95 (22.5)</td>
<td>96 (23.5)</td>
<td>95 (22.12)</td>
<td>94 (19.88)</td>
<td>95 (21.12)</td>
</tr>
<tr>
<td>24</td>
<td>97 (26.25)</td>
<td>95 (21.75)</td>
<td>90 (16.38)</td>
<td>94 (19.88)</td>
<td>87 (11.88)</td>
<td>83 (8.25)</td>
</tr>
<tr>
<td>48</td>
<td>98 (26.88)</td>
<td>93 (19.12)</td>
<td>91 (17.88)</td>
<td>95 (22.62)</td>
<td>84 (11.12)</td>
<td>79 (7.25)</td>
</tr>
<tr>
<td>72</td>
<td>93 (18.62)</td>
<td>93 (19.38)</td>
<td>83 (8.38)</td>
<td>89 (16)</td>
<td>72 (5)</td>
<td>58 (1.88)</td>
</tr>
<tr>
<td>96</td>
<td>90 (16)</td>
<td>81 (8.88)</td>
<td>90 (15.75)</td>
<td>75 (6.5)</td>
<td>78 (6.38)</td>
<td>61 (1.75)</td>
</tr>
</tbody>
</table>

For conidia incubated at 55 % RH, germination after 96 h was reduced to 75 %, which was significantly less than for shorter time periods. After 24 h or longer at the two lowest RH (44 % and 12.5 %), germination was significantly reduced below that of the control. Furthermore, incubation in 12.5 % RH for 72 and 96 h significantly reduced germination of conidia compared with 24 and 48 h.
4.4 Discussion

The effect of temperature on the survival of conidia of *A. rabiei* can be broadly summarised by the observation that as temperature increased above 25 °C survival decreased, and as relative humidity decreased below 55% the survival decreased. The longer the exposure to these extremes the less likely the conidia were to germinate. Although the three temperature experiments cannot be compared directly by statistical analysis, there was strong agreement between them with respect to the influence of incubation temperature on viability of conidia.

In experiments one and two, incubation at 40 ºC negatively influenced viability after 8 h and prevented germination for all the time periods longer than 8 h. Viability of conidia incubated for 48 h or longer at 35 ºC was reduced in both experiments two and three. Variability was observed between experiments two and three at temperature 35 ºC, with 1 % and 55 % of conidia germinating respectively. This variability was probably because these experiments were conducted separately and the controls were independent, meaning different inoculum plugs from the same stored isolates were used in each experiment. Until the present study, little or no data was available regarding the effect of temperature and relative humidity on the survival and subsequent germination of conidia of *A. rabiei*. However, studies of germination of conidia of *A. rabiei* and of survival of conidia of fungi similar to *A. rabiei*, such as such as *M. pinodes, B. cinerea* (Rotem & Aust, 1991) and *A. carbonarius* (Rotem & Aust, 1991, Leong et al., 2006), may provide insight into viability of *A. rabiei* and the role of conidial transportation and deposition in epidemics.

In 2007 Trapero-Casas and Kaiser reported that conidia of *A. rabiei* freshly harvested from 5 - 7 day old cultures on chickpea seed meal-dextrose agar (CDA) germinated on
RWA over a wide range of temperature, with the optimal for germination being 20 ºC after 6 h. Likewise, 20 ºC was the optimum for mycelial growth in earlier experiments by Kaiser (1973), Kovacevski (1936) and Nene and Reddy (1987) and for similar species, such as *M. pinodes* (Roger *et al.*, 1999). In the current study a similar response was observed, such that conidia exposed to 20 ºC on a dry membrane for up to 96 h subsequently had a germination rate above 90 %. In contrast to our findings where conidia survived temperatures up to 35 ºC for 8 h on a dry, inert medium, no conidia germinated on RWA at 35 ºC in the study by Trapero-Casas and Kaiser (2007). Satar (1933) reported that conidia of *A. rabiei* smeared onto chickpea seed and incubated for 5 months at 25 to 30 ºC (RH not specified) germinated at a rate of 50 %, whereas 5 % of those incubated at 35 ºC germinated. This led Satar (1933) to state that although periods of 35 ºC occur infrequently in India there was still a chance that dried conidia contaminating chickpea seeds would remain viable in these conditions. Although less than 5 % of conidia incubated at 35 ºC for 6 days germinated in the present study, both studies indicate that *A. rabiei* conidia can remain viable on inert media over an extended periods at 35 ºC. Australian isolates of the pathogen may be showing an adaptation to local conditions similar to that seen in India as there are occasional days of temperatures above 30 ºC during Australian springs when conidia are produced. In survival studies by Kaiser (1973), naturally infected chickpea stem and pod tissue harboured the fungus for over 2 years at temperatures between 10 and 35 ºC, showing that the fungus could survive in plant material for a long period at a broad range of temperatures. Kaiser (1973), however, did not study spore viability (conidia or ascospores) but re-isolated the fungus from the plant material onto PDA. Information about the ability of conidia to germinate after exposure to extremes of temperature, such as might occur in regions where chickpeas are grown, will help to understand if conidia can survive long distance
dispersal over a period of time and potentially have a role in initiating epidemics. The percentage survival influences the inoculum density, which is an important factor in determining disease severity (Trapero-Casas & Kaiser, 1992b) since the greater the number of germinating spores the greater the likelihood that the pathogen will infect after it lands on a host.

In the present study, conidia were able to germinate following incubation in a range of RH produced in the atmosphere via salt solutions (100 - 12.5 %) over a period of 96 h at 20 °C. In comparison, Trapero-Casas and Kaiser (2007) investigated the effect of water potential on germination of freshly harvested conidia using the agar isopiestic equilibration technique of Harris et al. (1970) as modified by Alderman and Beute (1986) to produce RH equivalent to 100, 98.5, 97.1, 95.7 and 94.3 % at 0 - 35 °C. Trapero-Casas and Kaiser (2007) reported that conidia did not germinate on RWA at water potential of -8 MPa (equivalent to 94.3 % RH) after 18 h, regardless of temperature, while only a few conidia germinated (0 % to 8 %) at water potential of -6 MPa (95.7 % RH) at 20 to 25 °C; the response of the two isolates tested differed slightly, particularly at the lower water potentials. The difference in the results of these studies is likely to be due to the approaches used to generate RH in the experimental system. Saturated salt solutions were used in our study to alter the RH in the atmosphere surrounding the conidia during incubation on membranes whereas Trapero-Casas and Kaiser (2007) altered the water (or osmotic) potential of agar immediately above and below the conidia. Furthermore, the present study focused on the effect of RH on survival such that conidia first were placed on an inert medium (membranes) then subsequently germinated on nutrient medium. Trapero-Casas and Kaiser (2007) studied the effects of RH on germination of conidia and ascospores where spores of D. rabiei were placed directly on agar, the RH of which was adjusted to set values. In studies discussed above, Kaiser (1973) also reported that the
pathogen survived on naturally infected chickpea stems at RH 0 - 30 % for over 2 years, where atmospheric RH was maintained using salt solutions in desiccators. He did not investigate the survival of conidia but showed that mycelium appeared to survive in plant tissue in conditions of low RH.

In this current study the impact of RH on conidial viability was studied at 20 °C, the optimal germination temperature. However, the effect of RH at a range of temperatures needs to be investigated. Unfortunately there was insufficient time in the current study to perform such an experiment. The combined effect of RH and temperature should be studied using a number of isolates and also ascospores to elucidate the influence of environmental conditions on spores as agents of dispersal. Trapero-Casas and Kaiser (2007) suggested that ascospores of *D. rabiei* appeared to be resistant to adverse environmental conditions. Citing unpublished data, they claimed that up to 15% of ascospores discharged in sterile Petri dishes and exposed to 20 - 23 °C and 15 - 30 % RH for a month germinated when RWA was poured into the plates.

The germination rate of conidia of *A. rabiei* isolate 141/99 was more than 50 % even after 4 days of exposure of dry conidia to 30 - 35 °C and also more than 50 % following incubation in RH as low as 12.5 % at 20 °C. Perhaps the ability of this South Australian isolate to tolerate low RH and high temperature would confer an advantage in South Australian conditions where warm temperatures combined with low RH are common (BOM, 2010). Rain events in Australia may be infrequent in the winter and spring months (June to October) when chickpea crops are grown. An adaptation to tolerate such conditions would be advantageous and may help to explain how epidemics can occur in climatic conditions in which rain events may be sporadic. This suggestion is supported by the finding of Trapero-Casas and Kaiser (2007) that the ability of spores (conidia and
ascospores) to tolerate intermittent dry periods during germination and infection may explain why the disease is so devastating in countries with temperate to semi-arid climates. Trapero-Casas and Kaiser (2007, 1992b) showed that periods of wet then dry conditions increased the severity of ascochyta blight compared with severity on plants that were continually wetted.

As discussed above, conidia of A. rabiei appeared to tolerate a relatively wide range of temperature and RH. It is feasible that conidia could be exposed to several days of extreme conditions before landing on a host and still retain the ability to germinate, infect and cause disease. Investigation of the infectivity of A. rabiei conidia after incubation is needed to test this assumption.

Individual isolates of A. rabiei differ considerably in terms of pathogenicity and characteristics such as growth rate, sporulation and appearance (Kaiser, 1973, Kaiser & Küsmenoglu, 1997, Zachos et al., 1963, Kovacevski, 1936, Trapero-Casas & Kaiser, 2007). The current experiments were conducted with a single isolate of A. rabiei from the South Australian chickpea growing region. Additional studies using a number of representative isolates from Australia and abroad are needed to understand how different isolates may survive in extremes of temperature and relative humidity. This would identify if there are differences among isolates corresponding to environmental conditions in their region of origin or if there is a natural variability in the A. rabiei population that enables some conidia to survive regardless of the conditions to which the fungus was previously exposed.

The ability of conidia of A. rabiei to tolerate a wide range of environmental conditions may be important in initiation of epidemics of ascochyta blight on chickpeas. Dispersal of
conidia typically occurs during rain events via raindrop splash (Fitt et al., 1989, Madden, 1997) but this study showed that they may subsequently survive for several days of variable conditions and, possibly, be able to infect a new host. While conidia are not recognised as being involved in long distance dispersal of *A. rabiei*, their ability to survive in adverse conditions raises the possibility that long distance dispersal is possible. Heavy rain events which cause liquid suspension in air (aerosols) have been known to incorporate spores and, in a heavy rain, event there is a possibility that the aerosols could trap *A. rabiei* conidia, transporting them long distances. Also, it is possible that if viable conidia are caught up in strong wind currents they could conceivably be transported long distances and potentially land on host plants, possibly causing infection and new disease foci.
Chapter 5 : Effect of wind and rain on the dispersal of A. rabiei conidia in a purpose-built wind and rain tunnel

5.1 Introduction

The natural elements of wind and rain are important in the dispersal of many plant pathogens. Rain splash is one of the most critical dispersal elements influencing short distance radial dispersal of plant diseases, as well as germination and the survival of spores (Fitt et al., 1986). When a raindrop strikes a surface the kinetic energy of rainfall is responsible for detachment and transport of matter such as soil particles, nutrients and spores (Hudson, 1993). When the surface is infected plant material bearing inoculum, the resulting droplets transfer the inoculum to adjacent healthy plants, thus spreading disease (Fitt et al., 1989). The amount of kinetic energy received by the impacted surface during a storm event depends largely on the rainfall intensity, the raindrop size distribution, and the storm duration, and to some extent on the wind speed (De Lima et al., 1992, Pedersen & Hasholt, 1995). Consequently, the dispersal of spores is linked to the intrinsic characteristics of rainfall and wind.

Wind dispersal is equally important but its function generally lies in transporting pathogens over long distances, to thousands of kilometres. This has been associated with pathogens such as Mycosphaerella musicola and M. fijiensis, which cause yellow and black sigatoka leaf spot of bananas, respectively (Burt, 1994, Stover, 1962) and with many species of rust fungi that have been dispersed across Australia (Watson & de Sousa, 1983) and across the Tasman Sea to New Zealand (Close et al., 1978). Long distance travel imposes obstacles such as desiccation, (UV) light and extremes of temperature, but
if the pathogen successfully overcomes these obstacles new foci can emerge in regions where resistance is non-existent (Agrios, 1997, Fitt et al., 1989). Mechanisms of release of fungal propagules have evolved to take advantage of wind and rain dispersal (Ingold, 1971), facilitating survival and subsequent epidemics. Two main types of spore dispersal, passive and active, are influenced by wind and rain. Passive dispersal occurs mainly when wind or rain picks up spores from raised surfaces and distributes them, meaning that the fungus has no mechanism for ejecting the spores (Lacey, 1996, Lucas & Dickinson, 1998). Puff and tap dispersal initiated by rain splash are examples of passively influenced spore dispersal, where either the puff of air formed prior to a raindrop landing or the vibration caused on the plant surface by the impacting raindrop results in the transportation of the spores (Fitt et al., 1989, Lacey, 1996, Lucas & Dickinson, 1998). Active dispersal is a mechanism that has evolved to exploit wind or rain action when environmental conditions are ideal for spore survival. Most ascomycetes (one of the most important group of plant pathogens) shoot their ascospores into the air actively, usually after wetting of the ascocarp by rain or dew (Lacey, 1996). Hygroscopic twisting is a form of dry dispersal by sporangia brought about by changes in RH that result in violent twisting of the sporangiophore and subsequent spore dispersal (Lacey, 1996).

Ascochyta rabiei is primarily transported passively by rain splash after pycnidia on plants ooze conidia during periods of cool, wet weather (Pandey et al., 1987). On wetting, often via rain in natural environments, the mucilage surrounding the spores dissolves leaving a suspension of spores in a thin film of water on the host surface (Fitt et al., 1989). Subsequent rain events via splash droplets transport the pathogen to bordering plants as described in section 2.8.2. Although A. rabiei conidia are primarily spread via splash to adjacent plants, windblown rain and driving winds can aid in longer distance dispersal. Studies by Zachos (1963), Kaiser (1992) and Kimber et al. (2007) have shown that wind
influences the spread of ascochyta blight in the direction of the prevailing wind but this knowledge extends only to how the disease spreads and not exactly how the pathogen, in particular the conidia, are dispersed. Trapero-Casas and Kaiser (2007) stated that many aspects of the biology of A. rabiei have been studied but information is lacking on the precise effects of environmental factors, such as wind and rain, on dispersal of conidia of A. rabiei. Therefore, this study was undertaken to quantify how rain and wind affect the dispersal of conidia of A. rabiei in controlled laboratory conditions using a purpose-built wind and rain tunnel (3.4.1) and in simulated field conditions. Investigated in this study was A. rabiei, the anamorphic stage of the pathogen, which is presumed to be the main cause of chickpea ascochyta blight in Australia. The aim of this study was to identify spore dispersal parameters for A. rabiei for use in calibrating the ascochyta blight simulation model (chapter 7).

5.2 Materials and methods

5.2.1 Tunnel procedure

Stubble of the susceptible chickpea cv. Howzat infested with A. rabiei was collected from Kingsford immediately after the field trial in 2006 - 07, as described in section 3.3.5, and stored for use as a source of conidial inoculum for experiments with the tunnel. After 2 - 3 months of storage, 5 g (dry weight) of stubble with prominent lesions were immersed in RO water for 5 minutes to initiate conidial ooze then placed in the tunnel (see section 3.4.1). Wind speeds were set at 1.4, 2.2, 3.3 and 4.7 m s⁻¹ and applied for 30 minutes at a time. When the experimental design required water, droplets were applied from 4 mm variable flow drippers inserted along the irrigation line inside the tunnel as described in section 3.4.1.
A total of 15 metal rods, 40 cm tall x 2 mm thick, cut from coat-hangers were placed in a grid pattern in the tunnel, i.e. at three points 10 cm apart, across the tunnel, at each of the following distances (2, 22, 44, 66 and 110 cm) from the stubble. Pieces of double sided sticky tape (3M™ Clear High Tack/Medium Tack Double Coated Removable Repositionable Tape, London, Ontario, Canada) 5 cm long x 2 mm wide were applied vertically to the rods at three positions above the base (1 - 6, 11 - 16 and 31 - 35 cm). A 1 cm gap was left at the base of each metal rod, allowing the rods to be fixed into Bostik Blu-Tack™ and held in an upright position (Figure 5.1). Wind speed in the tunnel before the start of each experimental run was measured, as described in section 3.4.1.
**Figure 5.1:** Operation of the tunnel, showing A) Metal rods in batches of five with double sided sticky tape at heights of 1 - 6, 11 - 16 and 31 - 36 cm; a 1 cm gap was left at the base to enable the rods to be placed upright in Blu-Tack™ in the tunnel. The tape was sliced between the rods with a scalpel blade and the rods separated. B) The tunnel with 5 g of wetted stubble (long arrow) placed at the beginning of the observation chamber, rods were placed at 2, 22, 44, 66 and 110 cm distances from the stubble, three rods across the tunnel, 10 cm apart, at each interval. Rods were held in place by Blu-Tack™.

### 5.2.2 Dispersal in wind and rain tunnel

#### 5.2.2.1 Dispersal by wind

An experiment was undertaken to determine the effect of wind on dispersal of conidia. Stubble prepared as described in section 5.2.1 was placed flat at the beginning of the tunnel observation chamber and replaced with freshly prepared stubble at each wind speed tested (see Figure 5.1B). Wind at either 1.4, 2.2, 3.3 or 4.7 m s⁻¹ was applied for 30 minutes to detach conidia from the stubble.
After 30 minutes of wind exposure, the rods were removed from the tunnel and the tapes were cut horizontally with a scalpel to produce 2 x 2.5 cm long pieces. Each tape was peeled off the rod with fine tweezers and transferred to a glass slide, with the conidia uppermost, and labelled to show the distance from the inoculum, height and replicate (r 1 - 3). Four drops of lactoglycerol without stain were applied to the sticky tape on the slide and a 22 x 50 mm cover slip applied. Air bubbles on the tape were tapped out with the blunt end of a dissecting needle. Slides were left for 24 hours to allow the lactoglycerol to dissolve the tape adhesive, allowing clear microscopic evaluation. All the conidia per tape were counted using a compound microscope at 400x magnification. The experiment was conducted three times at each wind speed.

5.2.2.2 Dispersal by wind and rain

Methods were as described in section 5.2.2.1, with the addition of rods at 44 cm from the source and water droplets to simulate rainfall. Water from four 4 mm variable flow drippers inside the tunnel was applied at a constant rate of 25 mL min\(^{-1}\) per nozzle (see section 3.4.1). A sample of 5 g of *A. rabiei*-infested stubble with oozing conidia was placed in the target zone on top of a metal grid and exposed to the wind (1.4 - 4.7 m s\(^{-1}\)) and falling water for a period of 30 minutes (Figure 5.1B).

The resulting splash was blown some distance along the tunnel and the spatial distribution of conidia was determined by catching and counting the conidia on traps as described in 5.2.2.1. The experiment was conducted five times for each wind and rain treatment.

5.2.2.3 Calculated kinetic energy from simulated rainfall in the tunnel

Kinetic energy of raindrops can be difficult to evaluate under natural conditions so it is common to use rainfall simulators (in the field or in the laboratory), which attempt to
control some of the variables encountered in nature (Ogden et al., 1997). Even with simulated rain, however, direct measurement of the kinetic energy is difficult because raindrop size varies enormously and isolation of individual drops is virtually impossible (Hudson, 1993), hence this often needs to be done indirectly (Fornis et al., 2005).

For the present study, a combination of direct and indirect approaches was used. In the first instance, a laboratory rainfall simulator was constructed as described in section 3.4.1, at the University of Adelaide, for which the delivery rate of kinetic energy was calculated in units of J m$^{-2}$ mm$^{-1}$ (see below). The kinetic energy was used to calculate an equivalent, natural rainfall intensity (mm h$^{-1}$) from a model based on similar climatic conditions (see below). The rainfall simulator consisted of a linear set of four evenly-spaced drippers (each adjusted to deliver 25 cm$^3$ of water per minute placed in the tunnel, 40 cm above a target zone (4 cm x 30 cm). The delivery rate of water (intensity, mm h$^{-1}$) was calculated to be 500 mm h$^{-1}$ based on the volume-rate of water delivery (100 cm$^3$ min$^{-1}$) and the target area on which it fell (120 cm$^2$). This was an excessively high rainfall intensity compared with natural rain, but since the droplets fell from only 40 cm they did not reach terminal velocity. The kinetic energy of all the water that fell was therefore calculated as $E_k = E_p = m g h$ (where $m =$ mass of water hitting the target area = 6 kg h$^{-1}$, determined from the delivery rate of water; $g =$ the gravitation acceleration constant, 9.8 m s$^{-2}$; and $h =$ the distance through which the water fell vertically, 0.4 m), assuming all potential energy in the simulated rainwater was released as kinetic energy upon impact with the target area. Hence the kinetic energy delivered to the stubble was found to be:

$$E_k = \frac{6 \text{ kg} \times 0.5 \text{ h} \times 9.8 \text{ m} \times 0.4 \text{ m}}{0.012 \text{ m}^2 \times 250 \text{ mm}} \approx 4 \text{ J m}^2 \text{ mm}^{-1}.$$
This kinetic energy was converted to a typical rainfall intensity based upon Kinnell’s (1987) relation for eastern Australia:

\[ E_k = E_{\text{max}} \left[ 1 - \alpha \exp(-\beta \text{Intensity}) \right] \quad \text{J m}^{-2} \text{mm}^{-1}, \]

where the intensity is expressed in mm h\(^{-1}\), \(E_{\text{max}}\) is the average maximum kinetic energy of typical rainstorms in eastern Australia (ca 28 J m\(^{-2}\) mm\(^{-1}\)), and the adjustable constants, \(\alpha\) and \(\beta\), have values of \(\alpha = 1\) (dimensionless) and \(\beta = 0.1\) (h mm\(^{-1}\)), respectively (after Kinnell 1987). Re-arranging and solving for the Intensity, we get:

\[ \text{Intensity} = \frac{1 - \left( \frac{E_k}{E_{\text{max}}} \right) \beta^{-1}}{-\ln \left( \frac{1 - \left( \frac{E_k}{E_{\text{max}}} \right) \beta^{-1}}{\alpha} \right)} \quad \text{mm h}^{-1} \]

Using the above values for \(\alpha\), \(\beta\), and \(E_{\text{max}}\) and the measured \(E_k = 4\) J m\(^2\) mm\(^{-1}\), an equivalent rainstorm intensity applicable to this experiment was found to be just under 2 mm h\(^{-1}\). This low intensity is not atypical of winter rain events in South Australia (Beecham & Chowdhury, 2008).

5.2.3 Dispersal in the field by wind and rain

An experiment was conducted in conditions of natural wind and rainfall at the Bureau of Meteorology Observation Centre (BOM) Kent Town, located 2 km north-east of the central business district of Adelaide. Three quadrats made from galvanized wire (900 x 13 x 0.55 x 10 m, Mitre 10, Glenunga, SA) cut to 1 m\(^2\) were placed at three positions noted as (a, b and c) within an 80 m\(^2\) area (Figure 5.2A) and were secured into the soil with a tent peg in each corner. True north was identified with a compass and marked with a
white pot tag placed in the soil next to each quadrat. Preceding a forecasted rainfall event, *A. rabiei* infested stubble (5 g) prepared as described in section 5.2.1 was placed in the centre of each quadrat. The stubble was held down via a metal coat hanger wire (2 mm x 10 cm) bent into a horse-shoe shape. Metal rods and tapes, as described in section 5.2.1, were placed in a grid pattern around the stubble (Figure 5.2B). One rod was set at the centre of stubble (0 cm) as the control and the rest were placed at 10, 30 and 50 cm in the directions N, NE, E, SE, S, SW, W, NW from the centre of the inoculum. Segments of sticky tape, as described in section 5.2.1, were placed at two heights (2 - 7 and 12 - 17 cm) to catch rain splashed and wind-dispersed conidia. The height 30 - 35 cm was not assessed in the field experiments due to the results obtained in the laboratory studies where very few conidia were caught at this height. The heights, 2 - 7 and 12 - 17 cm, were chosen since conidia were caught at these heights most frequently in the tunnel experiments.

A 2 cm gap was left at the base of the rods to allow penetration into the soil, enabling the rods to stay upright in strong wind events. A denture cup lid (Advanced Australian Packaging, Regency Park, SA) was skewered on top of each rod, through the centre, to reduce the risk of conidia being washed off the sticky tape by rain. The quadrat label (a, b or c), distance (10, 30 or 50 cm) and the direction of the rod (N, NE, E, SE, S, SW, W, NW) was written on the lid to keep track of where rods had been stationed after they were removed from the quadrat at the end of the experiment. Blu-tack™ was placed on both sides of the skewered denture cup lid to prevent it sliding down the wire onto the adhesive tape.

Rods were placed on the quadrat just prior to a rain event and the experiment was run during 30 minutes of consistent rainfall. This was achieved due to accurate interpretation
of rainfall events using the online BOM radar and the close proximity of the weather station site to the University of Adelaide, Waite Campus. This experiment was repeated over three days depending on the rain forecast. Weather data were collected from the onsite BOM Kent Town weather station, as described in 3.3.2, at 1 minute intervals.

At the end of an experimental run (30 minutes) rods were collected and the base inserted into a block of polystyrene to a depth of 2 cm to keep the rods upright and separate. These rods and blocks were then placed into a large plastic container with a lid and transported back to the Waite Campus, approximately 10 km away, for processing. The close proximity of the site allowed for quick sampling and processing.
Figure 5.2: Field site at the Bureau of Meteorology Kent Town: A) A metal quadrat (1 m²) in position with true North determined via a compass and marked with a white pot tag. B) Placement of metal rods with tape at various compass point directions (N, NE, E, SE, S, SW, W, NW) and distances (10, 30 and 50 cm) with denture cup lids at the top of the metal rods to reduce the risk of conidia being washed off the tape during a rainfall event. C) All three quadrats (a, b and c) placed within the 80 m² Kent Town weather observation site in close proximity to the automated weather observation system, that recorded weather data at 1 minute intervals. D) Google map showing the field site with surrounding trees, houses and fence-line. Also indicated (yellow) are the directions of the predominant prevailing winds and true North.
5.2.4 Statistical analysis of data

Statistical software Genstat 10th edition (Rothamsted Experimental Station, UK) was used to analyse the influence of wind, rain, distance and height on conidial dispersal. The data for all experiments were naturally log transformed to assist in analysis when working with small numbers of conidia. ANOVA was performed on the tunnel experiments and a regression analysis was performed on the data from the field experiment.

5.3 Results

5.3.1 Dispersal by wind

The effect of wind speeds of 1.4, 2.2, 3.3 and 4.7 m s\(^{-1}\) on the dispersal of A. rabiei conidia from infested stubble is shown in Figure 5.3. The ANOVA showed a significant three-way interaction between distance, tape height and wind speed (P <0.001) and all the individual factors were also significant (P <0.001). In general, most conidia were detected on tapes closest to the source of inoculum, and on the lowest parts of the rods. Virtually no conidia were detected on the highest tapes at 31 - 36 cm. The two slowest wind speeds dispersed conidia only 2 cm from the inoculum. As wind speed increased, conidia were detected at greater distances, and on tapes at the mid-range (11 - 16 cm) height. The largest numbers of conidia were found on tapes at 2 cm from the source, at a height of 1 - 6 cm after the higher wind speeds of 3.3 and 4.7 m s\(^{-1}\) (333 and 140 conidia, respectively) and at 11 - 16 cm height after wind speed of 3.3 m s\(^{-1}\) (135 conidia). At wind speed of 3.3 m s\(^{-1}\), the numbers of conidia were always significantly greater than at the two lower wind speeds, irrespective of distance or height. This wind speed (3.3 m s\(^{-1}\)) also resulted in a greater number of conidia trapped than wind speed 4.7 m s\(^{-1}\) at five of the six height \(x\) distance combinations; three of these differences were significant. The only situation in
which wind speed of 4.7 m s\(^{-1}\) resulted in a greater number of conidia trapped than at 3.3 m s\(^{-1}\) was at 66 cm x 11 - 16 cm height.

**Figure 5.3:** The effect of wind speed (1.4, 2.2, 3.3 and 4.7 m s\(^{-1}\)) on dispersal of *A. rabiei* conidia in a purpose-built wind and rain tunnel. Conidia were trapped at various heights (1 - 6, 11 - 16 and 31 - 36 cm) and distances (2, 22 and 66 cm) along the tunnel. Data represent the log of the mean number of conidia observed on the sticky tape for three replicates per position. Significance is determined by L.S.D (0.05) = 1.3.

Data for trap height 31-36 cm are not presented as few conidia were trapped and many counts were 0.
5.3.2 Dispersal by wind and rain

The effect of wind speed (1.4, 2.2, 3.3 and 4.7 m s\(^{-1}\)) combined with irrigation at 25 mL min\(^{-1}\) on the dispersal of *A. rabiei* conidia is shown in Figure 5.4. The ANOVA showed a significant three-way interaction between distance, wind speed and tape height (P <0.001) and all individual factors were again significant (P <0.001). In general, the highest concentration of conidia was detected on tapes closest to the source of inoculum and at the lower tape heights. The number of conidia declined with increasing tape height and distance from the source. As the wind speed increased, conidia were detected at greater distances and higher tape positions (11 - 16 and 31 - 36 cm). The distance of 22 cm from the stubble was the furthest that conidia were caught on the highest tapes (31 - 36 cm) and these were only found at the higher wind speeds of 3.3 and 4.7 m s\(^{-1}\), with 2 and 3 conidia observed, respectively. The maximum numbers of conidia were trapped at a distance of 2 cm and a height of 1 - 6 cm from the source, with a total of 1486 conidia trapped at wind speed 1.4 m s\(^{-1}\). The number of spores trapped sharply decreased with tape height, with 36 and 1 conidia observed, respectively at the next two tape heights (11 - 16 and 31 - 36 cm). At the highest wind speeds, 4.7 and 3.3 m s\(^{-1}\), conidia were transported the furthest distance, 66 cm, with 5 and 1 conidia caught at a height of 1 - 6 cm, respectively, and a single conidium caught at 11 - 16 cm, respectively. At wind speed 2.2 m s\(^{-1}\), conidia were transported to 44 cm, with 9 conidia observed at 1 - 6 cm and at 11 - 16 cm a single conidium conidia was observed. The lowest wind speed of 1.4 m s\(^{-1}\) dispersed conidia up to 22 cm, with 71 conidia caught at 1 - 6 cm but no conidia were observed at the other tape heights.
**Figure 5.4:** The effect of wind speed (1.4, 2.2, 3.3 and 4.7 m s\(^{-1}\)) with the addition of water droplets from variable flow drippers set at 25 mL min\(^{-1}\) on dispersal of *A. rabiei* conidia in a purpose-built wind and rain tunnel. Conidia were trapped at various heights (1 - 6, 11 - 16 and 31 - 36 cm) and distances (2, 22, 44 and 66 cm) along the tunnel. Data represent the log of the mean number of conidia observed on the sticky tape for five replicates per position. Significance is determined by L.S.D (0.05) = 1.1.

### 5.3.3 Dispersal in the field environment

The effect of wind direction on the dispersal of conidia was non-significant (P = 0.053). The presence of a high number of zero counts restricted the data set and did not allow for conventional analysis. Data for the 30 cm and 50 cm distances were removed so that only
the data for 0 and 10 - 15 cm were analysed. Conidia did travel a total distance of 30 cm but no spores were found on tape at a distance of 50 cm from the source. The mean wind direction was WNW in event one and close to N for events two and three, so it was expected that SE and S directions would have had the largest conidia counts. This, however, was not the case as means for S and SE did not dominate the directional list and means were fairly similar across all the directions sampled (Table 5.1). Event one showed a downward dispersal effect but this was cancelled out by event two and limited counts in event three. Height of tapes was a factor with three times more conidia caught at 2 - 7 cm (0.487) than 12 - 17 cm (0.161). Pilot studies were performed to catch conidia at 31 - 36 cm in height but no conidia were observed on any of the tapes. Rainfall (mm h\(^{-1}\)) intensity was calculated to be 2.8 mm h\(^{-1}\) for event one, 2 mm h\(^{-1}\) for event two and 0.8 mm h\(^{-1}\) for event three. There was a strong relationship between the number of spores trapped with increased rainfall, with a total of 2000 conidia caught at event one, 1,000 at event two and only 100 at event three.

**Table 5.1:** Passive trap directions in the field with mean recorded conidia counts for each direction and the centre (control). Directions S and SE (shown in bold) indicate prevailing wind directions where larger spores counts were expected.

<table>
<thead>
<tr>
<th>Direction</th>
<th>Mean (ln x+1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centre</td>
<td>4.753</td>
</tr>
<tr>
<td>E</td>
<td>0.743</td>
</tr>
<tr>
<td>N</td>
<td>0.537</td>
</tr>
<tr>
<td>NE</td>
<td>0.709</td>
</tr>
<tr>
<td>NW</td>
<td>0.475</td>
</tr>
<tr>
<td>S</td>
<td>0.394</td>
</tr>
<tr>
<td>SE</td>
<td><strong>0.840</strong></td>
</tr>
<tr>
<td>SW</td>
<td>0.423</td>
</tr>
<tr>
<td>W</td>
<td>0.863</td>
</tr>
</tbody>
</table>
5.4 Discussion

It is often assumed that splash-dispersed conidia travel relatively short distances but rarely is the actual distance and number of conidia of a species quantified in real terms. Understanding the influence of wind and rain on conidial splash dispersal is, perhaps, a key element in plant pathogen dispersal. It is important to have such precise knowledge when trying to understand the “behaviour” of a pathogen, especially in identifying how it may spread and cause epidemics, thus giving the ability to predict its spread through modelling techniques. In this study, conducted using controlled conditions, conidia of *A. rabiei* were dispersed at least 66 cm in wind alone and also with wind in combination with rain splash. There were slight differences in the distance over which conidia were distributed following different treatments of wind and wind plus rain but, in general, the distance was influenced by wind speed, with higher wind speeds dispersing spores further and the addition of raindrops dispersing spores to higher (vertical) tape positions.

In controlled experiments involving both wind and wind plus rain in the present study, inoculum density decreased as distance increased from the foci, such as seen with the example of a plume of smoke given by Lacey and West (2006), where spores are dispersed closest to the source. Chen *et al.* (2003) showed similar trends for conidia of *A. brassicicola* on cabbage. In controlled experiments, Chen, *et al.* (2003) found that conidia could be dispersed up to 25 cm in the presence of wind-driven rain at 1 m s$^{-1}$ and up to 50 cm at 3 m s$^{-1}$. Few conidia were found at greater than 75 cm with higher wind speeds (7.5 m s$^{-1}$), and as distance from the foci increased, spore numbers greatly decreased. This trend was also observed by Pedersen *et al.* (1994) where conidia of *A. fabae* were exposed to higher wind speeds (2.5 to 5 m s$^{-1}$) in the presence of rain were distributed further in droplets, 17 cm at 2.5 m s$^{-1}$ and 22 - 26 cm in 5 m s$^{-1}$, but the number of conidia
dispersed to 15 cm was reduced by half in still air. Simulated rain splash with wind for 30 minutes in the tunnel in this study also highly influenced the quantity of inoculum. In the presence of rain, five times more conidia were dispersed than with no rain. It seems likely that consistent rain could have produced sufficient and continuous energy to dislodge more conidia than would have been dislodged by wind alone. Chen et al. (2003) found that as rainfall was applied at greater intensity, more conidia were caught in funnels traps, suggesting that increased rainfall energy dislodged more conidia. In the wind experiment where only initial wetting was applied to stubble, only some conidia would have oozed out of the ostiole to be available for dispersal. Further dispersal of conidia may have ceased due to the lack of continual rain energy needed to disperse all of the conidia exposed on the surface of the stubble. The wind-alone experiments did however show some dry dispersal of conidia with only initial wetting of the pycnidia, which has not been observed for this stage of the pathogen before. In current literature it has been identified that only ascospores are distributed long distances up to hundreds of metres to kilometres from the source and are able to cause new infection (Trapero-Casas & Kaiser, 1992a, Trapero-Casas et al., 1996, Kaiser, 1997, Gamliel-Atinsky et al., 2005,). These experiments show that conidia through, dry dispersal, could be involved in long distance travel. If dry dispersal was aided by turbulent winds it is possible conidia could be lifted into the turbulent boundary layer where they could be distributed long distances (McCartney & West, 2007) and cause new infection, previously only noted with ascospores.

The finding that conidia travelled 66 cm in a gentle wind adds to our knowledge of epidemiology and pathogen spread. Khan et al. (1999a) showed that chickpea seed in south-eastern Australia had harboured inoculum of A. rabiei since 1992 and in 1997 - 98 the build up of seed-borne inoculum contributed to the major epidemic seen in chickpeas
planted at the time. With the knowledge that *A. rabiei* undergoes rapid polycyclic spread within crops through splash dispersal of asexual conidia (Davidson & Kimber, 2007), and that conidia can travel over half a metre in the wind speeds tested and potentially further in stronger wind speeds in presence or absence of continual rainfall, it becomes clear how prolific and devastating this pathogen would have been, at a time when inoculum was plentiful, susceptible plants were sown and prolonged rainfall occurred.

Simulated rain splash in the wind plus rain experiments also influenced the height to which conidia travelled, with conidia trapped at the intermediate height of 11 - 16 cm, few at 31 - 36 cm, but none were trapped at 31 - 36 cm with wind alone. This suggested that in the presence of rain, conidia may be dispersed at heights equal to or above canopy height depending on plant age. In this study, most conidia were caught vertically between 0 and 16 cm, as was found by Chen *et al.* (2003), Shaw (1987) and Brennan *et al.* (1984), who reported that vertically dispersed conidia were mostly caught at 0 - 10 cm, with fewer caught at higher positions.

In terms of rainfall intensity, more intense rainfall in the field experiments resulted in greater conidial dispersal. The numbers of conidia trapped on rods placed around the stubble, especially those trapped in events 1 (2100 conidia and rain of 2.8 mm h$^{-1}$) and 2 (just over 1000 and rain of 2 mm h$^{-1}$), are comparable with those observed in the tunnel when exposed to simulated rain of 2.2 mm h$^{-1}$.

Wind alone transported conidia a total distance of only 2 cm at the lowest wind speed of 1.4 m s$^{-1}$ compared with 22 cm with rainfall at the same wind speed. This could indicate that conidia are splash dispersed up to ~ 20 cm, as 1.4 m s$^{-1}$ may have little effect on spore dispersal due to its reduced energy, 1.4 m s$^{-1}$ being classed as light air (BOM,
This short distance dispersal is similar to that seen in other experiments involving rain-splashed conidia in still air. Schoeny et al. (2008) showed that conidia of *M. pinodes* (ascochyta blight on peas) travelled often between 1.6 and 6.5 cm but did reach up to 64 cm in simulated rainfall events of 2 mm, compared to the distance of 22 cm reported here for *A. rabiei*. Chen et al. (2003) showed that conidia dispersed by wind-blown splash droplets distributed 25 cm in 1 m s\(^{-1}\) wind-speed, which the authors classed as still air, a distance similar to that found here for *A. rabiei*. Pedersen et al. (1994) showed that dispersal gradients were very steep in still air, with rainfall-dispersed conidia travelling 12 - 15 cm. This was mirrored by the work of Travadon et al. (2007), where *Leptosphaeria maculans* conidia were dispersed 2 - 4 cm in still air with rainfall. Conidial dispersal in rain in the absence of wind needs further investigation. The tunnel used at the Waite Campus permitted rods to be placed in one direction only and could not be used to detect radial dispersal. Further experiments using a rain simulator situated directly above the stubble with the ability to trap conidia at a radius of up to 1 m would provide more information on radial dispersal without wind. In studies of splash dispersal of *M. pinodes* reported by Schoeny et al. (2008), a purpose-designed Microprocessor Controlled Spray System was used to simulate rain events in still air. In this study rainfall was generated by an oscillating nozzle positioned at a height of 3.8 m. Rainfall was produced at a constant speed with a sweep angle of 180°, providing a rainfall intensity of 40 mm h\(^{-1}\) which was worked out to be 2 mm when calculating the kinetic energy from the 3.8 m fall height. Such a spray system may be worth investigating for future research in radial dispersal of *A. rabiei* conidia.

The experiments in the tunnel in this study were limited to wind speeds from the 1 m s\(^{-1}\), light air, likened to wind directions shown by smoke, to 4.7 m s\(^{-1}\), gentle breeze, likened to leaves in constant motion, according to the Australian Bureau of Meteorology Beaufort
wind scale (BOM, 2007). It would have been beneficial to test stronger wind speeds to see if they resulted in further spore dispersal, and a modified irrigation line for splash dispersal but this was beyond the scope and budget of the project.

Dufault and Isard (2010) recently described a portable rainfall simulator for evaluating wet deposition of plant pathogens. The simulator can be transported to the field and easily assembled to stand 3 m above the canopy, the nozzles provide uniform spray over a rectangle or square 2 m² area and the pressure of the water produces drops that are within 2% of their terminal velocity. The pathogen of interest can be injected directly into the water flow from the simulators if needed. These types of devices would be ideal for future studies of the splash dispersal of *A. rabiei* and other pathogens in simulated field conditions and should be sought if studies are continued.

Before running the wind tunnel experiments the inoculum’s load per g of stubble was not quantified. It may be necessary to quantify the number of conidia per pycnidium in future experiments to get an estimate of the initial inoculum load. Perhaps lesion of various sizes on stubble of cultivars with different resistances should be examined to establish the number of pycnidia and conidia they bear. This information would allow an estimation of the number of conidia available for liberation at the beginning of an experimental run. In future experiments instead of using 5 g of stubble it may be more useful to use a defined number of lesions of a similar size which would, presumably, have a similar number of pycnidia and conidia. The regression analysis for the field data did not provide statistical support for the assumption that conidia travel in the direction of the prevailing wind. However, the analysis was compromised by the small data set. This field experiment was designed to investigate radial dispersion of conidia and to compare results with the tunnel experiments in the laboratory. Although vacuum traps and those with spinning arms, such
as the roto-rod trap, which are often used in air sampling experiments, would sample larger volumes of air, stationary traps may be a better indication of the probability in nature of a conidium landing on a passive host.

The presence of spores of other fungi, pollen, insects and dust on the tape exposed in the field made microscopic evaluation difficult. These particles occupied precious trapping space and may have reduced the number of conidia trapped, confounding observations, in contrast to the wind and rain tunnel in which only the spores of interest were caught on the tape. Lacey and West (2006) note that the passive sampling method is less efficient for small particles according to Stokes law, as large particles settle more quickly and thus more frequently. Furthermore, the perimeter fence, trees and buildings around this field site are likely to have influenced wind in this experiment. Therefore, we cannot deduce from our findings that dispersal in the direction of the prevailing wind is a non-significant factor in dispersal of conidia of *A. rabiei* in the field. Rather, this experiment could be repeated in an open field, even if the weather data collected might be less accurate than those available at the BOM site.

Further developments in the field of aerobiology and polymerase chain reaction (PCR) techniques would facilitate identification and analysis of wind and rain splashed diseases (West *et al.*, 2008). PCR techniques for early surveillance of wind and rain borne pathogens are being developed at SARDI and the University of Adelaide (B Vogelzang, SARDI, *pers. com.* November 2010). Such techniques will be useful in quantifying spores trapped on tape in field and laboratory conditions, and will allow more tapes to be processed in shorter times to provide larger data sets and, ultimately, a better understanding of the factors affecting spore dispersal.
Chapter 6 : Epidemiology of ascochyta blight of chickpeas in
South Australian field conditions

6.1 Introduction

Ascochyta blight of chickpeas is a devastating disease that has caused significant loss in production in many major chickpea growing countries, including Australia (Nene et al., 1996). The disease was first observed in Australia in 1995, as described in section 1.3, although retrospective seed testing showed it to have been present at low levels across the chickpea growing regions of South Australia and Victoria since 1992. The disease had been confused with Phoma blight. It is thought that the recurrence of ascochyta blight originated from a re-introduction of the pathogen into Australia (Khan et al., 1999a). It was speculated that heavy rains in 1997 were responsible for the following severe epidemic in 1998 as described with wind and rain causing further proliferation in the coming seasons. Production of chickpea in south-eastern Australia was severely affected and has still not recovered to this day.

Conidia are the only spore type to have been reported in South Australia and Victoria (Khan et al., 1999a, Galloway & MacLeod, 2003). This stage is promoted by cool, cloudy, humid weather conditions, 15 - 25 °C and greater than 150 mm of rainfall per growing season (Nene, 1982). Conidia of A. rabiei are primarily distributed by rain splash to nearby plants but can, presumably, be spread over longer distances in the presence of strong winds and wind driven rain. Little information exists on the effect of wind speed and rain on dispersal of conidia from a focus, which is defined as a site of local concentration of diseased plants or disease lesions (Jeger, 1983) and subsequent
proliferation of the pathogen in the field. Much attention has been paid to the sexual stage due to its long distance spread by wind (section 1.8.3). In Spain, Israel, Turkey and the United States, the sexual stage is responsible for new foci and primary infection hundreds of metres to kilometres away from the original infected crops (Kaiser, 1997, Gamliel-Atinsky et al., 2005, Trapero-Casas & Kaiser, 1992a, Trapero-Casas et al., 1996). It has been observed in many studies that the environmental conditions of temperature, rainfall and wind influence disease development and spread (Nene et al. 1987; Trapero-Casas et al. 1992b). Kimber et al. (2007) studied the dispersal of A. rabiei from disease foci using seed as the primary inoculum source in small plots (5 m by 1.5 m) of highly susceptible cultivars. They showed that disease spread across small distances (< 10 m) from these foci in the direction of the prevailing winds. The current study, which builds on the research of Kimber et al. (2007), was conducted to investigate the epidemiology of ascochyta blight of chickpea in three cultivars of varying susceptibility to ascochyta blight, in South Australian field conditions, particularly disease severity on individual plants over time and space from a central focus outwards. The design and sampling method used in this study was intended to provide epidemiological data that could be incorporated into the modelling component of this thesis (Chapter 7).

6.2 Materials and methods

6.2.1 Field sites

The epidemiology of ascochyta blight of chickpea was studied using the cvs Howzat (MS), Almaz (MR) and Genesis 090 (R), as described in section 3.3.1. Trials were sown in three consecutive years (2006 - 2008). The field sites were located at research stations at Kingsford (2006 - 2007) and Turretfield (2008), and at Waite Campus (2006 - 2007) as described in section 3.3.2. The trials in 2006, intended as pilot studies, were affected by
drought and there was little disease. Furthermore, the method of scoring plant infection along transects in 2006 at Kingsford (shown in Appendix B) differed from that used in 2007 and 2008, where 1 m² quadrats were used. Thus analysis of the 2006 data is not presented in this chapter. The Waite Campus field site was abandoned in 2007 because of the inability to control weeds and because the mesh enclosure above and around the chickpeas and other plant species, designed to exclude birds, interfered with the dispersal of conidia by wind and wind-blown water (irrigation) (Appendix B). Weather data for Kingsford and Turretfield were collected from the BOM automatic weather station (AWS) as per section 3.3.2.

On 12 June 2007, three plots were sown at Kingsford Research Station, one each of cvs Howzat (MS), Almaz (MR) and Genesis 090 (R). Dimensions, seeding rate, fertiliser, rhizobium inoculant and chemical applications were as described in section 3.3.2. The resistant cv. Genesis 090 (R) was sown as the middle plot to prevent or minimise inter-plot spread of *A. rabiei* by wind and rain. The central seedling in each plot was inoculated on 25 July 2007 with infested stubble as described in section 6.2.2.

On 5 June 2008, the field trial (Figure 6.1) was sown at Turretfield research station, 5 km north of the Kingsford site, and the central plants were inoculated with infested stubble on 20 June 2008 (as described in section 6.2.2).
**Figure 6.1:** Aerial photograph of Turretfield trial in mid-September 2008, showing cv. Almaz (MR) (top plot), cv. Genesis 090 (R) (middle plot) and cv. Howzat (MS) (lower plot). Each plot was sown to approximately 9 x 9 m with 1.75 m gap between plots. Large patches of disease are visible in the centre to SE corner of the Almaz plot and the SE to NE corner of the Howzat plot. Cereal surrounds the plots (Photograph by John Heap, SARDI).

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**NOTE:**
This figure is included on page 84 of the print copy of the thesis held in the University of Adelaide Library.

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### 6.2.2 Inoculation procedure used in 2007 and 2008

*A. rabiei* was introduced to each plot as follows. Four pieces of infested stubble with approximately five prominent lesions per piece were selected from the previous field trial, as described in section 3.3.3. The stubble pieces were placed into the soil in a vertical fashion, at the centre of each plot, approximately 2 cm south (upwind) of a newly emerged chickpea seedling (Figure 6.2). This was performed preceding a rain event, to initiate release of conidia from the pycnidia and infection of seedlings.
Plots were observed, at weekly intervals after the date of inoculation, for disease expression. Disease was assessed visually each week starting in August, when disease was first observed, until the end of the growing season in November 2007 and 2008.

**Figure 6.2:** Infested stubble pieces, each with approximately five prominent lesions, placed vertically in the soil, 2 cm south of an emerged seedling, in the centre of the plot, just prior to a rainfall event. Three white pot labels beside the seedlings, marked the location of the inoculum.

### 6.2.3 Disease assessment

In 2007 and 2008, at the newly emerged seedling stage, each plot was subdivided into 1 m² observation quadrats, marked out at each corner with white pot labels (Appendix B). Each plot was assigned a number, such that quadrat 1.1 was the focus and quadrat numbers increased in a clockwise direction away from the focus to the south of the plot, as shown in Figure 6.3. Each plot was assessed in a structured way, beginning at the perimeter (outermost, least diseased middle quadrat, designated 6.1) and moving in a clockwise direction to the centre of the plot (quadrat 1.1) which contained the disease.
focus. Boots were sprayed with 70% ethanol before moving from one quadrat to the next. This sampling method was designed to reduce the potential for mechanical spread of the pathogen. A total of 40 plants in each quadrat was assessed for disease severity (proportion of tissue affected per plant) using a percentage scale developed by Gowen et al. (1989) (Table 6.1).

**Figure 6.3:** Quadrat numbering in each cultivar plot in 2007 and 2008 field trials. Quadrats, 1 m², were assigned numbers as follows: 1.1 for the centre quadrat, 2.1 to 2.8, in a clockwise direction for the next quadrats; 3.1 to 3.16 in a clockwise direction for the next quadrats; 4.1 to 4.24 in a clockwise direction for the next quadrats; 5.1 to 5.32 in a clockwise direction for the next quadrats in a clockwise direction for the (outermost) quadrats. Blue arrows (dashed) from 5.1 to 1.1 and back to 5.1 indicate entry and exit points used when assessing disease. Blue arrows show the pattern in which disease was assessed, proceeding in a clockwise direction.
Table 6.1: The disease severity (proportion of tissue affected per plant) was estimated using the rating scale of Gowen et al. (1989) for each plant in each 1 m² quadrat.

<table>
<thead>
<tr>
<th>% infection</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>No infection - small lesions</td>
</tr>
<tr>
<td>11-20</td>
<td>Some stem lesions - minor stem breakage in upper foliage</td>
</tr>
<tr>
<td>21-30</td>
<td>1-2 branches broken - several girdling stem lesions low down on some branches</td>
</tr>
<tr>
<td>31-40</td>
<td>Large basal stem lesions or several branches broken near to main stem</td>
</tr>
<tr>
<td>41-50</td>
<td>Half foliage dead or partly severed</td>
</tr>
<tr>
<td>51-60</td>
<td>&gt; Half foliage dead or dying, young shoots still actively growing from base</td>
</tr>
<tr>
<td>61-70</td>
<td>Most foliage dead - some healthy stem tissue with lateral buds</td>
</tr>
<tr>
<td>71-80</td>
<td>Most foliage dead, no healthy lateral buds in leaf axils</td>
</tr>
<tr>
<td>81-99</td>
<td>Most foliage dead, decreasing areas of living stem tissue</td>
</tr>
<tr>
<td>100</td>
<td>Plants completely dead</td>
</tr>
</tbody>
</table>

6.2.4 Selection of quadrat data for spatial analysis of plant disease severity 2007 and 2008

Spatial analysis was performed on disease severity data averaged for 40 plants per plot from selected quadrats and three dates were selected, near the beginning of disease
expression and towards the middle and end of the season in both years for each trial, viz. 11 September 2007, 5 October 2007 and 2 November 2007, and 5 September 2008, 3 October 2008 and 2 November 2008. Data for Genesis 090 (R) were not analysed spatially as there was no disease in the plots in either year. Spatial analysis was performed in four directions, N, E, S and W, with data for disease severity analysed over a distance of 0 - 4 m; the disease foci (0 m) to close to the outer edge of the plot (4 m) for each selected date. Zero distance was quadrat 1.1 for all directions and then proceeded to 1 m, 2 m, 3 m and 4 m as follows: North direction; quadrats 2.5, 3.9, 4.13 and 5.17. East direction; quadrats 2.7, 3.13, 4.19 and 5.25. South direction; quadrats 2.1, 3.1, 4.1 and 5.1. West direction; quadrats 2.3, 3.5, 4.7 and 5.9 (Figure 6.4).

**Figure 6.4:** Quadrats selected for spatial analysis of data for cvs Howzat and Almaz, 2007 and 2008. Disease severity was analysed spatially over 0 - 4 m in the directions N, E, S and W from quadrat 1.1 (disease focus quadrat). Zero distance was at quadrat 1.1 for each direction and then proceeded to 1 m, 2 m, 3 m and 4 m as follows. N direction (green) consisted of quadrat 2.5, 3.9, 4.13 and 5.17. E direction (yellow) consisted of quadrat 2.7, 3.13, 4.19 and 5.25. S direction (grey) consisted of quadrat 2.1, 3.1, 4.1 and 5.1 and W direction (pink) consisted of quadrat 2.3, 3.5, 4.7 and 5.9.
6.2.5 Selection of quadrat data for temporal analysis of plant disease severity in 2007 and 2008

The disease severity data for cvs Howzat and Almaz were analysed temporally, to show the rate of change in disease severity over time, in 2007, starting on 15 August 2007, when disease was first observed, until 2 November 2007, at the end of chickpea growing season and again in 2008, from 3 August 2008, when disease was first observed, until 2 November 2008. For the temporal analysis, up to 11 quadrats were selected across the plot depending on the presence of disease. Disease was observed across the whole plot in cv. Howzat in both years, so a representative sample of quadrats was selected for analysis (Figure 6.5).

Figure 6.5: Selection of quadrats for temporal analysis in plots of cv. Howzat in 2007 and 2008. Disease was seen across the whole plot and quadrats highlighted in green were selected to compare disease severity in different directions and distances across the plot.

Figures 6.6 (A and B) show quadrats selected for Almaz in 2007 and 2008, respectively. Fewer quadrats were selected in the Almaz plots than in Howzat due to the lack of disease spread across the whole plot logistic regression analyses were performed to describe the
rate of change in disease severity over time in the selected quadrats. No temporal analysis was performed on Genesis 090 (R) as no disease was observed in this cultivar in either year. Quadrats used in the logistic regression analysis (Figures 6.5 and 6.6) were chosen depending on the presence of disease on plants, as quadrats with no diseased plants could not be included in statistical analysis.

**Figure 6.6:** Selection of quadrats for temporal analysis in plots of cv. Almaz in 2007 (A) and 2008 (B). Disease was limited across the plot thus quadrats highlighted in green were selected to compare disease severity in the directions and distances shown across the plot.
6.2.6 Statistical analysis and Java programming

Statistical software, Genstat 10th edition (Rothamsted Experimental Station, UK), was used to analyse temporal and spatial severity of ascochyta blight. A logistic regression, \( y = \frac{1}{1 + \frac{1}{y_0} \exp(-ct)} \) was used to describe both the effect of time and space on individual plant infection per 1 m\(^2\) quadrat (Madden et al., 2007, Zhang et al., 2004), with the percent plant infection data transformed using the logit function to fit a normal binomial distribution. The use of 1 m\(^2\) quadrats, instead of transects, was adapted from Diggle et al. (2002). Using 1 m\(^2\) quadrats allowed for analysis using spatio-temporal modelling (see chapter 7), where the model separates plots into 1 m\(^2\) units. The approach of Diggle et al. (2002) gives a better understanding of how disease is expressed through the whole plant population in each plot rather than from a representative sample of plants selected along a transect. The estimated values of the slopes and the constants from the fitted logistic regression models were statistically compared using two tailed t-tests at \( P < 0.05 \) (Freund, 1984) and the method was adapted from Zhang et al. (2004). The slope (c) values presented in the spatial analysis indicates the disease severity over directions N, S, E and W at distance (0 - 4 m) and in the temporal analysis (c) indicates the rate of change in disease severity over time. In the spatial analysis a smaller slope (absolute value) of the logistic curve indicates more severe disease in a given direction and in the temporal analysis a smaller slope (absolute value) indicates a slower rate of change in severity. In both logistic regressions, the constant, y0, is often called the intercept, as it is the value of y (\% severity) when x (time/distance) is zero (Genstat 10th edition). The slopes and constants generated by the logistic regression analysis were back transformed to natural numbers using the inverse logit function in Genstat 10th edition (A. Glaser, pers. com. February, 2011).
Java version 6 for Microsoft Windows (1995 - 2010, Oracle) was used to create a program to plot the Windrose graphs for Roseworthy AWS 2007 and 2008. The data from the Roseworthy AWS, which included hourly rainfall, wind speed and wind direction, were uploaded into the Java program. The values for hourly wind data which coincided with rainfall events greater than 0 mm were converted from polar coordinates (degrees, m s\(^{-1}\)) to cartesian (x, y) coordinates. The x, y coordinates were then plotted on the graphs as red dots, with a line attaching the cartesian coordinate (0,0) to represent the wind vector. If wind was not associated with rain it was not plotted.

6.3 Results

6.3.1 Spatial analysis of ascochyta blight on cvs Howzat and Almaz in 2007 and 2008

The slopes and constants of the logistic regressions, and the back transformed data, for disease severity in each direction (N, S, E and W) from the disease focus (quadrat 1.1, 0 m) to a distance of 4 m in the cvs Howzat and Almaz assessed on each of the three dates in 2007 are shown in Table 6.2 a and b. The logistic curves are presented in Appendix C (Figures 1 to 6). Based on 199 observations, a t-statistic of greater than 1.96 indicated significant differences (P < 0.05) between constants and slopes of these disease severity curves. In cv. Howzat, significant differences were observed on 11 September 2007 for the regression slopes comparing E - N, S - N and S - E, while the constants were not significantly different. The logslopes, which describe the disease gradients, indicated that significantly less disease occurred in plants to the N and W than in the E and S direction. The W direction had a high standard error. In this complete data set, a high standard error was generally associated with limited disease spread, in that disease had not spread over the 4 m but was most severe at the focus. The data for cv. Almaz on the 11 September
2007 showed no significant differences in slopes and constants since very little disease occurred at this stage.
Table 6.2 (a): Spatial analysis by logistic regression of disease severity over 4 m from the disease foci in one plot each of chickpea cvs Howzat and Almaz in four directions, viz. North, East, South and West on 11 September 2007, 5 October 2007 and 2 November 2007. Significant differences in logslopes for directions North, East, South and West were determined using paired t-tests and student t-distribution Table (P < 0.05) from 199 observations. Comparisons where differences were significant are shown in bold and shaded in the main body of the table and standard error (SE ±) of the residuals are shown for the log data.

(a): The logistic slope was back transformed into natural values using the logit function in Genstat and presented in the slope column. The logslope describes disease severity over distance, with a smaller logslope (absolute value) indicating greater disease spread in that direction.

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* t-values in grey shade and blue bold are significant at P < 0.05
(b): The logistic constant was back transformed into natural values using the logit function in Genstat and presented in the constant column. The regression constant describes the value of y (percentage severity) when x (distance) is zero.

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Data collected for cv. Howzat on 5 October 2007 generated regression slopes that were significantly different when comparing S with N and E. Disease was significantly more severe on plants due N and E of centre quadrat (1.1) with the regression slopes significantly less than due S. The regression constants for N were also significantly less than the constants for E, S and W. At this date, signs of disease first occurred in cv. Almaz and significant differences in regression slopes were seen between S and E. More severe disease occurred in the E than the S direction while no disease had spread in the N and W directions in this plot at this date.
Data collected on 2 November 2007 showed significant differences in all directions in cv. Howzat except for S - N. The E and N regression slopes were again significantly different, with more severe disease over the 4 m distance due E of quadrat 1.1. Comparison of S and E showed that severe disease occurred further due E of quadrat 1.1. Comparing slopes generated for W with the other directions showed that disease occurred on significantly more plants due E, N and S than W of quadrat 1.1. The regression constant differed significantly for comparisons between W and the other directions for cv. Howzat. The slope and regression constants showed no significant differences in cv. Almaz at this date.

The slopes and constants of the logistic regression, and the back transformed data, for disease severity in each direction (N, S, E and W) from the focus (quadrat 1.1, 0 m) to a distance 4 m for disease severity in the cvs Howzat and Almaz assessed on three dates in 2008 are shown in Table 6.3 a and b. The logistic curves are presented in Appendix C (Figures 7 to 12). Based on 199 observations, a t-statistic of greater than 1.96 indicated significant differences (P < 0.05) between constants and slopes of these disease severity curves.

In cv. Howzat on 5 September 2008, regression slopes were significantly different for the directional comparison between S - E (Table 6.3a). Disease was significantly more severe due S of centre quadrat 1.1 than to the E. No disease had spread in the north or west directions. The regression constant for cv. Howzat showed no significance in any directional comparisons on 5 September 2008 (Table 6.3b). Regression slopes for cv. Almaz showed no significant difference with regard to all directional comparisons at this date, however, the regression constant was significantly lower for W than E and S directions.
Regression slopes generated for 3 October 2008 for cv. Howzat showed significance when comparing all directions (Table 6.3a). Disease was significantly more severe due S of quadrat 1.1 than all other directions. Severe disease occurred significantly further in the E than N and W. The comparisons for significance between the regression constants showed E was more severe than all other directions on 3 October 2008 (Table 6.3b). The regression slopes generated for cv. Almaz on 3 October 2008 showed significant differences for all directional comparisons, such that more severe disease occurred due E of quadrat 1.1. Disease was significantly more severe due S of quadrat 1.1, with a smaller regression slope generated than for N, while disease was also significantly more severe due N of quadrat 1.1 than W. The regression constant for cv. Almaz showed significant difference for the W - S comparison at this date.

The regression slopes generated for the cv. Howzat on 2 November 2008 showed significance in all but W - N direction comparison (Table 6.3a). Disease was significantly more severe due S of quadrat 1.1, indicated by a positive regression slope, showing that disease severity at this date no longer declined with distance and all plants exhibited the same disease severity. Disease was also severe due E of quadrat 1.1 than W and N. The regression constant showed significant difference in the W - E and W - S directions for cv. Howzat although all plants were more than 90% affected (Table 6.3b). In cv. Almaz disease was more severe due E of quadrat 1.1 than all other directions. S showed disease significantly more severe than due W and N. Disease was also significantly more severe due N than W. The regression constant was only significantly different for the comparisons between W - N and W - S in cv. Almaz, where W had a lower constant.
Table 6.3: Spatial analysis of disease severity over 4 m from the disease foci in one plot each of chickpea cvs Howzat and Almaz in four directions, viz. North, East, South and West on 5 September 2008, 3 October 2008 and 2 November 2008. The regression logslopes were compared for directions North, East, South and West. Significance was determined by student t-distribution Table (P < 0.05) from 199 observations. Comparisons where differences are significant are shown in bold in the main body of the table and standard error (SE ±) of the residuals are shown for the log data.

(a): The logistic slope was back transformed into natural values using the logit function in Genstat and presented in the slope column. The slope describes disease severity over distance, with a smaller slope indicating more severe disease occurred further in that direction.

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T-values in grey shade and blue bold are significant at P < 0.05
(b): The logistic constant was back transformed into natural values using the logit function in Genstat and presented in the constant column. The regression constant describes the value of y (percentage severity) when x (distance) is zero.

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t-values in grey shade and blue bold are significant at P < 0.05

6.3.2 Temporal analysis of change in disease severity in cv. Howzat in 2007

The regression slopes for change in disease severity in the selected quadrats of the cv. Howzat from 15 August to 2 November 2007 are shown in Table 6.4. Pairwise comparisons indicate which quadrats were significantly different from each other for the rate of change in disease severity over time. Based on 479 observations, a t-statistic of greater than 1.96 indicated significant differences (P < 0.05) among quadrats and these are shown in bold in the body of the table. The rate of change in disease severity was significant for just under half of the quadrat comparisons. The rate of change in the centre
(quadrat 1.1) was faster than the intermediate quadrats to the NE (quadrat 3.11), SE (quadrat 3.15) and N (quadrat 4.13) and significantly slower than in 3.3 (intermediate SW), 5.29 (outer SE) and 5.21 (outer NE). The quadrats to the intermediate NE, SE, N and S sides of the centre plot had more steady increase in disease severity over time than those to the intermediate and outer NW and SW and outer NE and SE. Minimal disease was noted in the westerly and outer areas at the last assessments period, which resulted in the steeper regression slopes noted above (Figures 1, D and K, Appendix D).

6.3.3 Temporal analysis of change in disease severity in cv. Almaz in 2007

The regression slopes for change in disease severity in the selected quadrats of the cv. Almaz from 15 August to 2 November 2007 are shown in Table 6.5. Pairwise comparisons indicate which quadrats were significantly different from each other for the rate of change in disease severity. Based on 479 observations, a t-statistic of greater than 1.96 indicated significant differences (P < 0.05) among quadrats. Rate of change did not differ significantly in any of the quadrats compared.

6.3.4 Temporal analysis of change in disease severity in cvs Howzat and Almaz in 2007

The regression slopes for disease severity over time in the quadrats selected to compare cvs Howzat and Almaz (2007) are shown in Table 6.6. Pairwise comparisons indicate which quadrats were significantly different for the rate of change in disease severity. A t-statistic greater than 1.96 for 479 observations indicated significant differences (P < 0.05) among quadrats which are shown in bold in the body of the table. The rate of change in disease severity in quadrats 1.1 and 2.7 (adjacent to the centre plot in an easterly direction) in cvs Howzat and Almaz differed significantly over time. For both these quadrats, the slope for cv. Howzat was significantly steeper than that for cv. Almaz,
indicating that disease severity increased faster in Howzat. Comparisons of the outer quadrats (4.24, 5.25 and 5.30) showed no significant difference between the cultivars, in terms of change in disease severity over time in 2007.

6.3.5 Temporal analysis of change in disease severity in cv. Howzat in 2008

The regression slopes for change in disease severity in the selected quadrats of the cv. Howzat from 3 August to 2 November 2008 are shown in Table 6.7. Pairwise comparisons indicate which quadrats were significantly different from each other for the rate of change in disease severity. Based on 559 observations, a t-statistic of greater than 1.96 indicated significant differences (P < 0.05) among quadrats and these are shown in bold in the body of Table 6.7. Rate of change in quadrat 1.1 was significantly faster than quadrats 3.11, 3.7, and 4.13 located to the intermediate NE, NW and N, respectively. Rate of change in quadrat 1.1 was significantly slower than in quadrats 3.15 and 4.1 and 5.29, which are located to the intermediate S, SE and outer SE, respectively.

In these quadrats disease was not observed until midway or near the end of the assessments periods and then a large rapid spread of disease was observed, creating larger regression slopes than in the centre, (Figure 3, E, F and K in Appendix D). Rates of change for all other quadrats were not significantly different from the centre quadrat.

6.3.6 Temporal analysis of change in disease severity in cv. Almaz in 2008

The regression slopes for change in disease severity in the selected quadrats of the cv. Almaz from 3 August to 2 November 2008 are shown in Table 6.8.

Pairwise comparisons indicate which quadrats were significantly different from each other for the rate of change in disease severity. Based on 559 observations, a t-statistic of greater than 1.96 indicated significant differences (P < 0.05) among quadrats. Quadrat 1.1
had a slower rate of change in disease over time than the other quadrats as indicated by its smaller regression slope. The centre quadrat (1.1) was significantly slower in terms of rate of change in disease than all the other quadrats. This was attributed to all the other quadrats showing a rapid increase in disease in the later assessments periods (Figure 4, A to E, Appendix D).

Disease severity increased significantly faster in the outer SE (5.29) than the outer NE (5.21 quadrat). Disease severity also increased faster in the intermediate NE (3.11) than the intermediate SE (3.15).

6.3.7 Temporal analysis of change in disease severity in cvs Howzat and Almaz in 2008

The regression slopes for disease severity over time in the quadrats selected to compare cvs Howzat and Almaz (2008) are shown in Table 6.9. Pairwise comparisons indicate which quadrats were significantly different for the rate of change in disease severity. A t-statistic of greater than 1.96 for 559 observations indicated significant differences (P < 0.05) among quadrats and these are shown in bold in the body of the table. The results showed that the rate of change in disease severity was significantly greater for cv. Howzat than for cv. Almaz in all cases except quadrant 3.11.
Table 6.4: Temporal analysis of disease severity in cv. Howzat in 2007. Disease severity on each of 40 plants per quadrat was rated on a scale from 0 to 100 %, beginning when disease was first observed on 15 August 2007 until the end of the chickpea season on 2 November 2007. The slope (rate of change in disease severity) is shown for each quadrat. Note that quadrats are arranged in ascending order of log slope value and are compared across the quadrat row and down the quadrat column. The logistic slope was back transformed into natural values using the logit function in Genstat and presented in the slope column. Significant differences between pairs of quadrats for logslope were determined by student t-distribution tables (P < 0.05) from 479 observations. The significantly different quadrats are shown in blue bold in the main body of the table and standard error (SE ±) of the residuals are shown for the log data. A smaller logslope value indicates a slower rate of change in disease severity.

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* denotes quadrats already compared within the body of the table, (I) denotes intermediate quadrat distance approximately 1 – 2 m from the centre quadrat (1.1), (O) denotes outer-most quadrat approximately 4 - 5 m distance from centre quadrat (1.1), (C) denotes centre quadrat.
Table 6.5: Temporal analysis of disease severity in cv. Almaz in 2007. Disease severity on each of 40 plants per quadrat was rated on a scale from 0 to 100 %, beginning when disease was first observed on 15 August 2007 until the end of the chickpea season on 2 November 2007. The slope (rate of change in disease severity) is shown for each quadrat. Note that quadrats are arranged in ascending order of degree of slope and are compared across the quadrat row and down the quadrat column. The logistic slope was back transformed into natural values using the logit function in Genstat and presented in the slope column. Significant differences between pairs of quadrats for logslope were determined by student t-distribution tables (P < 0.05) from 479 observations. The standard error (SE ±) of the residuals are shown in the body of the table for the log data. A smaller logslope value indicates a slower rate of change in disease severity.

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* denotes quadrats already compared within the body of the table, (I) denotes intermediate quadrat distance approximately 1 – 2 m from the centre quadrat (1.1), (O) denotes outer-most quadrat approximately 4 - 5 m distance from centre quadrat (1.1), (C) denotes centre quadrat
**Table 6.6:** Comparison of the rate of change in disease severity in cv. Almaz and Howzat in 2007. Data for disease severity on each of 40 plants in quadrats 1.1, 2.7, 4.24, 5.25 and 5.30 (which exhibited disease) were selected for comparison. Plants were rated for disease severity on a scale of 0 – 100 %, beginning when disease was first observed on 15 August 2007 until the end of the chickpea season on 2 November 2007. The slope (rate of change in disease severity) is shown for each quadrat and cultivar. The logistic slope was back transformed into natural values using the logit function in Genstat and presented in the slope column. Significant differences between pairs of quadrats for logslope were determined by student t-distribution tables (P < 0.05) from 479 observations. The significantly different quadrats are shown in blue bold in the main body of the table and standard error (SE ±) of the residuals are shown for the log data. A smaller slope value indicates a slower rate of change in disease severity.

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<th>Slope Almaz</th>
<th>SE ±</th>
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<th>t-values in grey shade and blue bold are significant at P &lt; 0.05</th>
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<td>1.140</td>
<td>75.77</td>
<td>0.009</td>
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(I) denotes intermediate quadrat distance approximately 1 – 2 m from the centre quadrat (1.1), (O) denotes outer most quadrat approximately 4 - 5 m distance from centre quadrat (1.1), (C) denotes centre quadrat
Table 6.7: Temporal analysis of disease severity in cv. Howzat in 2008. Disease severity on each of 40 plants per quadrat was rated on a scale from 0 to 100 %, beginning when disease was first observed on 3 August 2008 until the end of the chickpea season on 2 November 2008. The slope (rate of change in disease severity) is shown for each quadrat. Note that quadrats are arranged in ascending order of degree of slope and are compared across the quadrat row and down the quadrat column. The logistic slope was back transformed into natural values using the logit function in Genstat and presented in the slope column. Significant differences between pairs of quadrats for logslope were determined by student t-distribution tables (P < 0.05) from 559 observations. The significantly different quadrats are shown in blue bold in the main body of the table and standard error (SE ±) of the residuals are shown for the log data. A smaller slope value indicates a slower rate of change in disease severity.

| Quadrat | Direction from centre | logSlope | Slope  | SE ±  | 3.15 | 3.11 | 3.7  | 3.3  | 4.13 | 4.1  | 5.29 | 5.21 | 5.13 | 5.5  |
|---------|----------------------|----------|--------|-------|------|------|------|------|------|------|------|------|------|------|------|------|
| 3.7     | NW(I)                | 0.064    | 51.60  | 0.004 | *    | *    | *    | -3.05| 1.11 | -18.07| -8.60 | -7.14| -5.00| -5.00|
| 3.3     | SW(I)                | 0.078    | 51.94  | 0.003 | *    | *    | *    | *    | 3.77 | -12.49| -6.39 | -1.89| -0.96| -0.96|
| 5.13    | NW(O)                | 0.082    | 52.04  | 0.003 | *    | *    | *    | *    | *    | *    | *    | *    | *    | *    | 0    |
| 5.5     | NS(O)                | 0.098    | 52.46  | 0.005 | *    | *    | *    | *    | *    | *    | *    | *    | *    | *    | *    |
| 3.11    | NE(I)                | 0.103    | 52.57  | 0.004 | *    | *    | *    | -3.84| -5.57| -2.94 | -18.90| -9.98| -9.38| -7.52| -7.52|
| 4.1     | S(I)                 | 0.104    | 52.61  | 0.004 | *    | *    | *    | *    | *    | *    | *    | *    | *    | 1.93 | 13.13| 12.92|
| 3.15    | SE(I)                | 0.104    | 52.61  | 0.004 | *    | *    | *    | 18.64| 17.65| 13.19| 18.15| 2.12 | 3.36 | 13.55| 13.48| 13.48|
| 5.29    | SE(O)                | 0.109    | 52.72  | 0.003 | *    | *    | *    | *    | *    | *    | *    | *    | *    | *    | 5.80 | 5.10 |
| 1.1     | C                    | 0.167    | 54.16  | 0.010 | -13.82| 7.39 | 4.81 | 0.73 | 5.68 | -13.34| -6.28 | -1.36| -0.29| -0.29|
| 5.21    | NE(O)                | 0.188    | 54.68  | 0.005 | *    | *    | *    | *    | *    | *    | *    | *    | 1.00 | 0.00 | 0.00 |
| 4.13    | N(I)                 | 0.206    | 55.12  | 0.007 | *    | *    | *    | -18.65| -8.99| -6.09 | -5.85 | -5.85|

* denotes quadrats already compared within the body of the table, (I) denotes intermediate quadrat distance approximately 1 – 2 m from the centre quadrat (1.1), (O) denotes outer most quadrat approximately 4 - 5 m distance from centre quadrat (1.1), (C) denotes centre quadrat

**t-statistic in grey shade and blue bold are significant at P < 0.05**
Table 6.8: Temporal analysis of disease severity in cv. Almaz in 2008. Disease severity on each of 40 plants per quadrat was rated on a scale from 0 to 100 %, beginning when disease was first observed on 3 August 2008 until the end of the chickpea season on 2 November 2008. The slope (rate of change in disease severity) is shown for each quadrat. Note that quadrats are arranged in ascending order of degree of slope and are compared across the row and down the column. The logistic slope was back transformed into natural values using the logit function in Genstat and presented in the slope column. Significant differences between pairs of quadrats for logslope were determined by student t-distribution tables (P < 0.05) from 559 observations. The standard error (SE ±) of the residuals are shown in the body of the table for the log data. A smaller slope value indicates a slower rate of change in disease severity.

<table>
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<tr>
<th>Quadrat</th>
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<th>Slope</th>
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<td>51.49</td>
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<td>51.76</td>
<td>0.005</td>
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* denotes quadrats already compared within the body of the table, (I) denotes intermediate quadrat distance approximately 1 – 2 m from the centre quadrat (1.1), (O) denotes outer most quadrat approximately 4 - 5 m distance from centre quadrat (1.1), (C) denotes centre quadrat.
Table 6.9: Comparison of the rate of change in disease severity in cvs Almaz and Howzat in 2008. Data for disease severity on each of 40 plants in quadrats 1.1, 3.15, 3.11, 5.29, and 5.21 (which exhibited disease) were selected for comparison. Plants were rated for disease severity on a scale of 0 – 100 %, beginning when disease was first observed on 3 August 2008 until the end of the chickpea season on 2 November 2008. The slope (rate of change in disease severity) is shown for each quadrat and cultivar. The logistic slope was back transformed into natural values using the logit function in Genstat and presented in the slope column. Significant differences between pairs of quadrats for logslope were determined by student t-distribution tables (P < 0.05) from 559 observations. The significantly different quadrats are shown in blue bold in the main body of the table and standard error (SE ±) of the residuals are shown for the log data. A smaller slope value indicates a slower rate of change in disease severity.

(I) denotes intermediate quadrat distance approximately 1 – 2 m from the centre quadrat (1.1), (O) denotes outer most quadrat approximately 4 - 5 m distance from centre quadrat (1.1), (C) denotes centre quadrat
6.3.8  Wind and coinciding rain event data for the seasons 2007 and 2008 collected by the Roseworthy AWS

Figures 6.8 (A) and (B) show the total rainfall, average temperature and average RH for the months of June to October for the 2007 and 2008 seasons, respectively.

**Figure 6.8:** Total rainfall (mm), average temperature (°C) and average relative humidity (%) per month recorded by the Roseworthy AWS (ID: 023122, Latitude: -34.51, Longitude: 138.68, Altitude: 65.0 m) situated approximately 10 km from the chickpea trial site in 2007 (A) and 13 km from the trial site in 2008 (B). Data were recorded continuously at 30 minute intervals for the months of June to October.
Rainfall patterns differed over the two years, especially in the months of July and August which were substantially wetter in 2008 than in 2007, with a 13 and 58 mm difference, respectively. The average temperature ranged between 10 and 15 °C from June to August and increased to between 20 and 25 °C from September to October in both years. RH was between 75 % and 85 % from June to October in 2007 but in 2008 decreased from 75 - 80 % in June to August to 70 % in September and 55 % in October.

The windrose graph in Figure 6.9 (A) shows that over the 2007 season, coincidental rainfall and wind events were evenly scattered around the compass directions, indicating that these events were multi-directional throughout the season. The strongest winds, however, up to 20 m s\(^{-1}\), were observed in the E to SE and were consistently stronger than those in the N, S and W directions. Figure 6.9 (B), which shows data for the month of August, indicates the NE, E and SE directions had a greater number of strong wind events with coinciding rainfall than other directions, with wind speeds ranging from 8 to 10 m s\(^{-1}\). Figure 6.9 (C), for the month of September, again shows the NE, E and SE corners had a greater number of strong wind events with coinciding rain events, with wind above 10 m s\(^{-1}\) in the E direction. Figure 6.9 (D), for the month of October, shows the wind events that occurred in the NW direction were just over 5 m s\(^{-1}\), while wind in the NE direction was strongest at speeds up to 8 m s\(^{-1}\).
**Figure 6.9:** Wind speed (m s\(^{-1}\)) and direction of wind during rainfall events in 2007 as recorded by the AWS at Roseworthy, 10 km from the Kingsford field site. Each point (red dot) represents a rainfall event and its associated wind speed (0 - 20 m s\(^{-1}\)) and direction (N, S, E, W). Combined wind and rain events for A, the year of 2007; B the month of August; C, the month of September; D, the month of October.
The windrose graph in Figure 6.10 (A) shows that over the 2008 season, coincidental rainfall and wind events were evenly scattered around the compass directions, and so were multi-directional throughout the season. Wind speeds and coinciding rain events were over 10 m \( \text{s}^{-1} \) in the N to the E directions. The strongest wind speed with coinciding rainfall was almost 15 m \( \text{s}^{-1} \) in the E and the weakest, 2.5 m \( \text{s}^{-1} \) in the NW. Figure 6.10 (B), for the month of August, shows wind speed and coinciding rainfall events had a similar pattern to Figure 6.10 (A), with wind speeds reaching just over 5 to 10 m \( \text{s}^{-1} \) across the N and S directions.

The weakest winds were in the NW, at 2.5 m \( \text{s}^{-1} \). Figure 6.10 (C), for September, shows the strongest wind speeds with coinciding rainfall events were in the NE, E and SE directions, often above 10 m \( \text{s}^{-1} \) and some even stronger events up to 15 m \( \text{s}^{-1} \) in the E. Figure 6.10 (D) shows that wind speed and rainfall events for October were weaker than in previous months, with wind speeds often less than 5 m \( \text{s}^{-1} \). The strongest wind events with coinciding rainfall were seen in the NE and SW, with one NE event above 10 m \( \text{s}^{-1} \).
Figure 6.10: Wind speed (V) and direction of wind during rainfall events in 2008 as recorded by the AWS at Roseworthy, 13 km from the Turretfield site. Each point (red dot) represents a rainfall event and its associated wind speed (0 - 20 m s\(^{-1}\)) and direction (N, S, E, W). Combined wind and rain events for A, the year of 2008; B the month of August; C, the month of September; D, the month of October.
6.4 Discussion

Logistic models have been successfully used to describe polycyclic diseases, such as ascochyta blight of chickpeas, since the 1960s (Van der Plank, 1964). The use of such models in this study helped to describe the rate of change of disease severity and spread from foci of ascochyta blight in plots of cultivars of chickpea that differed in susceptibility. The use of the standard errors of the residuals from the regression analysis, rather than the correlation, permitted assessment of the fit of the individual parameters and not the average fit of the regression (Dytham, 2003).

This study highlighted how disease severity changed over distance and over time from central foci in South Australian conditions and improved our understanding of how environmental factors affect the dispersal of ascochyta blight of chickpeas. Wind, rain and cultivar resistance influenced the direction and rate of spread of the disease. Disease spread in an outward direction from the foci and was more severe downwind, influenced
by strong winds with coinciding rainfall events. The findings over the two seasons also revealed that the amount of rainfall was a major influence in the rate of the increase of severity and distance of spread.

6.4.1 Spatial analysis

In cvs Howzat and Almaz over both years, disease was generally most severe downwind, S and E of the focus. Windrose graphs, Figures 6.9 and 6.10, showed that the strongest most predominant winds that coincided with rainfall events were from the north and north-west to the east and south-east, indicating that the pattern of spread of disease was influenced by wind-driven rain. The increased severity downwind (E of the foci) at Kingsford 2007, influenced by coinciding rainfall, resulted in a directional spread (easterly). This was similar to that reported by Kimber et al. (2007), who showed that 70 - 100 % of plants approximately NE (downwind) of the foci in chickpea plots (5 x 1.5 m) at Kingsford in 2001 were diseased (measured as incidence).

Kimber et al. (2007) also described an independent study in Israel in which lesion severity was assessed across transects, using scoring methods and directions similar to those reported here. The prevailing wind (to the east in the Israeli study) caused disease to spread nearly 4 m in cultivar Ayala (MS) and just over 2 m in the cultivar Hadas (MR). In the current study, disease spread 4 m in cultivar Howzat (MS) and was sporadic across 4 m from the focus in Almaz (MR), indicating a similar pattern of disease spread in cultivars of equivalent resistance ratings.

Disease in cultivar Howzat spread from one quadrat to the next in a continuous fashion in 2007 and 2008. Such successive disease spread was described by Cubero (1984) as patches of disease, first at the foci, which slowly spread across the whole plot over the
season. The uniform spread in cv. Howzat in the present study is likely due to the susceptibility of this cultivar. The continuous disease spread at further distances and increased severity in 2008 which was not observed in 2007 reflects the influence of the greater rainfall. The marked differences in rainfall and disease progress over the three seasons, 2006 - 2008, strongly suggests that rainfall indeed influenced the spatial distribution of disease.

The year 2006 was one of the driest years in South Australia since records began, with many BOM stations recording around 50 % of their average annual totals. Furthermore, 2006 was the third warmest year since 1910 (BOM, 2010). The reduced rainfall and hot weather limited the spread of ascochyta blight to small localised areas within the plot investigated in the pilot study (Appendix B). Rainfall during the growing season (April to October) at Kingsford during 2007 was also 31 % below the long term average (BOM, 2010). The rainfall in July and August 2008 was approximately 13 mm and 58 mm higher than the same months in 2007, respectively (Figure 6.8 A and B).

Ketelaer (1988) calculated that a monthly rainfall of at least 40 mm and a monthly average temperature of at least 8 °C are needed to allow the onset of an epidemic of chickpea ascochyta blight. In 2008 there was above 40 mm of rainfall in July and August, which are pivotal months at the start of the disease cycle for development of pycnidia and dispersal of secondary conidia and, therefore, the onset of an epidemic in southern Australia. The above average rainfall in July would have been important in disease initiation in this study. The first disease symptoms were expected to occur in August following these rains and, in fact, were observed on 3 August 2008, and it is expected that secondary spread would have taken place during the record rainfall event in August.
Consequently, the larger amount of rain during these months resulted in more disease expression in 2008 than in 2007.

Figure 6.8 (A and B) shows that the average temperatures for the months of August, September and October were similar in 2007 and 2008 (between 10 - 25 °C) but RH was slightly higher in 2007 (between 75 - 80 %) than in 2008 (between 60 - 80 %). These temperature and RH combinations are within the range that is conducive for disease development, formation of pycnidia, spore germination and infection (Kaiser, 1973, Trajano-Casas & Kaiser, 1992b, Navas-Cortés et al., 1998, Trajano-Casas & Kaiser, 2007) and the only major difference between the two years was the rainfall events. This study thus indicates the overall importance of rainfall in causing an epidemic. The windrose showed that wind was responsible for the directional spread and rain for the rate of the severity increase. Likewise, Kaiser (1992) also showed that combined wet, windy conditions were vital in a rapid disease spread across plant populations and this was confirmed by Kimber et al. (2007). Zhang et al. (2005) indicated the importance of “within day” rain events in releasing pycnidiospores of *M. pinodes* from infested stubble and the importance of rainfall in disease development, with wind influential in the directional spread of the sexual stage. In a study on ascochyta blight of field peas using trap plants to demonstrate dispersal of airborne inoculum, Schoeny et al. (2007) also indicated that primary inoculum availability was increased by rainfall and cumulative degree days.

Uniform spread was observed in cv. Almaz in 2008, which may have resulted from larger amounts of inoculum influenced by rain, which possibly overcame the moderate resistance of this cultivar. This response has been observed by Chongo et al. (2003),
where moderate resistance to ascochyta blight of chickpea was overcome in areas of intermediate to high disease pressure with conducive weather conditions.

The pattern of spread in cv. Almaz was sporadic in 2007, with disease 1 m from the focus and then 4 m, skipping the quadrats at 2 and 3 m in the E direction. In the S direction, a similar dispersion pattern was seen, with disease at 1 m and again at 3 m. This intermittent spread in 2007 may reflect the moderate resistance of cv. Almaz and low inoculum levels, influenced by the reduced rainfall. The pattern observed did indicate that some plants within a population could become selectively infected, giving a sporadic distribution. Partial resistance also delays the onset and severity of disease (Davidson & Kimber, 2007), which may be evident on some plants earlier than others, which also explains the patchy distribution observed.

When comparing cultivars and disease severity over distance, a steeper regression curve was seen in the susceptible cv. Howzat than in the more resistant Almaz. Zhang et al. (2004) and Kimber et al. (2007) reported a similar effect in studies of pea and chickpea crops; for example, Kimber et al. (2007) stated that the susceptibility of a cultivar to A. rabiei markedly affected the distance to which disease spread from the source of primary infection, influencing the size of the resulting foci.

In relation to our field trial plantings and disease spread the likelihood of conidia landing on a plant not directly next to the focus was dramatically decreased, especially at the beginning of the season when foliage was sparse. Row spacing was set at 17.5 cm in the field trials and is commonly set at 18 cm to 1 m in commercial plantings (Gentry, 2010). Thus, the likelihood of a conidium landing on the host at an 18 cm distance with no influence of wind but in the presence of rain is likely as A. rabiei conidia travelled a
maximum of 22 cm at wind speed 1.4 m s$^{-1}$, which is classed as a breeze (relative to no wind) in the wind tunnel experiment described in chapter 5 a conidium is unlikely to travel greater than 1 m. Schoeny et al. (2008) reported that inter-row spaces for peas are generally greater than 18 cm. *M. pinodes* conidia travel up to 64 cm in 2 mm of rain in still air and so there is a high probability they will be transported 18 cm to plants in neighbouring rows likely but they are unlikely to go over 1 m. *A. rabiei* then, may, infect crops with inter-row spacing commonly used in chickpeas without the aid of wind, potentially infecting the plants at greater distances than those just directly adjacent. However, the sparse canopy early in the season may still be a barrier to infection. Wind is obviously a factor in the transport of conidia, as shown in chapter 6, and in the presence of stronger wind the conidia of *A. rabiei* can travel across larger distances, up to 0.5 m at 4.7 m s$^{-1}$. Schoeny et al. (2008) also stated that wind and turbulence would influence dispersal of *M. pinodes* conidia over a greater distance than was shown in their studies. Although there are natural differences in the structure of chickpea and field pea plants, the concept of a denser canopy providing a larger area for spores to land on and cause infection is universal. Nevertheless, it would be valuable to replicate the work of Schoeny et al. (2008) using potted chickpea plants to obtain information about the optimum planting density that would be a barrier to splash dispersal and infection.

Future studies to investigate the influence of adjusted row spacing in the field on ascochyta blight development should also be performed in Australia. In Canada, Chang et al. (2007) studied the effect of planting Kabuli and Desi cultivars at 6 – 76 plants per m$^2$ with row spacing either 20, 30 or 40 cm. Seeding rate, row spacing and their interaction showed a smaller effect on severity of ascochyta blight than did the cultivar. Nevertheless, a positive relationship was found between plant density and ascochyta blight, and conidia appeared to be more efficient at crossing narrow row spacing or when
plants were tightly packed together, especially as a denser canopy resulted in less airflow, greater RH and conditions conducive to fungal growth.

### 6.4.2 Temporal analysis

The temporal analysis for cv. Howzat in 2007 showed that the rate of the increase in disease severity in the intermediate N (4.13), NE (3.11) and SE (3.15) quadrats was slower than in the centre (1.1). This was expected, since the centre quadrat (focus) was the source of inoculum. Plants next to the focus become infected quickly due to the localised splash dispersal, which distributes more of the pathogen closer to the source, as was shown in experiments reported in chapter 6 which identified the potential for thousands of conidia to be dispersed close to the inoculum (2 cm). The outer NE (5.21), SE (5.29) and intermediate SW (3.3) quadrats showed a faster rate of infection than the centre quadrat. This was attributed to the late but rapid expression of disease, which was supported by Figure 6.9 (A), which shows that strong winds coincided with rainfall events (downwind) NE and SE directions in 2007, likely transporting inoculum to those outer quadrats. The observation that disease did not appear in quadrats (5.21), (5.29) and (3.3) until later in the season, then increased rapidly, may be related to decrease in resistance as the crop matured (Basandrai et al., 2007, Nene & Reddy, 1987, Chongo & Gossen, 2001). In relation to the importance of prevailing wind, the most outer quadrats 5.21 (NE) and 5.29 (SE) were in the path of the strongest prevailing wind events. Quadrat 3.3 (SW) was also in the path of many moderate wind events, possibly influencing severity increase in that quadrat.

Interestingly, Basandrai et al. (2007) noted that symptom expression was most characteristic in 8 - 9 week old plants as the crop matured. This time frame was close to when the severity increase was observed in previously uninfected plants in outer quadrats.
in this study and perhaps symptoms were missed in earlier observations because they were less obvious due to the plant age.

Temporal analysis of disease in cv. Howzat in 2008 showed that the rate of change was significantly faster in the centre quadrat (1.1) than the intermediate N (4.13), NE (3.11), and NW (3.7) quadrats. Again, it was expected that rate of change would be greatest in the centre quadrat (focus). However, the rate of change was slower in the centre than the intermediate S (4.1), and SE (3.15) and the outer SE (5.29) quadrats. Figure 3 E, F and K, Appendix D, shows that disease occurred later in these quadrats but then spread rapidly, also supporting the claim that plant resistance declines with age (Basandrai et al., 2007, Chongo & Gossen, 2001, Nene & Reddy, 1987).

In both years, wind speeds greater than 10 m s\(^{-1}\) seem to have distributed ascochyta blight to the outer quadrats, with windrose for 2007 and 2008 (Figures 6.9 and 6.10) indicating the strongest winds (> 10 m s\(^{-1}\)) downwind, NE and SE where the fastest rates of infection relative to the focus were observed. Additionally, winds of 5 to 10 m s\(^{-1}\) seemed to influence intermediate distribution (1 - 1.5 m) from the focus, where severity increased rapidly in the intermediate quadrats, such as in 3.3 observed in 2007.

As discussed under spatial analysis (section 6.4.1), another factor that may have contributed to this increased severity later in the season could be the increase in canopy density and the increased likelihood that the conidia will land on the plants. Schoeny et al. (2008) observed the effect of the density of canopies at 49, 81 and 121 plants per m\(^2\) on the spread of ascochyta of field pea. Looser canopies reduced the likelihood of \textit{M. pinodes} conidia landing on a plant. A similar phenomenon may apply here, with looser chickpea canopies reducing the likelihood of \textit{A. rabiei} conidia landing on a plant. Later in
the season, with the combination of prevailing winds, the chance of spores landing further from the focus is increased due to the thicker canopy and this may have contributed to the increased severity observed later in the season and in outer quadrats.

Temporal analyses for cv. Almaz 2007 showed no significant difference in the rate of change of disease over the season, which was attributed in part to the moderately resistant rating of this cultivar and the moderate rainfall in 2007. In 2008 the intermediate NE and SE and outer NE and SE quadrats had faster rates of infection than the centre. This indicates that the wetter season in 2008 provided more occasions for the spread of the inoculum. Perhaps the rainfall also caused the ongoing development of vegetative growth, providing more opportunity for infection in the outer quadrats. The strongest winds with coinciding rainfall in the NE and SE direction (Figure 6.10 B to D) may have influenced inoculum dispersal in Almaz. Increased canopy density or perhaps the resistance decreasing as the plants reached maturity may have contributed to the rapid increase in disease in the outer quadrats than in the centre in this cultivar.

When comparing the rate of change of disease between quadrats 1.1, 2.7, 4.24, 5.25 and 5.30 in cvs Howzat and Almaz in 2007, cv. Howzat showed a significantly faster rate of change of disease at the focus (1.1) and in the quadrat closest to the focus (2.7). Rate of change of disease in all outer quadrats (4.24, 5.25 and 5.30) did not differ significantly between cultivars, possibly because of limited disease due to less rainfall and reduced canopy density in 2007. The lack of significance in these quadrats (4.24, 5.25, and 5.30) may indeed be due to a lack of observations and thus a larger standard error was given for cv. Almaz. Clearly, disease on cv. Almaz was less severe than cv. Howzat (as shown by Figure 1 (M and O) and Figure 2 (C, D and E in Appendix D)).
When comparing the rate of change between quadrats 1.1, 3.15, 3.11, 5.29 and 5.21 in cvs Howzat and Almaz in 2008, cv. Howzat showed a significantly faster rate of change of disease in all comparisons apart from 3.11. The trend (Figure 3 (D) and Figure 4 (B) (Appendix D)) in 2008 was likely due to the greater rainfall. It is again obvious that disease was much less severe in cv. Almaz than in cv. Howzat in all the quadrant comparisons.

6.4.3 Logistic regression model evaluation

When fitting the observed data to logistic models, it was considered important to select an appropriate model based on the known biology of the pathogen rather than simply on the shape of the curve (APS, 2011). It is quite possible that some of the data may have fitted other models more accurately. In the spatial analysis, there were significant differences between certain directions and constants, for example in Tables 6.2 and 6.3 b. The most plausible explanation for the differing estimates of the single value of severity at the focus is that the model was a poor fit. This suggests that another model may have been more appropriate in these cases. Logistic regression, however, gave the best fit for the majority of observations and uniformity of analysis allowed direct comparisons of disease severity among the quadrats.

In any investigation, random variability in observations may occur and this study was no exception (see plotted models in Appendix C and D). This was especially evident where the disease on some individual plants was slight but in the same quadrant others were very severely affected. In statistics, more data points allow better overall estimate of the disease severity. In quadrant 1.1, disease occurred from the start of the season and was observed until the end of the season, giving a less variable estimate of severity because of the greater number of data points. Some outer quadrats showed disease expression later in
the season and, thus, gave fewer data points and the possibility of poorer estimates, shown by larger standard error. Although not perfect, however, it is possible to estimate the apparent infection rate with just a few data points.

6.4.4 Future work

A larger study than this one, including replication of the plots with the various cultivars, would give a better understanding of changes in disease severity. Such replication may reduce the standard error observed in some of the quadrats where few disease observations occurred. However, chickpea seed was limited due to drought in 2006 and 2007 so replication was not possible. Assessment of every plant in each 1 m² quadrat may not be practical in replicated plots, especially when disease becomes severe, and the transect observation method might be more suitable.

The effect of plant age and resistance to ascochyta blight observed by Basandrai et al. (2007) and Chongo and Gossen (2001) should be studied in future field trials and laboratory experiments but using local isolates and cultivars, which would contribute to understanding the influence of plant maturity on disease development in Australian conditions. This would identify if plant age was a factor in these trials. Field trials performed with the inoculum placed in the plots at various stages of plant development (seedling, post seedling, vegetative, flowering and podding) may help to understand this phenomenon. Laboratory experiments using single conidial isolates applied to chickpeas at the different stages of development described above could indicate variation in pathogenicity within the Australian population, as well as the effect of the pathogen on chickpea of varying stages of growth.
The ability of conidia to adhere to the host and cause infection is a factor often overlooked in plant pathogen interactions. At present, it is not known what percentage of the conidia of *A. rabiei* dispersed adhere to the host. Wetness of the plant, the medium (water or no water) through which the spore travels, and the phenology of the plant would all play important roles in the ability of conidia to adhere. The success rate could be very low and large amounts of conidia may be needed to allow a single conidium to infect the plant. Chang *et al.* (2007) showed that chickpea plants with compound leaves have less ascochyta blight than those with unifoliate leaves, perhaps because the latter retain more moisture, which helps adhesion and infection. Knowledge of this aspect of the disease cycle would increase our understanding of long distance dispersal and infection, especially when perhaps only a few spores may reach a host.

Although irrigation did not reflect the natural spread of disease in the trial at the Waite campus in this study, overhead irrigation was used effectively by Kimber *et al.* (2007) and is a common practice in Israel to make conditions conducive to ascochyta blight of chickpea. As such, overhead irrigation of chickpea crops in Queensland in Australia (Ryley & Ferguson, 2011) may allow infection and spread of ascochyta blight and this needs further investigation.

The confirmation that rain promotes epidemics of ascochyta blight indicates that planting strategies could be useful for reducing the severity of epidemics in chickpeas in wetter seasons in South Australia. Delayed sowing practice could reduce the number of rain events to which the crop is exposed and so reduce the number of infection cycles. In Australia, this cultural practice is recommended to reduce the exposure of young plants to high levels of primary inoculum in field peas and chickpeas (Bretag *et al.*, 2000, Pande *et al.*, 2005).
6.4.5 Conclusion

The epidemiological information described by logistic models provided an improved understanding of factors that influence development and spread of chickpea ascochyta from a focus. These findings indicate that wet conditions are a pivotal factor in causing ascochyta blight epidemics, especially when other conditions (RH and temperature) are conducive to disease development. The findings extend the studies performed by Kimber et al. (2007) in South Australia, provide a more comprehensive understanding of ascochyta blight development in South Australian conditions and lead into the modelling work reported in chapter 7.
Chapter 7: Development of a model to simulate the spread of ascogyta blight of chickpea in the field

Manuscript prepared for submission to the journal Plant Pathology, except for coloured pictures, which will be adjusted to black and white for publication.

Development of a model to simulate the spread of ascogyta blight of chickpea in the field

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Abstract

Ascochyta blight, caused by Didymella rabiei, is a significant disease world-wide including southern Australia. To explore its intensity and pattern of spread, a weather-based spatio-temporal model was developed. The model, spread of Ascochyta rabiei in chickpea (SArC), is adapted from the previously published model, AnthracnoseTracer for lupins. The major parameters of the model were either derived from laboratory or field experimental data, or estimated through calibration with one year’s field data. The model was then subjected to qualitative and quantitative validation using the following year’s
field data; these data were not used for identifying parameters of the model. For quantitative validation, the performance of the model was analysed statistically using a confidence interval, correlation-regression approach and a deviation-based approach, and the model largely simulated the spread of the disease in fields in two chickpea cultivars of different resistance to ascochyta. Sensitivity analysis was then performed to show the relative sensitivity of the final model parameters. Given the strength of SArC model in the parameter estimation and calibration and validation, it has potential to be used as a tool in plant biosecurity and/or managing ascochyta blight in chickpea in farming systems.

Introduction

Ascochyta blight, caused by Didymella rabiei, is a significant disease in most of the world’s chickpea (Cicer arietinum L.) crops and is a major constraint to chickpea production in Australia (Pande et al., 2005). D. rabiei survives on infected seed and stubble, forming ascospores and/or conidia that initiate primary infection; ascospores are spread by wind and conidia by rain splash or wind-driven rain. Pycnidia develop on infected tissue, resulting in secondary spread through conidia that are dispersed by rain splash (Kaiser, 1997, Trapero-Casas & Kaiser, 1992b). In Australia, initiation and subsequent spread of this disease are attributed almost entirely to conidial infection and only one mating type (MAT 1-2) has been found to date (Phan et al., 2003, Leo et al., 2011). Dispersal of conidia occurs in wet and windy conditions (Shtienberg et al., 2006, Kimber et al., 2007); favourable conditions such as temperatures of 5-30 ºC optimum 20 ºC and relative humidity of > 95% are also required for the fungus to penetrate into and infect host tissues resulting in disease (Pande et al., 2005, Jhorar et al., 1997, Trapero-Casas & Kaiser, 1992a, Trapero-Casas & Kaiser, 2007).
Understanding the epidemiology of ascochyta blight with respect to interaction with host (e.g. degree of cultivar resistance) and environment (e.g. location-specific weather conditions) can help in formulating strategic and, to some extent, tactical management of the disease at a crop production level. It is impractical, given the limitations of resources and time, to investigate the epidemiology of a disease for each and every aspect of the host in all its growing environments. Thus, quantitative epidemiology from limited environments built into a model can aid in understanding epidemics in environments beyond its ‘domain of study’ (Salam et al., 2011).

Empirical models, which provide a fit to observed data but do not necessarily take into account all the biological processes that explain the relationship, have been applied in epidemiology of plant pathogens since the 1960s (Van der Plank, 1964, Madden et al., 2007). These models are valuable for determining a relationship between two or more variables and comparing the effects of treatments on biological processes (Madden et al., 2007, Payne et al., 2008). Logistic regression models are empirical models that have been used to describe the spatio-temporal development of A. rabiei on chickpea (Kimber et al., 2007) and Mycosphaerella pinodes on field pea (Kimber et al., 2007, Zhang et al., 2004). Shtienberg et al. (2005) also used empirical modelling to identify the influence of temperature and wetness period on pseudothecial maturity of Didymella rabiei on chickpea debris, to identify the timing of chemical application most likely to prevent primary infection by ascospores. Such statistical models characteristically produce outputs without reference to underlying physical or biological variables, hence they are sometimes known as “black box” or “input-output” models. Also, extrapolation often is not possible using these statistical modelling techniques (Jones et al., 2010). The other
broad class of models is known as mechanistic or simulation, as they explain causality between the variables by mimicking the system under consideration (Jones et al. 2010). Theories which identify key host and environmental interactions that influence the pathogen are devised at the beginning of development of these models, followed by mathematical representations, then validation, resulting in a working simulation model (Madden et al., 2007). Simulation models are generally dynamic, meaning that they predict changes in epidemics over time (Jeger, 1986) and are of interest to plant pathologists undertaking disease predictions.

Many successful simulation models of plant diseases use weather as the driver to enable predictions for varying environmental conditions. Salam et al. (2003, 2011) have produced such models for predicting risk of diseases in Australian conditions. The models can predict the onset of pseudohelial maturity and seasonal showers of ascospores of phoma stem rot (blackleg) of oilseed rape (canola, *Brassica napus*) caused by *Leptosphaeria maculans* (Salam et al., 2003) and severity and yield loss following release of ascospore of *Didymella pinodes*, cause of ascochyta blight (blackspot) of field peas (*Pisium sativum*) (Salam et al., 2011). The risk of disease based on the models is made available every year to stakeholders. However, to the best of our knowledge, a model that can simulate the spread of *Ascochyta rabiei* on chickpea in the field has not been developed in Australia or elsewhere.

Diggle et al. (2002) developed a simulation model (“AnthracnoseTracer”) for short distance rain-splashed dispersal of anthracnose of lupins, caused by *Colletotrichum gloeosporioides*, from infected seeds, the key epidemiology processes of which are similar to ascochyta blight of chickpea. In this study, we have adapted
“AnthracnoseTracer” to develop a spatio-temporal model for chickpea that simulates the spread of ascochyta blight at a field scale. The aims of this paper are to (i) describe the model, (ii) test the model with observed disease incidence in two chickpea cultivars of different ascochyta resistance, and (iii) perform sensitivity analyses.

Material and methods

The model, parameters, and estimation and/or calibration of parameters

The model, Spread of Ascochyta rabiei on Chickpea (SArC) has two broad components: (i) the initiation, growth and spread of the pathogen (Ascochyta rabiei) on the host (chickpea crop) and (ii) growth of the host associated with the state of the disease (having no other potential physiological constraints). The model operates in a production unit (e.g. a field) which is segregated into smaller units of 1 m² area, henceforth designated a “model-operation-unit”. The relational diagram of the model is shown in Figure 1. The model was written in Mathematica™ (Version 5.2, Wolfram Research Inc.).

SArC is driven by hourly weather variables, air temperature (°C), rainfall (mm), wind speed (m s⁻¹), wind direction (°), standard deviation of the wind direction (°) and the resistance of a chickpea cultivar to ascochyta blight (Table 1). Seedling density per model-operation-unit and initial infection point(s) in one or more model-operation-units are the initialisation variables of the model (Figure 1). The initial seedling density (SeedRate, an initialisation parameter) in a model-operation-unit is defined by the seeding rate of chickpea. A standard seeding rate of 45 seeds per m² was used as the value of this parameter (Day et al., 2006). It was assumed that 40 of these 45 sown seeds would germinate and each seedling would have one growing point initially. In the “AnthracnoseTracer” model, infected seeds are the source of disease initiation; an
infected seed produces an infected growing point at seedling emergence. Infested pieces of stubble replaced infected seeds in SArC. The location of an infected growing point is represented in the model as the row-column co-ordinate of the model-operation-unit in the field. A field is assumed to be facing north as in the conventional cartographical orientation and a column number is designated from south to north and a row number from east to west. An infected growing point is a simplified representation of a lesion, and sporulates after completion of a latent period (LP), a parameter of the model. The latent period is the time between infection and production of sporulating lesions.

The latent period was calculated from laboratory and field observations. A single *A. rabiei* isolate (7706c) was cultured from storage and a suspension of $6 \times 10^5$ spores per ml prepared as described by Kimber *et al.* (2006). Plants of chickpea cultivars Howzat and Almaz were artificially spray inoculated until dripping and plants then placed in a chamber maintained at approximately 100 % relative humidity (RH). The temperature was maintained at approximately 18-20°C. Plants were monitored daily until pycnidia containing conidia were first observed, 9 days (180 degree-days) later for cv. Howzat (moderately susceptible) and 13 (260 degree-days) for cv. Almaz (moderately resistant). In the field, the latent period, from the time of inoculation with infested stubble to observation of pycnidia containing conidia on infected seedlings, was approximately 13 days for both cvs which was translated into approximately 150 degree days using onsite weather data from the Bureau of Meteorology (BOM). Galloway and MacLeod (2002) reported the latent period of *A. rabiei* on chickpea cvs Tyson (susceptible), Sona (susceptible) and Kaniva (highly susceptible) in controlled conditions at 20°C to be 8-9 days (160-180 degree days), RH not specified. Trapero-Casas and Kaiser (1992b) found a latent period of 5.5 days (100 degree days) on cv. Burpee (highly susceptible) at 20°C and
100 % RH. Accordingly, a latent period of 150 degree days was adopted for the SArC model.

The model assumes that each sporulating growing point produces a number of “potentially infective” spores. A potentially infective spore is defined as a spore that has the capability to cause infection on an uninfected growing point in suitable environmental conditions. The number of potentially infective spores produced per sporulating growing point (or sporulating lesion) is the parameter SporeRate of the model (Figure 1). Like the parent model, “AnthracnoseTracer”, the SArC model does not consider in detail the dynamism of lesion formation. Furthermore, SporeRate is assumed to be constant, which implies that a sporulating growing point produces spores at a constant rate after formation. The SporeRate, an arbitrary number, differs between the chickpea cultivars depending on their level of resistance to ascochyta blight. In the parent model, this value was derived relative to that of a known lupin cultivar. In the SArC model, the value of SporeRate for two chickpea cultivars was derived through calibration. For this calibration, a set of values (0.15 to 0.60) with step 0.05 for cv. Howzat (Figure 2A) and for cv. Almaz (Figure 2B) parameter values of 0.050, 0.075, 0.08, 0.10 and 0.15 were chosen to encompass the potential range of sporulation rates. The model was run with each of the parameter values and the outputs were compared with observed field data (described below in validation of SArC model) through mean squared deviation (MSD) statistics (described below in MSD approach). The parameter value for each of the cultivars was finally estimated based on the closest agreement between the model outputs and observation (Figure 2 A and B).

The number of potentially infective spores spread from a model-operation-unit during a wet-hour is linearly related to the total number of sporulating growing points (SporuGP)
present in a given time. The model calculates the expected number of spores available for dispersion (NoSporeDisperse), in the given period, from a model-operation-unit as a random fractional value between 0-1, chosen from a Poisson distribution of the product of SporeRate and SporuGP.

Spread of *A. rabiei* spores, like that of other pathogens such as *Botrytis cinerea* or *Mycosphaerella pinodes* (Setti *et al.*, 2009, Saxena & Johansen, 1997, Taylor *et al.*, 2007), is initiated when favourable leaf wetness conditions are achieved. As leaf wetness data were not readily available, this is represented in the model in a simplified way by using a rainfall threshold of 0.1 mm in each hourly period (WHE) of model operation. In justifying using such a parameter value, in the parent model, Diggle *et al.* (2002) argued that 0.1 mm an hour or approximately 2 mm in a day would provide an adequate period of leaf wetness.

The spores expected to be dispersed are spread independently from each model-operation-unit that contains sporulating growing points (sporulating lesions). The model assumes that this dispersion originates from the centre of each model-operation-unit. The spores landing on the model-operation-unit may originate from the same model-operation-unit (m² quadrat) or from another model-operation-unit within or outside of the production unit (e.g. field). Dispersal of each potentially infective spore (DisperseSpore (angle, distance)) is a displacement vector with angle (θS) and distance (dS) components. This vector specifies the location at which the spore lands relative to its starting point. The actual dispersal is the vector sum of dispersal due to rain splash (DisperseSporeRain (angleRain,distanceRain)), and dispersal due to wind (DisperseSporeWind (angleWind,distanceWind)). The angle of dispersal due to rain splash (angleRain) is a
random number, with uniform probability from $0^\circ$ to $360^\circ$. Wind-induced dispersal angle (angleWind) is chosen from a normal distribution defined by the average wind direction ($^\circ$) in the hour and the standard deviation of wind direction as measured by a recording weather station. The distance component of dispersal due to rain splash (distanceRain in metres) is a random value chosen from a half-Cauchy distribution (Xu & Ridout, 1998) with median dispersal parameter RainDP:

\[
\text{distanceRain} = \text{RainDP} \left( \pi z/2 \right) \quad \text{(Equation 1)}
\]

where $z$ is a uniform random number between 0 and 1. The RainDP is the median of the distribution distance in metres over which spores may travel. The distance component of dispersal (distanceWind) due to wind is calculated in the same way as distanceRain, but the median dispersal parameter, WindDP, is multiplied by the average wind speed (m s$^{-1}$) for the hour. Examples of distributions for the distance component of displacement due to a rain splash, and due to wind at measured wind speeds, are shown in Figure 3, which compares the model output with observations from wind tunnel experiments performed in 2007.

Dispersal of A. rabiei conidia from infested chickpea stubble was examined using a wind tunnel developed at the University of Adelaide (INSKIP Dust and Fume Extraction Pty Ltd). Spores were trapped on strips of clear high tack/medium tack double coated removable repositionable tape (3M™), 5 mm long x 2 mm wide, on 2 mm diameter metal rods. Tape was placed vertically at three positions on the rods (1-6, 11-16 and 31-35 cm). Rods were then placed at various distances along the 1.3 m long by 0.5 m wide observation chamber of the wind tunnel. Wind speeds were set at 1.4, 2.2, 3.3 and 4.7 m
s\(^{-1}\) and applied for 30 minutes at a time. When the experimental design required water, droplets were applied from 4 mm variable flow drippers inserted along an irrigation line inside the tunnel. Tape pieces were peeled from the rods at the end of an experimental run using fine tweezers and the number of conidia per tape counted microscopically. The counts of conidia at the various distances were thus used to estimate the parameter “spore deposition probability”.

The probability that *A. rabiei* conidia will land on a given cm\(^2\) after distribution by wind and rain is shown in Figure 3. The probability of conidia landing on a cm\(^2\) area was high close to the source and greater wind speed increased the probability that conidia would land further from the source. Measured values taken at a wind speed of 1.4 m s\(^{-1}\) indicated that the probability of landing per cm\(^2\) area was highest over 0.35 m from the source, with a probability of 0.007 cm\(^2\), and declined to close to zero at 0.60 m. At 4.7 m s\(^{-1}\), the measured probability of impact per cm\(^2\) at 0.35 m was 0.010 and close to zero at 0.60 m. The model prediction for 1.4 m s\(^{-1}\) showed the probability of impact to be highest closer to 0.25 m with a probability of 0.020 cm\(^2\). This became nearly zero at 0.60 m. The prediction of the impact for 4.7 m s\(^{-1}\) was 0.020 cm\(^2\) at 0.40 m, but did not reach zero, indicating there is always a chance that a conidium will travel over 1.5 m.

It is unlikely that all potentially infective spores will produce infections because the density of receiving growing points in the target model-operation-unit is generally less than the total area of the model-operation-unit. In the model, the spore deposition probability (ProbableSporeDepo) is used to estimate successful infections from the number of potentially infective spores. The ProbableSporeDepo is calculated using an
exponential function similar to that commonly used for estimating the fraction of the incoming radiation intercepted by a crop canopy (Salam et al., 1994).

\[
\text{ProbableSporeDepo} = 1 - \exp (-\text{ProbableSporeLimit} \times \text{UninfectedGP} \times \text{InfectibilityMultiplier}) \quad \text{(Equation 2)}
\]

ProbableSporeDepo is the limit of the probability of deposition of potentially infective spores in units of susceptible growing point per m\(^2\) as the density of uninfected growing points (UninfectedGP InfectibilityMultiplier) approaches 0. The density of susceptible growing points on a square metre surface area is better expressed as susceptible growing point index, similar to leaf area index (LAI). In this study this was approximated by multiplying the uninfected growing points (UninfectedGP) by a multiplier (InfectibilityMultiplier) so that it simulated the pattern of LAI of the crop throughout the growing season. This multiplier (InfectibilityMultiplier) was approximated as 5, which closely matched the pattern of simulated LAI from APSIM (Agricultural Production Systems Simulator) model (module chickpea) runs for Roseworthy, South Australia, 2007 (courtesy of Dr. Imma Farre, Department of Agriculture and Food, Western Australia) (Figure 1 A, Appendix E). The number of susceptible growing points on each day is approximated as the product of InfectibilityMultiplier and the number of growing points formed on that day.

The growth of the chickpea plant is described by the development of growing points within each model-operation-section. Each seed sown, when germinated, produces one growing point which multiplies at a rate that is a function of temperature (GPREEPrate)
and is limited by proximity to a maximum growing point density (GPmax). The number of new growing points (NewGP) in an iteration period of one day, is calculated as:

\[ \text{NewGP} = \text{GPuninfected} \times \text{GPREPrate} \times \text{Temp} \times \left(1 - \frac{\text{GPuninfected}}{\text{GPmax}}\right) \] (Equation 3)

where GPuninfected is the total number of uninfected growing points at the time of iteration, and Temp is the average daily temperature in °C. The parameter GPREPrate is defined as the rate of increase of the number of growing points per degree-Celsius per day (Figure 4 A and B).

Ten plants each of cultivars Howzat (MS) and Almaz (MR) were randomly selected at weekly intervals and the growing points (the number of main stems and the terminal and lateral shoots that developed from the main stems) were counted. Growing points were counted weekly from the start of the growing season, 12 June 2007, until the end of the growing season, 2 November 2007. This gave the estimation of the GPREPrate and GPmax for each cultivar.

The number of growing points over time increased in a sigmoid curve for both cvs (Figure 4). Growing points on cv. Howzat (Figure 4 A) increased at a steady rate until a maximum of 5000 growing points m\(^2\) at 1500 degree days. The correlation between observed values and the model prediction was \(R^2 = 0.97\). Growing point density of cv. Almaz (Figure 4 B) increased exponentially to 1500 growing points m\(^2\) at less than 750 degree days, and reached a maximum of almost 6000 growing points m\(^2\) at 1500 growing
degree days. The observed values and the model prediction were closely correlated ($R^2 = 0.92$).

The model produced an output of the proportion of infected plants in a model-operation-unit, where $N$ is the seedling density at sowing and SporuGP is the number of sporulating growing points, as follows:

$$\text{Infected plants per model-operation-unit} = N - N(1-1/N)^{\text{SporuGP}}$$

Field data for model calibration and validation, and weather data for model operation

The spread of ascochyta blight on cvs Howzat, Almaz and Genesis 090 (Resistant) was studied in the field at Kingsford Research Station (S 34.55, E 138.78), South Australia in 2007 and Turretfield Research Station (S 34.55, E 138.82), South Australia in 2008. The trial in 2007 was termed the calibration plot as information was gathered from this plot to be used in calibration of the model. The trial in 2008 was termed a validation plot as it was only used to validate the model. Stubble with prominent lesions was used as a single source of inoculum placed centrally in each plot in 2007 and 2008. Plots were sown to dimensions 12 x 12 m in 2007 and in 2008, 11 m x 9.5 m, with a gap of 1.75 m between plots.

Plots were observed for disease expression at weekly intervals after placement of inoculum. Disease incidence was assessed each week starting in August, when disease was first observed, until the end of the growing season in November.
In 2007 and 2008, at the newly emerged seedling stage, each plot was subdivided into 1 m$^2$ observation quadrats, each of which contained 40 plants. Each plot was assigned a number, such that quadrat 1.1 was the focus and quadrat number increased in a clockwise direction away from the focus to the south of the plot, as shown in Figure 5. Each plot was assessed in a structured way, beginning at the perimeter (outermost, least infected middle quadrat, designated 5.1) and moving in a clockwise direction to the centre of the plot (most infected centre quadrat, 1.1) which contained the disease focus. Boots were sprayed with 70% ethanol before moving from one quadrat to the next. This sampling method was designed to reduce the potential for mechanical spread of the pathogen. An incidence score (0-100 %) was given as the percentage of the plants diseased of the total of 40 plants per m$^2$ quadrat.

The 2007 field data were used to calibrate the model and the 2008 data was used for model validation. To give uniform analysis the 11 x 9.5 m plot sown in 2008 was analysed over 9 x 9 m. The cultivar Genesis 090 was not modelled as no disease was observed.

Meteorological data were recorded at hourly intervals by an automated weather observation system (AWOS) (Telvent Australia Pty Ltd) at Roseworthy Research Station (S 34.51, E 138.68) situated 10 and 13 km from the site in 2007 and 2008, respectively. The data were accessed from the Climate & Consultative Services, Bureau of Meteorology (BOM), Adelaide, SA and downloaded via the Internet. The data collected comprised date and time, air temperature (°C), percentage relative humidity (% RH), average wind direction (°), standard deviation of wind direction (°), wind speed (m s$^{-1}$) and rainfall (mm).
Model validation and statistical analysis employed

The percentage of plants infected, the main output of the model, was subjected to qualitative and quantitative validation. In qualitative validation, observation and prediction for each m² unit were recorded on the lay-out of the plot; then each unit (m²) was coloured according to five arbitrary categories: Low (0-30% of diseased plants, green); Medium (31-60%, yellow); High (61-80%, purple); and, Very high (81-100%, red).

The quantitative validation was performed for the whole plot (Figure 6 A) and two sections of the plot, inner (orange) and outer (yellow) (Figure 6 B). For this validation, the performance of the model was analysed statistically using a confidence interval, correlation-regression approach (prediction versus observation) and a deviation approach (prediction minus observation). The confidence interval was calculated for each mean at 99.9% (Conover, 1998). The purpose of this analysis was to explore if the range of the true mean between an observation and the corresponding prediction overlapped at very high (99.9%) confidence level. Logistic regression-based statistics were employed, y = 1/[1 + [(1 – y0)/y0] exp(–ct)} (Madden et al., 2007, Zhang et al., 2004). The purpose of this analysis was to identify the differences, if any, between the observation and the model prediction, with the estimated values of the logslopes compared using two tailed t-tests at P < 0.05 (from the student t-distribution table, Freund (1984). This was performed for three dates, 5 September, 3 October and 2 November 2008, for cvs Howzat and Almaz in two scenarios comparing (i) the slopes of the observed and model prediction of the whole plot (Figure 6 A), and (ii) the outer and inner sections of the plot (Figure 6 B). Statistical software, Genstat edition 10.1 (VSNI international Ltd) was used for this analysis.
The deviation-based statistics that were employed were mean squared deviation (MSD) (Equations 5-8).

\[
MSD = SB + SDSD + LCS
\]

Equation 5

\[
SB = \left( \frac{1}{n} \sum_{i=1}^{n} (x_i - y_i)^2 \right)
\]

Equation 6

\[
SDSD = \left( \frac{1}{n} \sum_{i=1}^{n} (x_i - \bar{x}) - \frac{1}{n} \sum_{i=1}^{n} (y_i - \bar{y}) \right)^2
\]

Equation 7

\[
LCS = 2\left( \frac{1}{n} \sum_{i=1}^{n} (x_i - \bar{x}) \frac{1}{n} \sum_{i=1}^{n} (y_i - \bar{y}) (\bar{r} - r) \right)
\]

Equation 8

Where, x is the model output, y is the measurement, xi and yi are the simulated and measured values, respectively, for the i-th from n number of measurements \(\bar{x}\) and \(\bar{y}\) are the means of xi and yi (i = 1, 2...n), and r is the correlation coefficient between the simulation and measurement. MSD has three additive components: (i) squared bias (SB), the first term of the right side of Equation 2; (ii) squared difference between predicted and observed standard deviations (SDSD), the second term of the right side of Equation 2 and (iii) lack of positive correlation weighted by the standard deviations of prediction and observation (LCS), the third term of the right side of Equation 2. MSD measures the total deviation between predictions and observations. The lower the value of MSD, the closer the prediction is to the observation. SB indicates the mean agreement between the model and observation, whereas SDSD and LCS together show how closely the model predicts variability around the mean. There are two sources of this variability, the magnitude of
fluctuation among the n observations and the pattern of the fluctuation across n observations; SDSD and LCS quantify the model’s ability to describe the former and latter variability, respectively. Like the logistic regression approach, this was performed for both cvs Howzat and Almaz in two scenarios, (i) comparing the slopes of the observed and model prediction of the whole plot (Figure 6 A), and (ii) the outer and inner sections of the plot (Figure 6 B).

**Sensitivity analysis**

The parameters considered for the sensitivity analysis in the SArC model are presented in Table 2. These parameters explained development and dispersal of *A. rabiei* on chickpeas and were tested with values below and above the parameter set used in the model. A value 50% below and above of the model parameter set was applied for all but the latent period. The latent period was adjusted to a set value of 50 degree-days above or below the original value because an increase or decrease of a value by 50 degree-days represented varying latent periods reported in literature (Galloway & MacLeod, 2002, Trapero-Casas & Kaiser, 1992a).

**Results**

**Observation versus Simulation – a qualitative comparison**

Figures 7 and 8 (A to C) show the observed development of ascochyta blight on chickpea cvs Howzat and Almaz, in a pictorial scale, on each m² unit in the field, on 5 September, 3 October and 2 November compared with prediction from SArC. In 2008 the model slightly overestimated disease incidence in cv. Howzat at the beginning of the season and underestimated it at the end of the season (Figure 7 A to C). The spread of disease was influenced by the prevailing winds, blowing towards the SE direction. In contrast, disease
on cv. Almaz at the beginning of the season was slightly underestimated by the model but by the end of the season was slightly overestimated (Figure 8 A to C). Again, disease was influenced by wind blowing mainly towards the SE direction. The distribution of disease was patchy on 3 October (Figure 8 B) and 2 November (Figure 8 C); however, the model did not show any such patchiness in the prediction (Figure 8 A to C).

Observation versus Simulation – a quantitative comparison using confidence interval and logistic regression analysis

For both the cultivars, confidence interval (CI) statistics presented in Figure 9 (A to C) reveal that the 99.9% CI for the means for observation and prediction for each of the dates (5 September, 3 October and 2 November 2008) overlap, indicating a lack of significant difference between the observation and the model prediction.

The slopes of the logistic regression for disease incidence across the whole plot showed no significant difference (P > 0.05) between observation and prediction (Table 3) for cvs Howzat and Almaz.

Table 4 shows the slopes of the logistic regression for disease incidence, across the inner and outer sections of the plot, for the observation and prediction in cvs Howzat and Almaz. The model prediction for the inner section differed significantly (P < 0.05) from the observation on 3 October 2008 and 2 November 2008 for cv. Howzat. The same was true for the outer early (5 September 2008) and late (2 November 2008) observations. For the cv. Almaz, slopes observations and predictions were similar for both the inner or outer sections.
Observation versus Simulation – a quantitative comparison using MSD approach

Across the whole validation plot, the difference between observation and prediction for the early measured data (5 September 2008) is evident in Figure 10 (A), which shows the MSD for cvs Howzat and Almaz to be 320 and 153, respectively. Both deviations were largely attributed to LCS i.e. variability, in terms of pattern of fluctuation around the mean. With the subsequent data (on 3 October 2008), deviation between prediction and observation for both cultivars was also largely attributed to LCS. At the final assessment (2 November 2008), the MSD between observation and model prediction was attributed mainly to LCS for cv. Almaz, but to SDSD for cv. Howzat.

In the inner section of the validation plot (Figure 10B), the MSD analysis indicates that differences between observation and prediction for cv. Howzat were due predominantly to LCS. However, the squared bias (SB) also contributed to this deviation in the early observation (5 September 2008) (Figure 10B). In cv. Almaz, the MSD analysis indicates that, for all three observation dates, the difference between observation and prediction was mainly due to LCS; however, in the later two measurements (3 October and 2 November 2008) SB also contributed to MSD.

While the overestimation of plant infection in the outer section of the validation plot when the model was run for both cultivars with data for 3 October 2008 was attributed to LCS, the SB also contributed to a significant proportion of the overall MSD in cv. Howzat (Figure 10 C). With the 02 November 2008 data, the deviation (MSD = 987 for Howzat and 1121 for Almaz) between observation and prediction was similar for both the cultivars (Figure 10 C). Here, the attribution of the deviations (MSD) was not similar for
the two cultivars; the MSD components SDSD and LCS played a leading role in causing the deviation in cv. Howzat and Almaz, respectively.

**Sensitivity of parameter in SArC model**

Figure 11 shows the sensitivity of six parameters of the SArC model in simulating the spread of *A. rabiei* in the field. With respect to relative change in model output in terms of percent plant infection, three parameters appeared to be less sensitive in that they caused variation in model output of about 10% or less. Of these parameters, two (DistanceWind, DistanceRain) related to dispersion of spores by wind and rain, and the other (LP) was latent period. The parameter that drives the model with respect to the probability of deposition of potentially infective spores on a chickpea plant (ProbableSporeDepo) was moderately sensitive to the increased but not the decreased values. The same was true for the parameter SporeRate that denotes the number of potentially infective spores produced per sporulating lesion. The GPREPRate, i.e. the growth of the chickpea plant as described by growing point development, was the most sensitive parameter of the model (Figure 11). An increased and reduced value of this parameter resulted in about 70 and 25% reduction in model output, respectively.

**Discussion**

The SArC model, developed in this study, is the first model simulating the spread of the pathogen *Ascochyta rabiei* and ascochyta blight on chickpeas in a natural environment. The principles and structure of the model are based on the published model “AnthracnoseTracer” (Diggle *et al.* 2002) and other literature (Xu & Ridout, 1998). AnthracnoseTracer, which simulates the spread of anthracnose in lupins, was largely theoretical, therefore, a main focus in developing the SArC model was to acquire
experimental data for estimation and calibration of parameters. The other focus was to validate the model with extensive field data. These aspects have greatly strengthened confidence in the model with respect to its operational and predictive values.

Eight of nine parameters of the model were either estimated or calibrated with laboratory or field experimental data. The exception was the rainfall threshold which was taken from published literature, which reflects the minimum amount of leaf wetness required for germination of spores and successful penetration of host tissue (Diggle et al. 2002).

Three-step model validation, using independent data on percent plant infection recorded three times in the field from each square metre quadrat of a 9 by 9 m plot, was encouraging. Independent data were not used for any processes of development of the SArC model. A pictorial representation, the first step of the model validation employed in this study, was intended to provide a general impression, or snapshot, of the model’s capability of simulating the observation. A similar snapshot of model output was published previously for phoma stem canker of oilseed rape (Aubertot et al., 2006). The present qualitative validation showed that the model, in general, capably predicted the observed spread of ascochyta blight in Howzat and Almaz, two cultivars of chickpea that differed in resistance. This inference is supported in the first part of the two-step model validation, where it was observed that the 99.9% confidence intervals for the mean values of the observations and the predictions overlapped, indicating that the values were likely to be similar (Madden et al., 2007). The second part of the model validation, in which the statistical significance (t-statistics) of the slopes of logistic equations for the observation and prediction was tested, showed that the model agreed with observations for all comparisons on three dates of data recording for both the cultivars, when the average of
the data of the whole validation plot was used. This was also true for comparisons of the inner and outer sections of the validation plot for cv. Almaz, although there was some discrepancy between observation and model prediction for cv. Howzat. The small sample size with the many zeros present in the data, when the plot was analysed in two sections, is likely to have caused overestimation of the effect measure, indicated by the large regression slopes (Nemes et al., 2009). This indicates that this analysis may not be reliable in estimating accurately the slope due to the zeros values present.

A difference or deviation between the model prediction and reality (or observation) is expected, as reality is always simplified in a model, partly because our understanding of basic processes is limited, partly because this enables us to handle the model (Salam, 1992). Furthermore, models in biological systems are working hypotheses and it is not possible to prove hypotheses absolutely in science (Whisler et al., 1986). Thus, modelling is a continuous process aimed at improving predictability. To improve the accuracy of the model’s prediction, a first step should be to identify the discrepancies between prediction and observation. The MSD analysis in the third step of model validation provided the opportunity to locate the major cause of deviation between model prediction and observation. In this study, LCS appeared to be the major deviation between observation and prediction. The LCS denotes the lack of positive correlation weighted by the standard deviations of the prediction and observation; it is one of the three additive components of MSD. Together with SDSD, the LCS shows how closely the model predicts variability around the mean. There are two sources of this variability, the magnitude of fluctuation among the n observations and the pattern of the fluctuation across n observations; the LCS quantifies the model’s ability to simulate the latter variability. One of the reasons that the model could not simulate some of the observed
disease spread may be simplification of the infection process. Unusual patterns of disease spread were occasionally observed in the field. For example, severe disease occurred in the north-east section of the plot of cv. Almaz on 3 October and 2 November (Figure 8, B and C), which was not predicted by the model. The disease observed may have been due to mechanical spread or other phenomena such as gusts of wind. The model simulated less disease in the north-western corner of cv Howzat (Figure 7, B and C) than was observed. Again, the model did not simulate this event, thus further tweaking of the model may lead to closer simulation of these occurrences.

The model is a simplified version of spread and infection caused by A. rabiei in the field and does not encompass every detail of the interaction. For example, it does not take into account the dynamics associated with turbulent winds, continuously changing wind directions and topography, which would add complexity to the model. Gust of winds create turbulence near the leaf surface and can disperse spores upwards to be caught in laminar winds (Sache, 2000); if spores are transported high enough via gusts they can reach the laminar boundary level where they can travel many kilometres (Lacey & West, 2006). The pattern of spore dispersal was studied in a wind and rain tunnel with laminar flow of air, thus further research is needed to estimate dispersal in turbulent winds to model this phenomenon. Moderately resistance cvs of chickpea have also been shown to decrease in resistance as the plants age (Basandrai et al., 2007, Chongo & Gossen, 2001). Reduction of resistance as plants age may explain why the moderately resistant cv. Almaz showed unusual disease occurrence in the north-east section of the plot as the crop matured, thus resistance may need to be adjusted overtime within the model to increase accuracy.
In modelling, certainty in parameters is always an issue. Sensitivity analysis tests how responsive the model is to changes in certain parameters (Whisler et al., 1986). When a parameter is insensitive or less sensitive, for example parameters related to rain and wind-assisted dispersal and latent period in the SArC model, this implies that it is robust, so improving this parameter value will contribute least to performance of the model; also when applying the model in different environments, calibration of this parameter could be a lesser priority. Sensitivity analysis showed the SArC model to be very sensitive to growing point replication rate (GPRepRate) and spore replication rate (SporeRate). The value of the growing point replication rate was calculated using field experiments; therefore, there is little scope to improve this parameter. However, this sensitivity provides a caution that special attention may be required to assign the value of the parameter when the model is applied to an environment different from that in which it was tested. The other sensitive parameter, the spore replication rate, was derived from model calibration. This reflects the degree of resistance to ascochyta blight. It is an important parameter and future research should aim to quantify the productivity of conidia.

Given the strength of the SArC model in the validation and the scope identified for its improvement, it has potential to be used as a tool in plant biosecurity and/or managing ascochyta blight in chickpea. For example, in the field of biosecurity, it may be used to predict the spread of exotic plants pathogens such as anthracnose on lentils (Colletotrichum truncatum), and to study the implications if the disease were to occur in Australia. From a disease management point of view, the model might be used to identify the scale of potential damage, if any. It can also be used to examine the effect of cv. on disease spread and could help farmers decide which cultivars to sow.
This study has produced the first simulation model to predict the spread of *Ascochyta rabiei*. Although some aspects of the model need further investigation to determine applicability in other agroecological regions outside of South Australia, the model will be useful for chickpea growers as an ascochyta management tool and, with further development, could be used to predict exotic plant pathogens with similar dispersal methods.

**Acknowledgements**

We thank the Crop Improvement Group, South Australian Research and Development Institute (SARDI) for chickpea seed and site maintenance. This project formed part of the PhD program of Mr Steven Coventry, with support from the Cooperative Research Centre for National Plant Biosecurity, SARDI, Department of Agriculture and Food Western Australia and the University of Adelaide.

**References**


Table 1 Cultivar description, seed source and ascochyta blight rating of chickpeas used in the field trials at Kingsford in 2007 and Turretfield in 2008.

<table>
<thead>
<tr>
<th>Chickpea cultivar and description</th>
<th>Resistance rating a</th>
<th>Seed source b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Howzat - Desi type, medium to tall height, early flowering, medium size, light brown seed</td>
<td>MS</td>
<td>Balaklava, SA</td>
</tr>
<tr>
<td></td>
<td>MS</td>
<td>Turretfield, SA</td>
</tr>
<tr>
<td>Almaz - Kabuli type, medium height, late flowering, large cream seed</td>
<td>MR</td>
<td>Riverton, SA</td>
</tr>
<tr>
<td></td>
<td>MR</td>
<td>Turretfield, SA</td>
</tr>
<tr>
<td>Genesis 090 - Kabuli type, medium height, mid flowering, medium to small cream seeds</td>
<td>R</td>
<td>Riverton, SA</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>Turretfield, SA</td>
</tr>
</tbody>
</table>

a. MS = moderately susceptible, MR = moderately resistant, R = resistant

b. Seeds were sourced from chickpea breeding trials and commercial trials in South Australia (SA)
Table 2 Parameter sensitivity, comprising the parameter description, the unit, the original model parameter values, and the adjusted range (± 50 % or ± 50). The latent period of 150 degree days was adjusted by ± 50 based on latent periods found in the literature and through experiment.

<table>
<thead>
<tr>
<th>Biological parameter description</th>
<th>Unit</th>
<th>Model parameter value</th>
<th>Tested range</th>
<th>Low</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth of the chickpea plant as described by growing point development</td>
<td>GPREPRate</td>
<td>0.0065</td>
<td>± 50 %</td>
<td>0.00325</td>
<td>0.00975</td>
</tr>
<tr>
<td>The probability of deposition of potentially infective spores on a chickpea plant</td>
<td>ProbableSporeDepo</td>
<td>0.000065</td>
<td>± 50 %</td>
<td>0.0000325</td>
<td>0.0000975</td>
</tr>
<tr>
<td>Distance a spore is transported by rain</td>
<td>DistanceRain</td>
<td>0.015</td>
<td>± 50 %</td>
<td>0.0075</td>
<td>0.0225</td>
</tr>
<tr>
<td>Distance a spore is transported by wind</td>
<td>DistanceWind</td>
<td>0.015</td>
<td>± 50 %</td>
<td>0.0075</td>
<td>0.0225</td>
</tr>
<tr>
<td>The number of potentially infective spores produced per sporulating lesion</td>
<td>SporeRate</td>
<td>0.220</td>
<td>± 50 %</td>
<td>0.11</td>
<td>0.33</td>
</tr>
<tr>
<td>The period of time between infection and production of sporulating lesions</td>
<td>LP</td>
<td>150</td>
<td>± 50</td>
<td>100</td>
<td>200</td>
</tr>
</tbody>
</table>
Table 3 Logistic regression analysis of the observed incidence of ascochyta blight across the whole plot compared to the model prediction of incidence on 5 September 2008, 3 October 2008 and 2 November 2008 for chickpea cultivars Howzat and Almaz. SE ± of the residual are shown for the logit data. The regression slopes were compared for the model and the observation. Significance was determined by student t-distribution table (P < 0.05) from 80 observations.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Date</th>
<th>Model</th>
<th>logSlope</th>
<th>SE ±</th>
<th>t-statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Howzat</td>
<td>5/09/2008</td>
<td>Observation</td>
<td>0.07</td>
<td>0.12</td>
<td>-0.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prediction</td>
<td>0.10</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3/10/2008</td>
<td>Observation</td>
<td>0.21</td>
<td>0.09</td>
<td>-0.39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prediction</td>
<td>0.52</td>
<td>-0.80</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2/11/2008</td>
<td>Observation</td>
<td>0.00</td>
<td>18.32</td>
<td>-0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prediction</td>
<td>0.70</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>Almaz</td>
<td>5/09/2008</td>
<td>Observation</td>
<td>0.08</td>
<td>0.10</td>
<td>-0.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prediction</td>
<td>0.10</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3/10/2008</td>
<td>Observation</td>
<td>0.13</td>
<td>0.10</td>
<td>-0.59</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prediction</td>
<td>0.21</td>
<td>0.08</td>
<td></td>
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<tr>
<td></td>
<td>2/11/2008</td>
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<td>0.17</td>
<td>0.11</td>
<td>-0.56</td>
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<tr>
<td></td>
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<td>Prediction</td>
<td>0.24</td>
<td>0.08</td>
<td></td>
</tr>
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</table>

* indicates significant at P < 0.05
Table 4 Logistic regression analysis of the observed incidence of ascochyta blight across the inner and outer sections of the plot compared to the model prediction of incidence on 5 September 2008, 3 October 2008 and 2 November 2008 for chickpea cultivars Howzat and Almaz. The regression slopes were compared for the model and the observation. SE ± of the residuals are shown for the logit data. Significance was determined by student t-distribution table (P < 0.05) from 24 observations for the inner and 45 observations for the outer sections.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Date</th>
<th>Model</th>
<th>logSlope</th>
<th>SE ±</th>
<th>t-statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Outer</td>
<td>Inner</td>
<td>Outer</td>
</tr>
<tr>
<td>Howzat</td>
<td>5/09/2008</td>
<td>Observation</td>
<td>0</td>
<td>0.06</td>
<td>0.01</td>
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<tr>
<td></td>
<td></td>
<td>Prediction</td>
<td>0.09</td>
<td>0.11</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>3/10/2008</td>
<td>Observation</td>
<td>0.11</td>
<td>0.08</td>
<td>0.03</td>
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<tr>
<td></td>
<td></td>
<td>Prediction</td>
<td>0.07</td>
<td>1.64</td>
<td>0.02</td>
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<tr>
<td></td>
<td>2/11/2008</td>
<td>Observation</td>
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<td>0</td>
<td>0.01</td>
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<td></td>
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<td>Prediction</td>
<td>0.09</td>
<td>5.50</td>
<td>0.02</td>
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<tr>
<td>Almaz</td>
<td>5/09/2008</td>
<td>Observation</td>
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<td>0.08</td>
<td>0.01</td>
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<td></td>
<td></td>
<td>Prediction</td>
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<td>0.04</td>
<td>0.03</td>
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<tr>
<td></td>
<td>3/10/2008</td>
<td>Observation</td>
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<td>0.18</td>
<td>0.70</td>
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<td></td>
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<td>Prediction</td>
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<td>0.08</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>2/11/2008</td>
<td>Observation</td>
<td>0.15</td>
<td>0.23</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prediction</td>
<td>0.06</td>
<td>0.13</td>
<td>0.02</td>
</tr>
</tbody>
</table>

* indicates significant at P < 0.05
Figure 1 Relational diagram of the SARC (spread of *Ascochyta rabiei* on chickpea) model including the development and dissemination of *A. rabiei* in a chickpea field represented by the model. Ovals (blue) represent the parameters of the model identified through calibration or investigation; Bold (orange) ovals represent initialisation variables used to run the model; ovals (yellow) represent information used to drive the simulation process.
**Figure 2** Calibration of the parameter SporeRate for chickpea cultivars Howzat (A) and Almaz (B) for 15 September, 12 October and 2 November 2007. Values of 0.15 to 0.60, with a step of 0.05, were used in calibrating cv. Howzat. Values of 0.050, 0.075, 0.08, 0.10 and 0.15 were used in calibrating cv. Almaz. The parameters which showed closest agreement between model output and observations for each cultivar were applied to the model as the SporeRate parameter. SporeRate is assumed to be constant so that a sporulating growing point produces spores at a constant rate after formation. The SporeRate is an arbitrary number.
Figure 3 The measured and predicted probability that conidia of *A. rabiei* will land on a given cm² area in the presence of rain plus wind at 1.4 to 4.7 m s⁻¹. Measured data were obtained from wind and rain tunnel experiments and the prediction was calculated via the equation, distanceRain = RainDP (π z/2), where z a uniform random number on the interval between 0 and 1.
Figure 4 (A) and (B) The growing point density of chickpea cvs Howzat (A) and Almaz (B) per m\(^2\). Growing point development was recorded at Kingsford, South Australia in 2007. Growing points (main stem development and lateral shoot development) were calculated using growing degree-days (number accumulated degree-days above the base temperature taken from first infection observation). The line (-) indicates the model prediction and the symbol (*) indicates the measured values of growing points.
Figure 5 Disease assessment strategy for each cultivar in field trials. Quadrats, 1 m$^2$, were assigned numbers as follows: 1.1 for the centre quadrat; 2.1 to 2.8, in a clockwise direction for the next quadrats; 3.1 to 3.16 in a clockwise direction for the next quadrats; 4.1 to 4.24 in a clockwise direction for the next quadrats; 5.1 to 5.32 in a clockwise direction for the next quadrats. Dashed arrows from 5.1 to 1.1 and back to 5.1 indicate entry and exit points used when assessing disease. Solid arrows show the pattern in which disease was assessed, proceeding in a clockwise direction.
**Figure 6 (A)** Statistical design for the 9 x 9 m plot to compare the accuracy of the observed and the model prediction output for cvs Howzat and Almaz on the whole plot level (dotted shading) and **(B)** over two sections of the plot, the inner (dotted) and outer (checked shading) of the plot.
**Figure 7** The percentage of plants infected per m² in a 9 x 9 plot planted with cv. Howzat.

Observation and prediction on (A) 5 September 2008, (B) 3 October 2008 and (C) 2 November 2008.
**Figure 8** The percentage of plants infected per m² in a 9 x 9 m plot planted with cv. Almaz. Observation and prediction on (A) 5 September 2008, (B) 3 October 2008 and (C) 2 November 2008.
Figure 9 (A to C) Comparison of the observed (Obs) and modelled (Pre) mean plant infection (%) on 5 September, 3 October and 2 November 08, across (A) the whole plot, (B) the inner section of the plot and (C) the outer section of the plot. The bars indicate means and the lines indicate 99.9% confidence intervals.
Figure 10 (A to C) Comparison of mean squared deviation (MSD) and its components; lack of correlation (LCS), weighted by the standard deviations (SDSD), and squared bias (SB), for the observed and predicted plant infection (%) on 5 September, 3 October and 2 November 2008 in cvs Howzat and Almaz. Performed over (A) the whole plot, (B) inner section of the plot and (C) outer section of the plot.
Howzat Almaz

Mean squared deviation (MSD)

Date of observation per cultivar

LCS
SDSD
SB

0
300
600
900
1200
1500
1800
2100
5-Sep-08
3-Oct-08
2-Nov-08
5-Sep-08
3-Oct-08
2-Nov-08

Date of observation per cultivar

(C)
**Figure 11** Sensitivity analysis indicating parameters most affected by a change of ± 50% from the original parameter value.
Chapter 8 : General discussion

The aims of this project were achieved through: (i) identifying the influence of temperature and relative humidity (RH) on germination of *A. rabiei* conidia and their potential as long distance dispersal structures (ii) quantifying horizontal dispersion (in terms of distance and number) of conidia of *A. rabiei*, by exposing infested stubble to wind of 1.4 to 4.7 m s$^{-1}$ in the presence and absence of rainfall events of 2 mm h$^{-1}$ in a wind and rain tunnel; such environmental conditions being typical of South Australian winters; (iii) observing the influence of prevailing wind, rain and cultivar on the rate and distance of spread of ascochyta blight in chickpea in field experiments in three growing seasons; and (iv) developing and validating a weather-driven simulation model, to predict the spread of *A. rabiei* in the field in natural conditions.

Previously, the epidemiology of ascochyta blight and the factors favouring disease development on chickpeas had been investigated; however, gaps remained in the understanding of aspects of the development of the disease and specifically on prediction of its spread (Pande *et al.*, 2005). This study addressed some of those gaps and has revealed new information about the effect of the environment, in particular the influence of temperature, RH, rainfall and wind, on survival and dispersal of conidia of *A. rabiei* and the development of ascochyta blight on chickpea. Furthermore, a spatio-temporal model was developed and successfully tested to predict the spread of ascochyta blight in the field. The model is driven by hourly local weather data, namely air temperature (°C), rainfall (mm h$^{-1}$), wind speed (m s$^{-1}$), wind direction (°) and standard deviation of the wind direction (°).
Investigation of the influence of temperature and RH indicated that conidia of *A. rabiei* can withstand severe conditions and retain the ability to germinate. This was the first study to investigate the survival of *A. rabiei* conidia by initially applying conidia to a dry, inert medium (nitrocellulose membranes), exposing them to various conditions of temperature and RH, and subsequently observing the germination of conidia on RWA. This approach was aimed to simulate the conditions to which conidia may be exposed when transported via wind over long distances before landing on a host. Conidia of *A. rabiei* could germinate after being exposed to a maximum temperature of 40 °C for up to 8 h. Conidia stored on dry membranes at 20 and 25 °C, over a period of 2 to 192 h, germinated well (30 -100%) when applied to RWA. However, germination decreased to zero following incubation for 240 h. This indicates that transport by wind without moisture for long periods is detrimental to germination, but conidia transported for shorter periods may still germinate if subsequently exposed to suitable conditions. When conidia were exposed to 20 °C on membranes at 12.5% RH (the lowest RH tested) for 96 h, more than 50% germinated when transferred to RWA. Germination of between 90 and 75% was observed after 96 h at the other RH values tested (100 - 44%), and decreased with decreasing RH. Therefore, conidia could potentially survive long distance dispersal when exposed to extreme conditions of temperature and RH, and retain the ability to germinate. This implies that even a few conidia transported to adjacent fields of chickpea by wind could be capable of infecting the crop. If caught on strong wind currents, conidia may be distributed many kilometres and remain viable for at least one week, especially if conditions are optimal (20 - 25 °C with 85 - 90% RH). Long distance atmospheric transport of inoculum is presumed to be one of the causes of incursions of plant pathogens from distant sources (Aylor, 2003) and the findings of the present study support the idea that, in Australia, conidia may have infected crops many kilometres from...
the source of inoculum. It was originally proposed that seed infection may have caused an outbreak of the disease in Western Australia in 1998 and 1999 tens to hundreds of kilometres from the presumed source but this was considered unlikely as disease was observed on all the cultivars. In the absence of ascospores, conidia on wind-blown microtrash were initially considered to have provided the inoculum. Subsequently, small numbers of ascospores were identified on stubble by Galloway et al. (2003) and it is currently believed that ascospores may have been the cause of this outbreak (B. MacLeod, pers. com. May, 2011). The present study adds support to the original hypothesis of dispersal via conidia and could explain the dispersal of the pathogen over long distances in parts of Australia where ascospores have not been observed. While ascospores have been observed in laboratory conditions in Western Australia, it is probable that they originate from a low level of homothallic compatibility (Galloway & MacLeod, 2003) because, to date, only one mating type has been identified in Australia (Phan et al., 2003, Leo et al., 2011). This leads to the assumption that the asexual conidial stage is predominant. Microscopic spores are difficult to detect in the field, particularly at low concentrations, thus assumptions about movement of the spores over extended distances has relied on local appearance of disease symptoms (Aylor, 2003). Although dispersal by ascospores cannot be discounted, this study indicates that conidia may act as a means of long distance dispersal.

Spores disseminated by wind over long distances are often exposed to high levels of UV radiation in the atmosphere and tend to have high mortality rates (Lacey & West, 2006). Although the effect of different wavelengths of near UV light and blue light on germination of conidia of A. rabiei has been studied in the laboratory (Kaiser, 1973), there is a lack of information in the literature on the effect of natural UV on survival of A. rabiei conidia. Investigation of the effect of natural UV on conidial survival would help
to clarify if conidia could survive during long dissemination in air, perhaps in wind and
dust, and subsequently initiate epidemics far from their original source.

Investigation of the dispersal of conidia of *A. rabiei* in a purpose-built wind and rain
tunnel (Chapter 5) showed that conidia could travel at least 66 cm in the presence of wind
alone or with rain at wind speeds up to 4.7 m s\(^{-1}\). Rain caused more conidia to be
dispersed from pycnidia than did wind alone, because of the splash effect. Hundreds of
conidia were trapped close to the inoculum source due to wind alone, but thousands were
trapped in the presence of wind and rain. Rain also influenced vertical dispersal of
conidia, in that conidia were trapped 31 - 36 cm above the starting point whereas none
were trapped at this height in the presence of wind alone. This is the first report of
observation and quantification of conidia of *A. rabiei* on traps to determine the distance
and height to which these propagules travel in the presence of wind and rain. Such
information has improved our understanding of the dispersal of these structures.

Further study is needed on the dispersal of conidia by winds stronger than, and over
greater distances than those tested in this research. Evidence from wind tunnel
experiments and observations from field studies suggested that conidia may be distributed
considerably further by stronger winds. The limitation of the wind tunnel in producing a
maximum wind speed of 4.7 m s\(^{-1}\) meant that it was not possible to examine the effect of
wind up to 20 m s\(^{-1}\), which was observed in the field. Dispersal of conidia in rain of
intensity just under 2 mm h\(^{-1}\), which is typical of winter rain events in South Australia,
was also studied. Further experiments with rainfall events of greater and lesser intensity
would show the effect of varied rainfall events on distribution and dissemination of
conidia. Such research may identify the minimum rainfall intensity needed to induce
release of conidia from pycnidia and any relationship between intensity of rain and number of conidia released from infested stubble.

The techniques used in this study for trapping and counting spores were time-consuming and required substantial microscopic evaluation and a trained eye. Rogers et al. (2009) have developed PCR techniques to quantify airborne inoculum of *Sclerotinia sclerotiorum* trapped on wax-coated plastic tape used in Burkard spore traps. These new techniques are capable of detecting DNA from as few as two ascospores. Similar PCR techniques for early surveillance of wind and rain borne pathogens are also being developed at SARDI and the University of Adelaide, for pathogens such as *A. rabiei*, *L. maculans* and *M. pinodes* (B Vogelzang, SARDI, pers. com. January, 2011). These developments will, in the future, allow quantification of spores without the need for microscopic evaluation, which would significantly reduce the time required to identify and quantify spores in air samples. Such techniques would be useful in future experiments to study the effect of stronger winds and varying rainfall on spore dispersal.

The spread of ascochyta blight, initiated by wind and rain from a single focus, on several cultivars of varying resistance was studied over three years (2006 and 2008, Chapter 6). A novel approach of using infested stubble with lesions to initiate infection was used to simulate the natural infection process in 2007 and 2008. The rate of spread of disease and distance of spread were then analysed via logistic regression, which showed the influence of rainfall, wind and cultivar on disease progress.

This study suggested that the plant age and planting density may have been contributing to an increased rate of disease over time and space in the field experiments, particularly in the later part of the season. Studies by Basandrai et al. (2007) and Chongo and Gossen
(2001), investigating the effect of plant age on ascochyta blight resistance in chickpea, have confirmed that as moderately resistant and resistant chickpea cultivars age their resistance to ascochyta blight diminished. However, moderately susceptible and susceptible plants showed little difference in disease with plant age because they were already susceptible at all stages. From this study, the rate of increase in disease severity in the moderately susceptible cultivar late in the season may be a result of canopy density and not plant age. Studies have shown that canopy density of pea crops affects the ability of *M. pinodes* to infect plants, with a less dense canopy making it harder for conidia to land and infect peas, and this may be what was also observed in these trials. Perhaps there are three key factors contributing to the increase in disease throughout the season, namely plant age, canopy density and also the prevailing wind, which distributed the pathogen in particular directions. The influence of plant age and canopy density needs to be studied in chickpeas in Australian conditions and with Australian isolates. Future replicated field trials would identify the role these factors (plant age or canopy density) in the increased rate of infection over time and space in these trials, particularly in the later part of the season. Further to these experiments, the field trials need to be performed on a larger scale than was tested here to determine if the pathogen could have been distributed over greater distances than were tested here.

The accumulation of information from the laboratory and field experiments facilitated identification of the key parameters needed to modify the AnthracnoseTracer model (Diggle *et al.*, 2002) to develop the “spread of ascochyta rabiei in chickpea (SArC) model”. Field trials in 2007 allowed for calibration of the parameters of the model. Field trials in 2008 facilitated validation of the model by running it with local weather data and then comparing the predictions with the observed field data using statistical methods (logistic regression, confidence intervals (CI) and MSD statistics). It may be mentioned
that regression analysis is a tool commonly used in plant pathology (Madden et al., 2007). This approach often involves regression of the (y) measurement on the (x) observation, but the assumption that (y) is linearly related to (x) is not guaranteed (Kobayashi & Salam, 2000). The use of MSD statistics and its components, squared bias (SB), squared difference between standard deviation (SDSD) and lack of correlation (LCS), is not a common practice in plant pathology, but is a powerful technique, helpful to identify differences between the model and the predictions (Salam et al. 2003). MSD allows quantification of the deviation of the observed values from the model’s predictions, whereas regression is not ideal for this type of comparison. MSD thus allows comparison of the observation to the prediction and not just the fit of the model to the measurement as per the regression analysis.

The regression analysis, CI and the MSD showed the closeness of fit of the observed data to the model prediction, particularly across the whole plot. However, MSD helped to interpret the deviation via the three additive components, LCS, SDSD and SB. This made the differences in the estimation between the observation and model identifiable. Such knowledge then allows insight into measures to correct the deviation through further experimentation or other methods to improve the predictive ability of the model.

This type of predictive spatio-temporal model is, to our knowledge, the first to be developed for ascochyta blight of chickpea. The SArC model developed here is considered to be an improvement on the AnthracnoseTracer model, because it was developed through direct investigation of the parameters which influence disease spread and relies upon less literature and calibration than the AnthracnoseTracer model (Diggle et al. 2002). The output for the ascochyta model was designed to colour-code disease severity from cool (least infection) to warm (most infection). This coloured output helped
to visually identify areas of the most severe disease, potentially allowing targeted management strategies to be applied.

The model, which correlated closely to observations of disease incidence in South Australian conditions, particularly over the whole plot, is now being tested in different agro-geological zones with varying weather patterns, such as southern Queensland, where an outbreak of ascochyta blight occurred in 2009 (M Salam, DAFWA, pers. com. October 2010). Testing the model in an environment such as Queensland, where rainfall is generally more intense than South Australia and weather patterns differ, will help to determine if the parameters set for the South Australian conditions are applicable, and identify which, if any, parameters that may need to be modified to give the best fit for other zones.

The likelihood that conidia can travel across long distances and cause infection could be tested by applying the methods of observation and modelling described here, with much larger plots in a variety of conditions. These tests should be observed and modelled as per this study. The many factors involved in longer distance dispersal, including changing winds, turbulence and topography of the landscape, which may add complexity to the model need to be addressed. In this study, the wind tunnel and field experiments did not take into account the effects of turbulence. Wind tunnels produce laminar flow at lower wind intensities but such laminar air may only partially represent nature, thus continued field studies and modelling of turbulence are needed to understand this phenomenon, especially in relation to long distance dispersal. In a natural wind, turbulence is common and causes spores to be distributed like a plume, which dilutes downwind (McCartney & West, 2007). Turbulence makes it difficult to quantitatively define dispersal distance and this is why spore concentration or gradients are used to describe how spore density
changes away from the host (McCartney & West, 2007). Turbulence is likely to be important in picking up conidia of *A. rabiei* from the host, and incorporating them into the turbulent boundary layer above the canopy, where stronger winds may distribute them many kilometres. McCartney & West (2007) stated that most fungal spores probably travel across relatively short distances, but if incorporated into the planetary boundary layer they may travel across extensive distances. Turbulence is most common in unstable atmospheric conditions, which occur during sunny afternoons (McCartney & West, 2007), which are common in southern Australia.

Recently, David Savage, a PhD student of the Cooperative Research Centre for National Plant Biosecurity, investigated long distance dispersal modelling and its efficiency. Using only wind data and spore characteristics of *L. maculans*, Savage *et al.* (2011) constructed (directionally dependent) anisotropic kernels, which describe the expected distribution of spores over time, and which provide reasonable predictions of the spread of spores over long distance. This approach is an improvement over current (uniformly directional) isotropic kernels, providing a more informative prediction. Such dispersal kernels, once constructed, offer templates that describe dispersal, and they can be integrated into other epidemiological models (Travis *et al.*, 2010, Diggle *et al.*, 2002). Thus the kernels produced by Savage *et al.* (2011) could be used to improve the SARC model and adapt it for studying long distance dispersal in the future.

The SArC model offers a new tool for an integrated disease management approach to preventing outbreaks of disease. It can be utilised to influence decision making for planting times, spray application and cultivar selection, allowing prevention of epidemics of ascochyta blight of chickpea. Using local weather data, a simulation could be run with parameters for various cultivars to identify if disease is likely to affect a particular
cultivar and, if so, in what direction and at what severity, through a coloured output map. Growers could then judge which cultivar to sow or what spray options may be best to eradicate low, medium or high infection and where to apply targeted applications. Such a predictive tool would allow the farmer to improve the timing of operations such as sowing and spraying, thereby reducing unnecessary chemical application and associated costs as well as benefiting the environment.

Although cultivars with partial resistance to ascochyta blight, such as Genesis 090, are now being grown in Australia and the pathogen population currently exhibits very limited genetic diversity (Phan et al., 2003, Leo et al., 2011), it is still possible that epidemics may occur in future. Endemic genotypes of the pathogen may mutate to overcome resistance genes, or new genotypes of A. rabiei, including those belonging to the second mating type, may be introduced and become established in the country.

Australia is no longer an isolated continent, in a biosecurity sense, and is susceptible to incursions by new pathogens, including new genotypes of A. rabiei. The Cooperative Research Centre for National Plant Biosecurity and its early surveillance program, including modelling techniques to predict pathogen incursion and spread, are a vital step in protecting Australian agriculture and environment. Research being conducted in, or planned by, the Cooperative Research Centre for National Plant Biosecurity on long distance dispersal and novel surveillance techniques for exotic plant pathogens, will help to prepare for and to manage epidemics of endemic and exotic plant disease in Australia (Jackson & Bayliss, 2011). This SArC model, with further development, may be used as a tool in Australian biosecurity to predict the spread of other threatening exotic plant pathogens which are spread by wind and rain, such as Colletotrichum truncatum, which causes anthracnose on lentils, and C. lupini which causes anthracnose of lupins (Plant
Health Australia, 2009). Although the SArC model was developed for ascochyta blight and is best suited to crops such as peas and lentils, which differ from lupins in architecture, it could be applied in conjunction with the AnthracnoseTracer model to predict the spread of \textit{C. lupini}. The continued development of such predictive tools is vital to the security of the agricultural sector in Australia and internationally to prevent epidemics.

In conclusion, this thesis has contributed to knowledge of the factors that affect the spread of ascochyta blight of chickpea. It has also produced a working simulation model that can predict the spread of ascochyta blight in a chickpea crop using local weather data as the driver. This study paves the way for further development of a model that can be used in plant biosecurity to predict the spread of exotic plant pathogens by wind and rain.
References


Appendices

Appendix A (Media and Reagents)

**Streptomycin Potato Dextrose Agar**
- PDA (Difco) 39 g
- RO water 1000mL
- 10% Streptomycin Sulphate (Sigma) 1mL

**Reverse Osmosis Water Agar**
- Bacto-agar (Difco) 6g
- RO water 300mL
- 1% Streptomycin Sulphate (Sigma) 3mL

**Lacto- glycerol**
- Lactic acid (Sigma) 100mL
- Glycerol (Crown scientific) 100mL
- Water 300mL

Add to a glass beaker and stir well with glass stirrer

Culture media were autoclaved at 121 °C for 20 min and, in a sterile laminar flow unit, 1% streptomycin sulphate antibiotic was added to molten media, once cooled to approximately 50 °C. Agar media were dispensed into 9-cm diameter Petri dishes, approximately 15mL per plate, and allowed to cool and solidify.
Appendix B (Field sites, wind and rain tunnel studies and harvest data)

Figure B1: The University of Adelaide, Waite Campus, Birdproof enclosure with chickpea cv. Howzat sown in a 90 x 30 m plot. Spray irrigation (white circles) was applied to promote disease. Black netting visible above the trial.

Figure B2: Kingsford 2006, with three plots (12 x 12 m) sown with cv. Howzat. Red flags visible through the canopy, highlighted by white circles, showing some transect placement. Transects were placed in N, NE, E, SE, S, SW, W, NW directions.
**Figure B3:** A central seedling and sprayed with inoculum suspension (6 x 10^5 spores per mL^-1) at Kingsford Research Station, 2006. A transparent plastic sheet is pictured around the centre seedling and another was used to cover the seedling when inoculating.

**Figure B4:** White pot tags used in the field in 2007 and 2008 to mark out 1 m^2 quadrats.
Figure B5: Aerial photograph of Turretfield trial in mid-September 2008, showing cv. Almaz (MR) (top plot), cv. Genesis 090 (R) (middle plot) and cv. Howzat (MS) (lower plot). The black dotted line across the centre of each plot indicates how the plot was divided for harvest. Harvest took place at the end of November 2007 and 2008. The harvested seeds were then processed at the University of Adelaide, Waite Campus, South Australia and weighed.

Figure B6: Seeds weights obtained from harvested chickpea cvs Howzat, Genesis 090 and Almaz from Kingsford 2007 and Turretfield 2008. Plots in each year (2007 and 2008) were harvested, in two sections, E and W, as shown above in Figure B5.
Appendix C (Fitted and observed spatial graphs)

Figure C1: Fitted model (red) and observed relationship (blue crosses) for disease severity (percentage of individual plant infected) over 4 m in the (A) east, (B) north, (C) south and (D) west direction from the disease focus in cv. Howzat 11 September 2007.
Figure C2: Fitted model (red) and observed relationship (blue crosses) for disease severity (percentage of individual plant infected) over 4 m in the (A) east, (B) north, (C) south and (D) west direction from the disease focus in cv. Howzat 5 October 2007.
Figure C3: Fitted model (red) and observed relationship (blue crosses) for disease severity (percentage of individual plant infected) over 4 m in the (A) east, (B) north, (C) south and (D) west direction from the disease focus in cv. Howzat 2 November 2007.
Figure C4: Fitted model (red) and observed relationship (blue crosses) for disease severity (percentage of individual plant infected) over 4 m in the (A) east, (B) north, (C) south and (D) west direction from the disease focus in cv. Almaz 11 September 2007.
**Figure C5:** Fitted model (red) and observed relationship (blue crosses) for disease severity (percentage of individual plant infected) over 4 m in the (A) east, (B) north, (C) south and (D) west direction from the disease focus in cv. Almaz 5 October 2007.
**Figure C6:** Fitted model (red) and observed relationship (blue crosses) for disease severity (percentage of individual plant infected) over 4 m in the (A) east, (B) north, (C) south and (D) west direction from the disease focus in cv. Almaz 2 November 2007.
**Figure C7:** Fitted model (red) and observed relationship (blue crosses) for disease severity (percentage of individual plant infected) over 4 m in the (A) east, (B) north, (C) south and (D) west direction from the disease focus in cv. Howzat 5 September 2008.
**Figure C8:** Fitted model (red) and observed relationship (blue crosses) for disease severity (percentage of individual plant infected) over 4 m in the (A) east, (B) north, (C) south and (D) west direction from the disease focus in cv. Howzat 3 October 2008.
**Figure C9:** Fitted model (red) and observed relationship (blue crosses) for disease severity (percentage of individual plant infected) over 4 m in the (A) east, (B) north, (C) south and (D) west direction from the disease focus in cv. Howzat 2 November 2008.
Figure C10: Fitted model (red) and observed relationship (blue crosses) for disease severity (percentage of individual plant infected) over 4 m in the (A) east, (B) north, (C) south and (D) west direction from the disease focus in cv. Almaz 5 September 2008.
Figure C11: Fitted model (red) and observed relationship (blue crosses) for disease severity (percentage of individual plant infected) over 4 m in the (A) east, (B) north, (C) south and (D) west direction from the disease focus in cv. Almaz 3 October 2008.
Figure C12: Fitted model (red) and observed relationship (blue crosses) for disease severity (percentage of individual plant infected) over 4 m in the (A) east, (B) north, (C) south and (D) west direction from the disease focus in cv. Almaz 2 November 2008.
Appendix D (Fitted and observed temporal graphs)

**Figure D1:** Fitted model (red) and observed relationship (blue cross) for quadrats (A) 1.1, (B) 2.7, (C) 3.3, (D) 3.7, (E) 3.11, (F) 3.15, (G) 4.1, (H) 4.13, (I) 4.24, (J) 5.5, (K) 5.13, (L) 5.21, (M) 5.25, (N) 5.29 and (O) 5.30 in cv. Howzat taken from the temporal analysis of plant severity for 40 plants per quadrat, rated on a scale from 0 to 100 %, beginning when disease was first observed on 15 August 2007 until the end of the chickpea season on 2 November 2007. The Y axis indicates the proportion of severity from 0 to 100% and the x axis represents the date (serial value format).
**Figure D2:** Fitted model (red) and observed relationship (blue cross) for quadrats (A) 1.1, (B) 2.7, (C) 4.24, (D) 5.25 and (E) 5.30 in cv. Almaz taken from the temporal analysis of plant severity for 40 plants per quadrat, rated on a scale from 0 to 100 %, beginning when disease was first observed on 15 August 2007 until the end of the chickpea season on 2 November 2007. The Y axis indicates the proportion of severity from 0 to 100% and the x axis represents the date (serial value format).
Figure D3: Fitted model (red) and observed relationship (blue cross) for quadrats (A) 1.1, (B), 3.3, (C) 3.7 , (D) 3.11 , (E) 3.15 , (F) 4.1 , (G) 4.13, (H) 5.5, (I) 5.13 , (J) 5.25 and (K) 5.29 in cv. Howzat taken from the temporal analysis of plant severity for 40 plants per quadrat, rated on a scale from 0 to 100%, beginning when disease was first observed on 3 August 2008 until the end of the chickpea season on 2 November 2007. The Y axis indicates the proportion of severity from 0 to 100% and the x axis represents the date (serial value format).
Figure D4: Fitted model (red) and observed relationship (blue cross) for quadrats, (A) 1.1, (B) 3.11, (C) 3.15, (D) 5.21 and (E) 5.29 in cv. Almaz taken from the temporal analysis of plant severity for 40 plants per quadrat, rated on a scale from 0 to 100%, beginning when disease was first observed on 3 August 2008 until the end of the chickpea season on 2 November 2007. The Y axis indicates the proportion of severity from 0 to 100% and the x axis represents the date (serial value format).
Appendix E (Susceptible growing point index compared to leaf area index at field trials in South Australia, 2007)

Figure E1: The susceptible growing point index (SGI, red line) used in the SArC model at days after sowing (DAS) compared to the average Leaf Area Index (LAI, green line) for Agricultural Production Systems Simulator (APSIM) runs for chickpea at Roseworthy, South Australia 2007.