The relationship between natural rain intensity and Ascochyta blight in chickpea development

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Abstract

Ascochyta blight is one of the most devastating diseases of chickpea worldwide. In Australia, Ascochyta blight management strategy in a standing crop is solely based on applying protective fungicides before a forecast rainfall event. Despite this, studies on the likely interaction between variable natural rain amount, rain duration, environmental factors and Ascochyta blight development are rare. We used generalised linear mixed models to investigate the relationship between rain intensity, wind speed and Ascochyta blight development. Briefly, 7 g of infested chickpea residue were placed at the soil surface in a 1 m² plot, and three pots (3 trap plants per pot) of a susceptible chickpea cultivar were randomly placed on each side of the 1 m² plot (total 12 pots), preceding a forecast rainfall event. Trap plants were transferred to a controlled temperature room (20 °C) for 48 h (100% humidity) after rain events. After a 48 h incubation period, trap plants were transferred to a glasshouse (20 °C) to allow lesion development. The number of lesions on all plant parts were counted after two weeks. Lesions developed in rain amounts as low as 1.4 mm and rain durations as short as 0.7 h. The number of lesions significantly increased with increasing rain amount. There was positive effect of increasing rain duration and a negative effect of increasing wind speed. This study suggests that small rain amounts, shorter duration rains or a limited amount of primary inoculum are not barriers to conidial dispersal or infection.

Keywords: splash dispersal, spore dispersal, quantitative epidemiology, *Ascochyta rabiei*, rain splash, conidial spread

Introduction

Chickpea (*Cicer arietinum*) is the second most important crop globally with the production value of more than \$7.6 B between 2014–2016 (FAOSTAT, 2018). Ascochyta blight caused by *Ascochyta rabiei* (syn. *Phoma rabiei*) is the major biotic constraint to chickpea production worldwide (Pande et al., 2005, Nene, 1982). Yield losses from Ascochyta blight can be up to 100% in susceptible cultivars under favourable conditions (Nene, 1982). *Ascochyta rabiei* is known to survive on infested residue, infected seed and volunteer chickpea plants (Pande et al., 2005). The survival period on infested residue is up to 2 years when infested residues are at the soil surface, but viability is lost within 5 months when infested residues are 5–40 cm deep in soil (Kaiser, 1973). Infested residue and volunteer chickpea plants serve as the main source of inoculum for disease initiation, and even less than 1 % infested residue can result in serious yield losses under conducive conditions (Kaiser, 1992). In Australia, 100% control of seed borne infections can be achieved via commercial seed treatment; therefore infected seeds are not considered important in Ascochyta blight epidemiology unless growers are sowing untreated or poorly treated seed (Kevin Moore's unpublished data). *Ascochyta rabiei* is heterothallic, however only one mating type has been found in Australia (Leo et al., 2016, Mehmood et al., 2017). The sexual stage/spores of the fungus are absent in Australia, and thus conidia are the only spore type involved in the disease cycle (Coventry, 2012). Infection and disease development are favoured by temperatures between 10–30 °C with optimum 20 °C (Kaiser, 1973), relative humidity > 95 % (Nene, 1982), and wetness period of at least 6–10 h (Moore et al., 2016). To date, there is no resistant variety available against Ascochyta blight in Australia. All varieties are susceptible to moderately susceptible (<https://www.nvtonline.com.au/>).

Rain is required to spread *A. rabiei* conidia, heavy dew or high relative humidity alone will not cause a significant disease spread (Moore et al., 2016). Briefly, pycnidia imbibe moisture during rain and conidia within pycnidia are exuded through the ostiole in a cirrus by hydrostatic pressure. When rain drops strikes a cirrus, the kinetic energy in rain drops

break up the conidial mass and disperse conidia to nearby plants, thus spreading disease (Coventry, 2012). The amount of kinetic energy varies with the size of rain drops, rain duration and intensity (Sache, 2000). There is no information in relation to *A. rabiei* conidial spread, but larger raindrops have been reported to carry more wheat rust spores and vice versa (Sache, 2000). *Ascochyta rabiei* conidia are primarily spread by rain splash up to 1 m (Kimber, 2002), but a recent study showed that conidia travel at least 75 m in wind driven rain (Khaliq et al., 2020). Conidia can also be washed off from upper leaves to lower leaves in a wash off effect, or can be scrubbed from aerosols (liquid suspension of conidia in air) when falling rain drops pass through the aerosol and conidia are deposited on chickpea tissues (Coventry, 2012).

There are significant gaps in understanding the epidemiology of *Ascochyta* blight due to the historic emphasis on developing resistant cultivars and chemical control of the disease (Pande et al., 2005). Rain is an environmental variable with many intrinsic characteristics, such as amount and duration (Pruppacher & Klett, 1978). Spore dispersal in splash dispersed pathogens is linked to the intrinsic characteristics of rain (Madden et al., 1996). Studies on the relationship between the intrinsic characteristics of natural rain and *Ascochyta* blight development are rare; however, studies conducted on other plant pathogens can provide useful insights into understanding the relationship between the intrinsic characteristics of natural rain and *Ascochyta* blight development. A positive relationship between conidial spread and an increasing rain duration has been established for a splash dispersed pathogen, *Colletotrichum acutatum* (Ntahimperera et al., 1997). Therefore, a positive relationship between an increased rain duration and *A. rabiei* conidial spread is plausible. Since spread of splash dispersed pathogens depends directly on rain, a positive association between an increased rain amount and *A. rabiei* conidial spread is plausible. As *A. rabiei* conidia are mainly dispersed by a rain splash, a minimal or no association between conidial spread and wind speed is also plausible.

In Australia, chickpea growers' *Ascochyta* blight management strategy in a standing crop is solely based on applying fungicides before a forecast rain event. Almost all of the

fungicides used are protectants, which protects chickpea from *Ascochyta* blight infections for about 14–18 days when applied before a rainfall event (Pritchard, 2000). Protective fungicides are recommended as a main disease management strategy, and post infection fungicides have been found ineffective unless applied within 48 h of the rain starting (when paddocks are often too wet for ground rigs) (Moore et al., 2019). Australian chickpea farmers often have to start applying fungicides several days before a rain event because their paddocks are often larger than 1000 ha (a 24 m ground rig travelling at 20 kph will cover about 380 ha in 8 h period). However, protective fungicides applications are often missed due to the unavailability of spray contractors, machinery breakdown, insufficient time to spray crop prior to a rain event, and rain occurrence when it is not predicted (Moore et al., 2019). As little is known about the rainfall threshold for *A. rabiei* conidial release and spread, crops often get sprayed when they did not need to be, resulting in unnecessary use of fungicides. Rainfall threshold is the minimum amount of rain required for conidia dispersal, and to result in sufficient leaf wetness for germination and successful penetration of host tissues (Diggle et al., 2002). Rainfall threshold and disease intensity in turn are directly related to the inoculum density, i.e., less rain can cause more crop damage when inoculum density is high (Kaiser, 1992). Growers therefore need information on the likely interaction between rain duration and amount and inoculum density that triggers fungicide spraying to make cost effective *Ascochyta* blight management decisions.

A previous study used large (10 x 2 m) chickpea plots with 100% plant infections to get an accurate estimate of the distance *A. rabiei* conidia travel in wind driven rain (Khaliq et al., 2020). They found that greater inoculum density accurately determined conidial dispersal at increasing distances (0–75 m) from the inoculum source. Lesions were recorded on trap plants in rain amounts as low as 0.8 mm (Khaliq et al., 2020). To get more insights into approximate rainfall threshold, studies should use limited inoculum density and emphasis should be placed on capturing variable rain amount and duration.

Studies aimed at determining the relationship between rain and splash dispersal have largely based on generating rain using a rain simulator. The simulated rain does not

necessarily represent the intrinsic characteristics (duration and frequency of occurrence) of natural rain (Madden et al., 1996). Despite rain being the main driver of *Ascochyta* blight spread in Australia (where ascospores are absent), and despite being the sole determinant of making decisions related fungicide application, the relationship between the intrinsic characteristics of natural rain and *Ascochyta* blight development has not been investigated. This research was conducted to investigate the relationship between rain intensity and *Ascochyta* blight development

Methods

Experimental design and spore trapping

This study was conducted at the agriculture field site (Ag Plot) at the University of Southern Queensland, Toowoomba, Queensland from March through to December 2020. The Ag Plot site was chosen because of the mesh enclosure to exclude birds. The experiment was halted between April to July due to COVID-19 restrictions. The relationship between rain intensity and *Ascochyta* blight development was determined by trapping *A. rabiei* conidia on 21 day old chickpea plants, placed around the inoculum source, during rainfall events of variable intensities (henceforth called rainfall events). Commercial disease-free chickpea seed was used for growing chickpea plants. Infested residue pieces were sourced in the previous year from volunteer chickpea plants in a wheat paddock. Briefly, 7 g of infested chickpea residue was placed at the soil surface in a 1 m² plot and three pots (three trap plants per pot, total 12 pots) of a susceptible chickpea cultivar 'Kyabra' were randomly placed on each side of the 1 m² plot preceding a forecasted rainfall event. The inoculum load on the infested residue pieces was not quantified, but efforts were made to select infested residue with approximately the same number of lesions. Emphasis was placed on capturing smaller rainfall events to get more insights into the approximate rainfall threshold. After exposure to a rainfall event, trap plants were transferred to a controlled temperature room set at 20 °C for 48 h (100% humidity). After 48 h incubation period, trap plants were transferred to a glasshouse with temperature set at 20 °C to allow lesion development. Plants were watered

daily with a hand sprinkler and extreme care was taken to prevent splash dispersal to nearby plants. The number of characteristic Ascochyta blight lesions were counted on all trap plants after 14 days. The 1 m² plot was cleaned thoroughly and the previously used infested residue were replaced with fresh residue (to account for inoculum depletion) to trap conidia in the next rainfall event. The process was repeated for a total of 7 rainfall events and the date and time trap plants were deployed and removed from the Ag Plot, and the date lesions were counted on trap plants were recorded (Table 1).

For the entire duration of the experiment, meteorological data were recorded by an automated weather station 'WeatherMation' (<https://www.weathermation.net.au/WMLLogin.aspx>) located within 500 m of the experimental location. The data collected were comprised of date and time, daily average air temperature (°C), total daily rain (mm), daily average relative humidity (%), daily average wind speed (ms⁻¹) and daily average wind direction (°).

Statistical analyses

The influence of total rain, rain duration and wind speed on the total number of lesions recorded on trap plants per pot during each rainfall event was determined by fitting generalised linear mixed models using the `glmmTMB()` function of the 'glmmTMB' package version 1.0.2.9000 (Brooks et al., 2017) in the R programming language version 4.1.0 (R Core Team, 2021).

A quasipoisson family was used because preliminary analyses showed the data to be overdispersed. The overdispersion was tested using Bolker's custom function 'overdisp_fun' (Bolker, 2021). The predictors total rain, rain duration and wind speed were included as fixed effects and the predictor rainfall event (total 7 rainfall events) was included as a random effect to directly compare the influence of total rain, rain duration and mean wind speed on the number of lesions recorded. The best fit model was selected using Akaike's Information Criterion (AIC) (Akaike, 1974). Model diagnostics was performed using DHARMA package version 0.4.1, which uses a simulation-based approach to determine whether the data met the model assumption (Hartig, 2019).

Data were processed and visualised in the R programming language using the contributed packages 'tidyverse' version 1.3.1(Wickham et al., 2019), 'lubridate' version 1.7.10 (Grolemund & Wickham, 2011), 'gridExtra' version 2.3 (Auguie et al., 2017) and 'clifro' version 3.2.5(Seers & Shears, 2015). Mean wind speed was calculated using a 'circular.averaging' function from 'SDMTools' version 1.1.221(VanDerWal et al., 2014).

Results

The total number of lesions recorded on trap plants varied greatly between rainfall events (Table 2, Figure 1). The number of lesions significantly ($p < 0.05$) increased with increasing rain amount. The highest number of lesions was recorded during rainfall event 4 (Figure 2). Lesions were recorded in a rain amount as low as 1.4 mm and a rain duration as short as 0.7 h (Table 2). There was a statistically non-significant positive influence of an increasing rain duration. The number of lesions recorded tended to increase with an increasing rain duration (Figure 1). For instance, more lesions (597) were recorded during rainfall event 4 than rainfall event 5 (568), although same rain amount occurred during both rainfall events (Table 2). Conversely, the number of lesions recorded tended to decrease with increasing wind speed (Figure 1), although the influence was statistically non-significant. This was particularly evident during rainfall events 2 and 3 where twice as many lesions (32 vs. 15 lesions) were recorded during rainfall event 2, although higher rain amount (3.4 mm vs 2.8 mm) and duration (1.7 vs 0.7 h) was observed during rainfall event 3 (Table 2).

Mean wind speed, relative humidity and temperature varied during each rainfall event. Mean wind speed ranged from 2.9 to 4.6 m/s, mean relative humidity ranged from 70.3 to 90 % and mean temperature ranged from 10.3 to 21 °C (Table 2). Wind was not blowing in all directions during rainfall events. Wind was mostly blowing from E during rainfall events 1, 2, 3, 7 and from SW during rainfall events 4, 5 and 6 (Figure 4). DHARMA diagnostics showed that the data met the best fit model assumptions. The model fit for total rain, rain duration and wind speed is shown in Figure 4.

Discussion

To the best of our knowledge, this study is the first to investigate the effect of natural rain intensity and intrinsic attributes (wind direction and wind speed) associated with rain on *Ascochyta* blight in chickpea development. The number of lesions recorded on trap plants increased with increasing rain amount and duration, whereas the reverse was true for wind speed. That is, as wind speed increased, the number of lesions recorded on trap plants decreased.

The significant increase in the number of lesions recorded on trap plants with increasing rain amount is not surprising as increasing rain amount releases more conidia, which are then dispersed to neighbouring plants by a rain splash (Kimber, 2002), or to longer distances (at least 75 m) by wind driven rain (Khaliq et al., 2020). Rain not only releases conidia from pycnidia and disperses them, it also facilitates conidial germination, penetration and subsequent infection by keeping host tissues wet. *Ascochyta rabiei* requires a wetness period of at least 6–10 h to infect (Moore et al., 2016), and further disease development is very limited when relative humidity is less than 86 % (Navas-Cortés et al., 1998). Lesions were recorded on trap plants in rain amount as low as 1.4 mm. Previous studies recorded lesions on trap plants in rain amount as low as 0.8 mm (Khaliq et al., 2020) and 1 mm (Galloway & MacLeod, 2002), however 0.2 mm rain event was not sufficient to disperse conidia (Galloway & MacLeod, 2002). Conidial spread in low rain amounts suggests that conidial spread is more dependent on the occurrence of rain rather than the amount. A similar spore dispersal pattern has been observed for the sexual spores (ascospores) of *A. rabiei*, where ascospore discharge was more dependent on the occurrence of rain rather than the amount (Trapero-Casas et al., 1996). Of the different environmental factors affecting *Ascochyta* blight development, rain has been reported to have the major influence (Kimber et al., 2007). Moreover, moderate resistance to *Ascochyta* blight can be overcome when chickpea plants are exposed to intermediate to high disease pressure under cool (20 °C) and wet (> 90 % humidity) conditions (Chongo et al., 2003, Coventry, 2012).

The number of lesions recorded on trap plants tended to increase with an increasing rain duration. The positive effect of an increased rain duration on conidial spread was especially evident during rainfall event 4 where more lesions (597) were recorded than rainfall event 5 (568), although same rain amount (13.4 mm) occurred during both rainfall events. It is likely that longer duration rain provided sufficient kinetic energy for longer duration and dislodged more conidia without quickly depleting inoculum supply. Consistent with the positive effect of an increasing rain amount, longer duration rainfall events can further increase lesion development by providing an adequate amount of wetness period to facilitate conidial germination, penetration into host tissues and subsequent infection. Although there is no information in relation to *Ascochyta* blight in chickpea, the positive influence of an increasing rain duration on spore dispersal has been well established for other plant pathogens (Ntahimpera et al., 1997, Sache, 2000). Compared to quick thunderstorms, low intensity and intermittent rains provide the most conducive conditions for spore spread without depleting the inoculum supply (Sache, 2000). The positive influence of an increasing rain duration on the number of lesions recorded on trap plants was statistically non-significant in the current study, which can be attributed to small sample size.

Splash dispersal is linked with other environmental factors associated with rain, such as wind speed and wind direction (Khaliq et al., 2020). Increasing wind speed had a statistically non-significant negative influence on the number of lesions recorded on trap plants in the current study. The non-significant effect of wind speed on the number of lesions recorded on chickpea trap plants, distributed at the distances of 0, 10, 25, 50 and 75 m from infected chickpea plots, has been established previously (Khaliq et al., 2020). Wind speed also had no significant effect on the spatial and temporal *Ascochyta* blight progress from primary infection foci (i.e., four infested stubble pieces) placed at the center of a 20 m x 20 m plot (Khaliq, 2021). The non-significant effect of wind speed on both conidial and disease spread can be attributed the absence of the sexual spores of *A. rabiei* in Australia. Ascospores are primarily dispersed by wind up to hundreds of metres to kilometres (Trapero-Casas & Kaiser, 1992, Kaiser & Küsmenoglu, 1997), while conidia are mainly splash

dispersed to neighboring plants (Khan, 1999). However, the negative effect of increasing wind speed on the number of lesions recorded on trap plants in the current study is unlikely to reflect disease dynamics under field conditions, as 1 m² plot was used in the current study. It is likely that conidia did not fall on trap plants and dispersed up and through the air as the wind speed tended to increase. Farmers paddocks are usually large (> 1000 ha), so heavy disease outbreaks are plausible when rain is accompanied by wind. For splash dispersed pathogens, the effect of wind and rain on spore dispersal is not mutually exclusive. That is, rain provides the mechanism to remove spores and splash disperse them to shorter distances, while wind can transfer spores upward and across the field (Khan, 1999). It was hypothesized that if aerosols enter the turbulent boundary air, then wind can disperse conidia to distances that are associated with ascospores (Coventry, 2012). The question whether conidia would remain viable after being transported to longer distances by turbulent wind remains unanswered. Although Armstrong-Cho et al. (2004) found that *A. rabiei* conidia could withstand intermittent dry periods for up to 24 h after inoculation in controlled environmental condition.

Wind was not omni-directional and was mostly blowing from E during rainfall events 1, 2, 3, 7 and from SW during rainfall events 4,5 and 6. The influence of wind direction was not directly investigated in the current study as the model failed to converge when wind direction predictor was included in the model, suggesting that there were not enough degrees of freedom. However, the influence of wind direction on Ascochyta blight in chickpea has been well established. Wind direction is responsible for the directional spread of the disease when conidia in aerosols are blown in a particular direction. Studies conducted on the spatial and temporal disease progress from primary infection foci showed that disease was more severe in the downwind direction of the primary infection foci (Kimber et al., 2007, Coventry, 2012). The tendency of wind to blow in all directions resulted in a uniform disease spread across plots (Coventry, 2012).

Our study shows that small rain amounts, shorter duration rains or a limited amount of primary inoculum is not a barrier in conidial dispersal. Previous epidemiological studies

have shown that limited inoculum is not a constraint in the development of an epiphytotic under conducive conditions (Coventry, 2012, Kaiser, 1992, Kimber et al., 2007). It has been shown that less than 1% primary infection can have devastating effects on susceptible cultivars under cool and wet conditions (Kaiser, 1992); and single infection foci (four infested stem pieces placed at the center of the plots) were enough to spread disease across whole plots in a rainy season (Coventry, 2012). It is difficult to determine an exact rainfall threshold for spraying fungicides, as rainfall threshold can vary with rain intensity, the time of the day, inoculum density and the level of resistance of the cultivar. A successful disease management in chickpea should involve an integrated disease management approach, including paddock hygiene, maintaining 1–4 years rotation, sowing the best available resistant cultivars, seed treatment and strategic use of fungicides (Lyon, 2017). The strategic use of fungicides involves spraying before the occurrence of first rain event after crop emergence, three weeks after crop emergence, or at the three branch crop growth stage, whichever occurs first (Lyon, 2017). In high-risk (high rainfall) areas, it is recommended to spray fungicides every 14–21 days throughout the growing season (Pritchard, 2000). In low-risk areas, crop should be monitored throughout the growing season, especially 10–14 days after a rain event, and fungicides should be applied if *Ascochyta* blight is detected (Lyon, 2017).

Further experiments with continuous or uninterrupted rain events at different times of day and/or night will give more insights into the effect of rain intensity and time of the day/night on the disease development, although such experiments will be difficult to conduct with natural rain. Leaf wetness sensors may be used to measure the duration of leaf wetness produced by different amount of continuous or uninterrupted rain.

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Conflict of interest

None to disclose.

Data availability and code availability

All raw and generated data used in the statistical analyses and data visualisation have been made available as a part of a research compendium for reproducibility. Please see the fully reproducible and version-controlled code at <https://github.com/lhsanKhaliq/rainy>. The archived code and data are available at <https://doi.org/10.5281/zenodo.5077955>

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Table 1 Date and time trap plants deployed to and removed from agriculture field site (Ag Plot) of the University of Southern Queensland, Toowoomba, Queensland, with the date chickpea trap plants were assessed for lesions for each rainfall event. Lesions were counted at approximately fortnightly intervals after 48 h of incubation period

Rainfall event	Date deployed	Time deployed	Date removed	Time removed	Assessment date
1	2020/02/24	19:10	2020/02/25	15:30	2020/03/19
2	2020/02/28	17:00	2020/03/01	9:10	2020/03/20
3	2020/03/03	20:10	2020/03/04	16:50	2020/03/22
4	2020/07/14	13:00	2020/07/29	16:00	2020/08/12
5	2020/06/08	15:00	2020/08/10	10:00	2020/08/25
6	2020/08/15	7:10	2020/08/17	11:00	2020/09/02
7	2020/11/24	8:00	2020/11/26	16:00	2020/12/10

Table 2 Hours trap plants deployed in the agriculture field site (Ag Plot) of the University of Southern Queensland, Toowoomba, Queensland, amount and duration of rain, mean relative humidity, mean wind speed, mean temperature and total lesions recorded on chickpea trap plants during each rainfall event

Rainfall event	Total rain (mm)	Rain duration (hours)	Mean relative humidity (%)	Mean wind speed (m/s)	Mean temperature (°C)	Total lesions
1	1.4	0.8	89.6	4.2	19.6	2
2	2.8	0.7	83.2	3.7	21	32
3	3.4	1.7	90	4.5	20.3	15
4	13.4	7.0	76.8	2.9	10.5	597
5	13.4	3.3	82.9	3.7	10.6	568
6	11.8	2.7	82.9	3.9	10.3	81
7	1.4	0.7	70.3	4.6	21.2	5

Fig. 1 Total number of lesions per pot for 12 pots recorded for seven rainfall events as indicated by the point colour. Total rainfall (mm) is displayed on the x-axis with wind speed (m/s) on the y-axis. The point size relates to the duration of the rainfall d during the event (hour). The number of lesions increased as the rainfall duration and amount both increased but decreased with increasing wind speed.

Fig. 2 Box plots showing total number of lesions per pot for 12 pots recorded on chickpea trap plants during 7 different rainfall events of differing durations. The highest number of lesions were recorded during rainfall event 4, 121 in one pot.

Fig. 3 Wind roses showing daily average wind speed and direction during each rainfall event.

The notation '[]' represents a 'closed interval' indicating an interval is inclusive of both lower and upper values, whereas the notation '(]' represent 'half open interval' indicating an interval is exclusive of the lower value but inclusive of the upper value

Fig. 4 Generalised linear mixed models showing the influence of total rain, rain duration and average wind speed on the number of lesions recorded on chickpea trap plants.