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FULL RESEARCH PAPER

The role of seedling infection in epiphytotics of ascochyta blight on chickpea

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Abstract *Didymella rabiei*, the causal agent of ascochyta blight, survives on infected seeds and seedlings. Diseased seedlings originating from infected seeds occasionally serve as the source for primary infection in chickpea crops. Experiments carried out independently in Australia and in Israel provided quantitative information on the temporal and spatial distribution of ascochyta blight from initial infections and on the relationship between the amount of initial infection and the intensity of subsequent epiphytotics for cultivars differing in susceptibility to the pathogen. Disease spread over short distances (<10 m) from individual primary infections, was governed by rain and wind, and was up to five times greater down-wind than up-wind. Cultivar response to *D. rabiei* significantly affected the distance and area over which disease spread and the intensity of the disease on infected plants. At onset of the epiphytotic, the relationship between disease spread and time was exponential ($P < 0.05$; $R^2 > 0.95$) and the area of the resulting foci was over 10 times greater in susceptible cultivars than in resistant cultivars. Regression equations showed the relationship between disease severity and the distance from the focus-plants was inverse-linear for all cultivars tested ($P < 0.05$). A simulation model based on the experimental data revealed that even if primary infection is infrequent (less than 1% of plants), the consequences are potentially devastating when susceptible cultivars are used. The epidemiological information and simulation model generated by this study provide an increased understanding of the development of an epiphytotic in which the primary foci of disease originate from infected chickpea seedlings.

Keywords *Ascochyta rabiei* · Gram blight · Quantitative epidemiology

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Introduction

Chickpea (*Cicer arietinum*) is an important food legume crop worldwide, with annual production

exceeding 7 million metric tonnes (FAO, 2005). Ascochyta blight, caused by *Didymella rabiei* (anamorph *Ascochyta rabiei*), is one of the most important foliar fungal diseases of chickpea worldwide, and has been recorded in at least 35 countries (Nene, Shiela, & Sharma, 1996).

Pycnidiospores and ascospores provide inoculum of ascochyta blight, the former over short distances, the latter are wind-borne (Kaiser, 1992; Nene & Reddy, 1987). Primary infection that originates from pycnidiospores occurs when seeds derived from ascochyta blight-affected crops are used, or may arise from infected plant debris when there is nil or short rotation between consecutive chickpea crops. In such cases, disease is observed soon after emergence and on basal parts of the plants (Mitsueda, Hussain, Bashir, & Ahmad, 1997). Generally, seed transmission of related diseases on pulse crops is infrequent, in the order of a few seeds in a thousand (Gossen & Morrall, 1986), but the frequency of transmission is positively correlated with the proportion of infected seeds sown (Kimber, Scott, & Ramsey, 2006). The spread of the pathogen from infected seed to seedlings, referred to here as transmission, is non-systemic, in that contact must be made between the emerging growing point and lesions on the seed coat or cotyledons (Dey & Singh, 1994). As pycnidiospores are disseminated short distances by rain-splash, primary infections that originate from infected seedlings occasionally result in distinct foci randomly scattered about the field; plants in the centre of each focus are more severely diseased than those at its margins. Pseudothecia are initiated on plant debris in autumn; ascospores mature by late winter—early spring, are dispersed long distances by wind, and generally infect the upper foliage.

Following primary infection, disease intensification is governed by environmental conditions. Ascochyta blight is favoured by temperatures of 5–30°C (optimum of 20°C) and wetness periods of 10 h or more (Khan, 1999; Reddy & Singh, 1990; Trapero-Casas & Kaiser, 1992). Rainfall is the critical factor in most epiphytotics (Kaiser, 1992; Luthra, Sittar, & Bedi, 1935; Nene & Reddy, 1987). Though primary infection foci in a field may be limited and isolated, the pathogen is spread by raindrops hitting sporulating lesions on foliage and shattering into small droplets, disseminating masses of pycnidiospores to neighbouring foliage, particularly in windy conditions (Pande et al., 2005).

Ascochyta blight epiphytotics were recorded in southern Australia in 1998 and 1999 and yield losses exceeded 50%, with many crops destroyed. The cost to the chickpea industry in 1998 is estimated at AUD$20 million. Consequently, growers abandoned cultivating chickpea and the area sown decreased from ca 100,000 ha in 1998 and 1999 to ca 1,000 ha in the following years (Pulse Australia, 2005). At the time of these epiphytotics, there was no evidence to suggest that the teleomorph was present and only one mating type (*MAT1-1*) had been identified (Khan et al., 1999). As growers practised 3–5 years rotations, it is likely that infected seeds served as the source of primary inoculum in these epiphytotics (Ramsey, Khan, & Scott, 1999). *D. rabiei* was subsequently detected in seed lots that had been harvested in 1992 (Khan et al., 1999). Quantitative information on the temporal and spatial distribution of ascochyta blight from initial infections and on the relationship between the amount of initial infection and the intensity of subsequent epiphytotics for cultivars with diverse response to the pathogen, is crucial. It would assist in understanding the development of the 1998 and 1999 epiphytotics in Australia through quantifying the role of primary infection in the subsequent development of an epiphytotic. Furthermore, this information is useful in developing effective disease management strategies.

This paper presents the results of two separate studies, conducted independently in Australia and Israel, that aimed to investigate the factors involved in the spatial and temporal development of ascochyta blight from individual, distinct, primary infections. The opportunity to combine the studies allowed us to quantify the role of seedling infection in subsequent ascochyta blight epiphytotics at the population (crop) level in the field and in simulation experiments.

Materials and methods

Spatial and temporal development of ascochyta blight from primary infections

Patterns of disease spread from infected seed (field experiment—Australia)

A field trial was conducted at the Kingsford Research Station, near Gawler (South Australia) in 2001 to determine the effect of cultivar and incidence of seed infection on disease development. Chickpea had not been grown in this field for at least 4 years. Seed of three chickpea cultivars; Desavic (highly susceptible), Dooen (highly susceptible) and Howzat (moderately susceptible), with no detectable *D. rabiei*, was used. The trial was sown on 25 June 2001 in a completely randomised factorial design, with four replications. Trial plots were 5 m long and 1.5 m wide, sown at 18 cm row spacing with approximately 45 plants m^{-2} and sowing depth approximately 5 cm. Plots of faba beans (cv. Fiord) of similar size were sown adjacent to trial plots and perpendicular at the end of the trial plots to provide a barrier and to reduce the spread of disease among chickpea plots. On 27 June 2001, 2 days after sowing, 0, 1 (centred) or 15 (as an evenly distributed 3 by 5 configuration) artificially infected seeds were placed by hand (5 cm sowing depth) into the appropriate treatment plots, to simulate a seed infection rate of approximately nil, 0.25 and 3.75% per plot, respectively.

Artificially infected seeds were prepared using a mixture of four isolates of *D. rabiei*, originating from four regions of south-eastern Australia. The isolates were grown on Potato Dextrose Agar (PDA) at 22°C for 2 weeks under blacklight and fluorescent light and suspensions of 2.5×10^7 pycnidiospores ml^{-1} were prepared as described by Kimber et al. (2006). Seeds of cvs Desavic, Dooen and Howzat were inoculated using techniques modified from Kaiser, Okhovat, and Mossahebi (1973). Briefly, seeds were surface sterilised in 1.0% sodium hypochlorite for 5 min then rinsed three times with sterile reverse osmosis water, placed on sterilised filter paper and allowed to dry for 3 h in a laminar flow cabinet. They were then submerged in the spore suspension and placed in an implosion-proof desiccation bowl (25 cm diam). The bowl was sealed and placed under vacuum using –70 kPa suction. The vacuum was applied and released several times, then applied for 3 h. Seeds were then drained on sterile muslin, placed on sterilised, dry, germination paper and air-dried for 24 h in the laminar flow cabinet.

Emergence in the nil treatment for each cultivar was counted 5 and 8 weeks after sowing (2 and 23 September 2001). Starting 5 weeks after sowing, plots were monitored for signs of diseased seedlings. Disease transmission was determined for each cultivar, calculated as the percentage of diseased seedlings that emerged from artificially infected seed sown. However, disease incidence was not related to experimental treatments ($P \geq 0.05$), hence at this point the 10 individual plots were used to derive a relationship between primary infections and epiphytotic development. The first seedling showing infection by *D. rabiei* (the primary focus of disease) within a plot was marked with a coloured peg, and the position was checked with the sowing records. The spread of disease from the primary focus within a plot was recorded weekly for 5 weeks by counting the number of surrounding plants showing ascochyta blight symptoms, which are referred to here as secondary infections. Starting from the foci, diseased plants were recorded within 1 m bands in a concentric pattern using a pivoting 8 m string with 1 m graduations. Spatial distribution of disease development within a plot was also recorded by drawing onto a template the area of the plot where plants with secondary infection were located.

As secondary infections had spread (within each experimental plot) in the direction of the prevailing wind, plants up-wind were seldom infected. Accordingly, disease incidence was calculated as the number of plants exhibiting ascochyta blight symptoms out of the total number of plants grown in the portion of the experimental plot located down-wind of the site of primary infection. The final assessment was made 12 weeks after sowing (20 October 2001).

Trap plants were used to monitor the possible introduction of disease via ascospores. Each week, a 30×35 cm tray of 12 punnets (0.55 l

each) filled with potting mix (University of California) was sown with Desavic, two seeds per punnet. Plants were grown in the glasshouse for 21 days before being moved to the field and placed within an open-mesh cage approximately 500 m west of the trial site. Each tray remained in the field for 1 week before being exchanged with the subsequent tray. The tray of trap plants was returned to the glasshouse, misted with sterile distilled water (SDW) and placed in a plastic bag for 4 days, to provide conditions conducive for disease development, before being placed on the glasshouse bench. Plants were examined for symptoms of ascochyta blight 14 days later. Trap plants were placed in the field every week for the duration of the trial.

Weather data were collected using an Automatic Weather Station (Measurement Engineering Australia Pty Ltd, Australia). The station was located approximately 500 m from the trial site and recorded the following; air temperature, soil temperature, air relative humidity, rainfall and leaf wetness. Wind speed and direction were recorded at an automatic weather station located nearby, operated by the Australian Bureau of Meteorology.

Patterns of disease spread from infected seedlings (field experiment—Israel)

The spatial and temporal development of ascochyta blight from primary infection was studied in a field trial conducted at the Volcani Central Experimental Station located at Bet Dagan, on the coastal plain of Israel. Seeds of four cultivars; Sfaradit (highly susceptible), Ayala (moderately susceptible), Hadas (moderately resistant) and Bulgarit (highly resistant) were mechanically sown on 1 December 1992. Chickpea had not been sown in the trial area for at least 2 years. The distance between rows was 0.5 m and plants were 6–8 cm apart within rows. Each experimental plot was 15 m long and 9 m wide and plots were separated by a 2.5 m wide fallow area. An overhead irrigation system was used, as required, to provide conditions conducive to spread the pathogen during dry periods. Preparation of the land, fertilization and application of herbicides and insecticides were as recommended to chickpea growers in Israel, excluding the application of fungicides.

A local isolate of *D. rabiei* was grown on sterilized wheat seeds at 20°C for 14 days. Spore suspensions were prepared by adding SDW to the flasks, shaking them for several minutes and filtering through four layers of cheesecloth. The suspension was adjusted with a haemocytometer to 10^5 pycnidiospores ml^{-1} and Tween 20 (one drop per 100 ml) was added. The suspension was used to inoculate one plant in the centre of each experimental plot (the focus-plant). Inoculation was performed using an air-pressure sprayer, until run-off in the afternoon of 14 February 1993, after the plots had been wetted by overhead irrigation. The first disease symptoms on the focus-plants were observed on 26 February 1993. As this was the only source of inoculum in the experimental plots, a typical disease focus developed over time.

Plants in the experimental plots were inspected visually and the outer-most location of diseased plants was marked with coloured flags and recorded. Assessment began on 3 March 1993 and continued, at 3–5 day intervals, until 30 April 1993. These records were used to calculate the ascochyta blight-affected area (in m^2). On 14 May 1993, disease severity was assessed on a longitudinal transect of the resultant disease foci, directed from south-west to north-east. Three plants were sampled every 25 cm along the transect, and the number of stem lesions per plant (a measure of disease severity) was counted. For each cultivar, changes in disease severity over the distance from the focus-plants were quantified using linear regression. The independent variable in the analyses was the distance from the focus-plant (in metres, transformed to log units) and the dependent variable was the disease severity (number of stem lesions $plant^{-1}$). The ascochyta spread index (ASI) was determined by dividing the maximal distance of disease spread down- or up-wind (in cm) by the disease severity on the plants immediately adjacent to the focus-plants. ASI values provide estimates for the distance of spread attributed to a single stem lesion.

The role of seedling infection in subsequent ascochyta blight epiphytotics

The role of seedling infection in subsequent ascochyta blight epiphytotics was evaluated in two field trials conducted in Israel. Seeds of the moderately susceptible cv. Ayala, harvested in 2001 from ascochyta blight-affected fields, were mechanically sown on 4 January 2002 (experiment 1) and 8 January 2003 (experiment 2). The first experiment was carried out in Masuot–Yitzhak and the second at the Volcani Central Experimental Station at Bet Dagan. Chickpea had not been sown at either site for at least 3 years. In the first experiment, the distance between rows was 1 m and each experimental plot was 24 m long, in one row. In the second experiment, the distance between rows was 0.5 m and each experimental plot was 9 m long by 2 m wide. Plots in this experiment were separated by 1.5 m laterally and 2.5 m longitudinally. There were 10 experimental plots in the first experiment and 15 plots in the second experiment. Experimental plots differed in the number of primary infections. The number of seedlings exhibiting ascochyta blight symptoms ranged from 0 to 6 per plot. Starting at emergence, the seedlings were inspected weekly for ascochyta blight. The first disease symptoms were observed in experiment 1 on 3 February 2002 and in experiment 2 on 22 February 2003. At that time, the incidence of seedlings exhibiting primary infection was recorded. In Experiment 1, the spread of the disease from the primarily infected seedlings was recorded on 23 February 2002, after the plants had been exposed to one rain event of 28 mm. The size of the resulting foci (in m) was determined for each primarily infected seedling. In experiment 2, disease severity (the proportion of leaf area exhibiting symptoms) was assessed on 3 March 2003, after the plants had been exposed to four rain events (of 30, 9, 31 and 88 mm). Disease severity was assessed on a whole-plot basis. The coincidence between the intensity of primary infection and the subsequent intensity of ascochyta blight epiphytotics was derived using linear regression analysis; the independent variable in the analysis was the initial incidence of diseased seedlings and the dependent variables were disease incidence (in experiment 1) or disease severity (in experiment 2).

Data were used to simulate the role of primary infection in subsequent ascochyta blight epiphytotics, for cultivars with diverse responses to *D. rabiei*. First, the number of infected seedlings m^{-2} (IS) was determined using the formula: IS = -PI × S/100, where PI = primary infections (incidence of seedlings exhibiting ascochyta blight symptoms; %) and S = plant stand (number of seedlings m^{-2}). Then, estimates for the maximal area of individual disease focus (FA; m^2), recorded in the experiment conducted at Bet-Dagan in 1992–1993 (above), were used to calculate the proportion of ascochyta blight-affected area (IA; %) for the various categories of cultivar resistance, using a modification of the Abbott formula (Kosman & Cohen, 1996) as follows: IA = IS × FA × 100. Finally, IA estimates were corrected for overlapping of adjacent foci using the formula IA* = IA – ((IA/2) × (IA/2))/100. As adjacent foci are in contact with each other only in one of their dimensions, the IA values in the correction term of the Abbott formula were divided by 2. The following estimates were used for the various parameters: PI values ranged from 0 to 1%; S = 10 seedlings m^{-2}; FA for highly susceptible cultivars (FA_{HS}) was 18 m^2; for moderately susceptible, moderately resistant and highly resistant cultivars, FA_{MS}, FA_{MR} and FA_{HR} values were 13, 5 and 0.75 m^2, respectively. Yield loss (YL, %) was estimated by multiplying the proportion of affected area by loss factors (LF; %) designated for the various categories of cultivar resistance. LF values reflect the typical reduction in yield associated with ascochyta blight epiphytotics in our previous experiments (D. Shtienberg, The Volcani Center, Israel, unpubl.). In this report, the LF estimates used for the highly susceptible, moderately susceptible, moderately resistant and highly resistant cultivars (LF_{HS}, LF_{MS}, LF_{MR} and LF_{HR}) were 60%, 40%, 10% and 5%, respectively. In sensitivity analysis tests, values greater and smaller by 25% were assigned to the model's parameters. The relationships between IA* and YL and PI for each category of cultivar resistance were plotted and used for estimating the role of seedling infection in subsequent ascochyta blight epiphytotics and

determining the relative contribution of various measures to be used for disease suppression.

Data analyses

Data were analysed using a simple linear model—analysis of variance (ANOVA) and regression. ANOVA was performed in GenStat for Windows version 7.0, which takes into consideration the statistical design of each experiment. The assumptions made in the ANOVA were assessed by examination of associated diagnostic plots, such as plotting the residual versus fitted values. Regression analyses were performed using Microsoft Excel® for Windows. The dependent and independent variables are indicated in the text. For each analysis, the significance of the regression equation and the corresponding intercept and slope were tested and only those that were significant at $P < 0.05$ are presented.

Results

Spatial and temporal development of ascochyta blight from primary infections

Patterns of disease spread from infected seed (field experiment—Australia)

Emergence did not differ significantly ($P \geq 0.05$) among the cultivars tested. The average emergence counts recorded 8 weeks after sowing were 43.6, 45.2 and 46.4 plants m^{-2} for Desavic, Dooen and Howzat, respectively. The first seedling infected with *D. rabiei* was observed 5 weeks after sowing. In total, only 10 seedlings with primary infection were found. These occurred in 10 plots, one per plot (equivalent to 0.25% seeds emerged per plot), and were observed between 5 and 8 weeks after sowing. Disease transmission from artificially inoculated seeds to the emerging seedling was only 4.7% for cvs Dooen and Desavic, and 6.3% for cv. Howzat, from the total of 64 infected seeds sown by hand in the four replicate treatment plots for each cultivar. Seedlings showing primary infection were identified in three plots of Desavic, three of Dooen and four plots of Howzat. However, the relationship between the frequency of primary infection and rate of seed infection was insignificant ($P \geq 0.05$). In cvs Desavic and Dooen, approximately 40% of seedlings identified as the primary foci of disease had withered and died by 9 weeks after sowing. Plants identified as the primary focus of disease in cv. Howzat did not show the same deterioration, even at the later observations.

Plants showing symptoms of secondary infection were observed first adjacent to the primary foci, and all trap plants used to monitor ascosporic inoculum remained free from symptoms of ascochyta blight. The spread of disease from the primary infection foci in the 10 plots was analysed independently. Disease was initiated earlier in plots of cvs Dooen and Desavic (5–6 weeks after sowing) than in plots of cv. Howzat (8 weeks after sowing). Substantial secondary spread of disease occurred in nine plots (Fig. 1). On 6 October 2001, 3–5 weeks after primary infections were identified, 70–100% of plants north-east (down-wind) of the primary infection within these plots showed secondary infection. Although disease severity differed according to the susceptibility of each cultivar, there was no significant difference ($P \geq 0.05$) between cultivars in the rate of disease development over time. The relationship between disease spread and time was exponential for all cultivars (mean curve $Y = 0.337\ (\pm 0.048) \times 2.199\ (\pm 0.321)^X$ (parameters ± SE); $P < 0.05$; $R^2 = 0.981$) and in the period of 9–10 weeks after sowing, exceeded 25 plants exhibiting secondary infection per day.

Temporal and spatial spread of the disease for six representative plots are shown in Fig. 2. Disease spread in an outward pattern from the foci in all plots. A strong bias in the direction of spread was evident in all plots, such that the majority of plants showing secondary infection were approximately north-east of the disease foci, corresponding to the direction of prevailing winds. By 10 weeks after sowing the spread of disease in some plots was approximately 5 m from the foci. Two weeks later the disease had spread at least 10 m from the foci, as adjacent chickpea plots were affected.

Moderate wind fronts (20–25 km h^{-1}) from a west to north-west direction were recorded between 6 and 8 weeks after sowing, whereas winds of 15–20 km h^{-1}, from a south-west to west

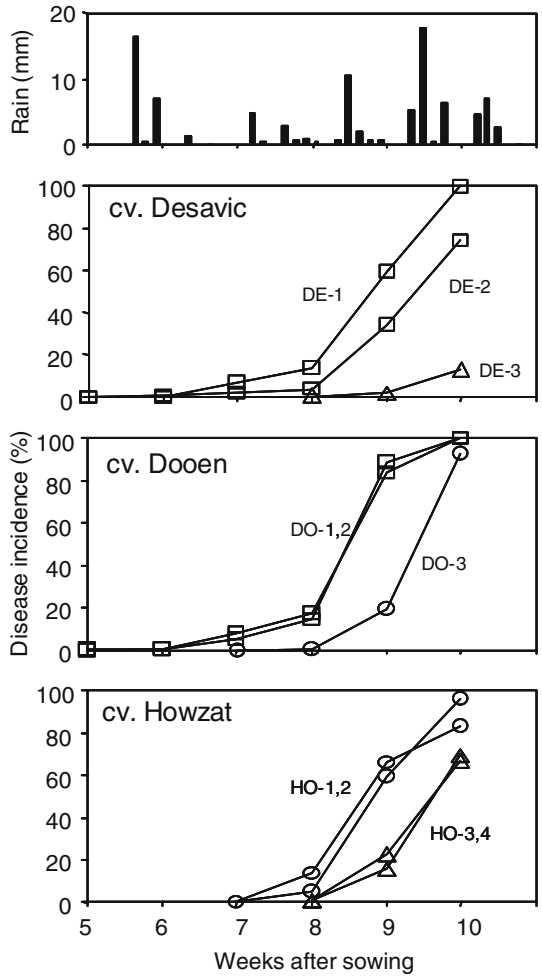

Fig. 1 Development of ascochyta blight over time in three chickpea cultivars susceptible to *D. rabiei*. Disease initiated from infected seedlings was recorded in ten 1.5 × 5 m experimental plots. Infected seedlings were observed 5 and 6 weeks (rectangles), 7 weeks (circles), or 8 weeks after sowing (triangles). The 10 plots are coded (cv. Desavic = DE, cv. Dooen = DO and cv. Howzat = HO) for comparison with Fig. 2. Bars in the upper graph indicate the time and quantity of rain events

direction, were recorded thereafter. Rainfall events between 10 and 28 mm were recorded several times during the experiment (Fig. 1). The average relative humidity was 80–90% from 8 weeks after sowing onwards.

Patterns of disease spread from infected seedlings (field experiment—Israel)

An increase in ascochyta blight-affected area was apparent about 7–10 days after each rain event or application of overhead irrigation; affected areas did not change during dry periods. About 2 months after inoculation of the focus-plants, the infected area in the plot of the hightly susceptible cv. Sfaradit was 18 m^2. By that time, infected areas in plots of the moderately susceptible (Ayala), moderately resistant (Hadas) and the highly resistant (Bulgarit) cultivars were 6, 2 and 0.25 m^2, respectively. Recording disease spread for cv. Sfaradit ceased in mid-April 1993, as the disease had reached the edge of the experimental plot. For the more resistant cultivars, assessment continued until early May 1993 (Fig. 3). The distance of disease spread down-wind was approximately five times larger than up-wind, for all four cultivars (Fig. 4).

Within each trial plot, ascochyta blight severity was related to the distance from the focus-plant. The regression equations describing the coincidence between disease severity and the distance from the focus-plants were inverse-linear ($P < 0.05$; R^2 values > 0.96); slopes of the regression equations denote the reduction in disease severity per unit increase in distance. Steeper slopes were observed corresponding to up-wind spread than for down-wind spread. For example, the up-wind slope for the highly susceptible cv. Sfaradit was –75.4 ± 7.8 (slope ± SE) stem lesions $plant^{-1}$ $log\ m^{-1}$ compared with –45.2 ± 2.1 stem lesions $plant^{-1}$ $log\ m^{-1}$ for the down-wind spread. The regression equations were $Y = 15.3–75.4X$ ($r^2 = 0.959$; $P = 0.02$) and $Y = 33.1–45.2X$ ($r^2 = 0.9680$; $P < 0.0001$), respectively (Fig. 5). Additionally, the steepness of the slopes decreased as host resistance increased. For example the down-wind slope for the highly resistant cv. Bulgarit was –8.6 ± 2.4 stem lesions $plant^{-1}$ $log\ m^{-1}$ (Fig. 5). There were also differences in the intercept (a measure for disease severity of the plants adjacent to the focus-plant) among the four cultivars: the highest value (68.2 stem lesions $plant^{-1}$) was observed for the highly susceptible cultivar and the smallest (4.9 stem lesions $plant^{-1}$), for the highly resistant cultivar. ASI values were 10, 11.1, 10.0 and 12.5 cm stem $lesion^{-1}$, for the highly susceptible, moderately susceptible, moderately resistant and highly resistant cultivars, respectively.

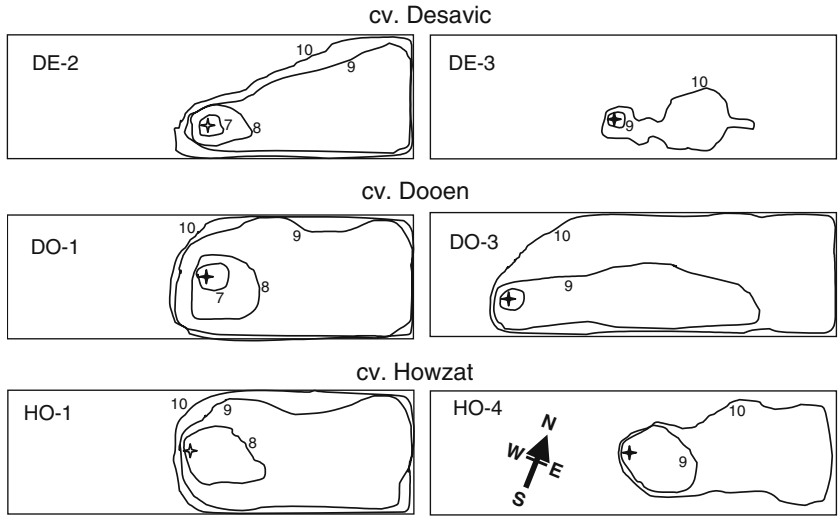

Fig. 2 Pattern of ascochyta blight spread from a focus, for three chickpea cultivars susceptible to *D. rabiei*. Disease originated from an infected seedling emerging from artificially inoculated seeds and was recorded within 1.5 × 5 m experimental plots, starting on 8 September 2001 (6 weeks after sowing), at weekly intervals, up to 10 weeks after sowing (6 October, 2001). Location of the primary infected seedling (focus) within each plot is indicated by an asterisk. Bands and adjacent numbers indicate the furthest distance from the foci where plants showing secondary infection were observed at subsequent weekly intervals. Disease first appeared 1 week before the first band. Plots are coded (cv. Desavic = DE, cv. Dooen = DO and cv. Howzat = HO) for comparison with Fig. 1

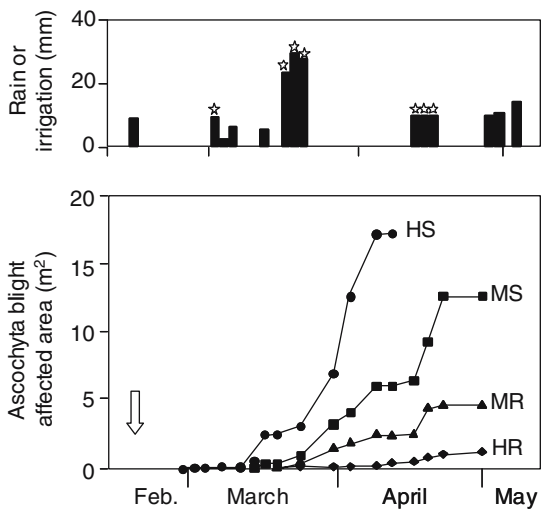

Fig. 3 Changes in ascochyta blight-affected area over time, for four chickpea cultivars differing in response to *D. rabiei*. Disease was initiated from focus-plants, artificially inoculated on 14 February 1993 (arrow). Cultivar response to *D. rabiei*: HS = highly susceptible (cv. Sfaradit); MS = moderately susceptible (cv. Ayala); MR = moderately resistant (cv. Hadas); and HR = highly resistant (cv. Bulgarit). Bars indicate the time and quantity of rain or over-head irrigation (indicated by asterisks)

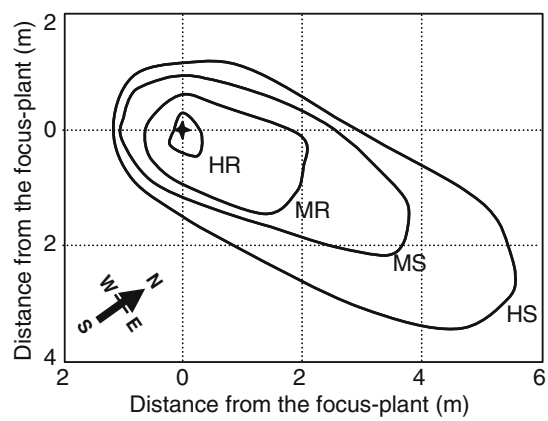

Fig. 4 Pattern of ascochyta blight spread from artificially inoculated focus-plants for four chickpea cultivars differing in their response to *D. rabiei*. The focus-plants (asterisk) were artificially inoculated 8 weeks prior to disease assessment. Each band represents the furthest distance from the foci where plants showed secondary infection in four 9 × 15 m experimental plots, which are overlaid for clarity. Cultivar response to *D. rabiei*: HS = highly susceptible (cv. Sfaradit); MS = moderately susceptible (cv. Ayala); MR = moderately resistant (cv. Hadas); and HR = highly resistant (cv. Bulgarit)

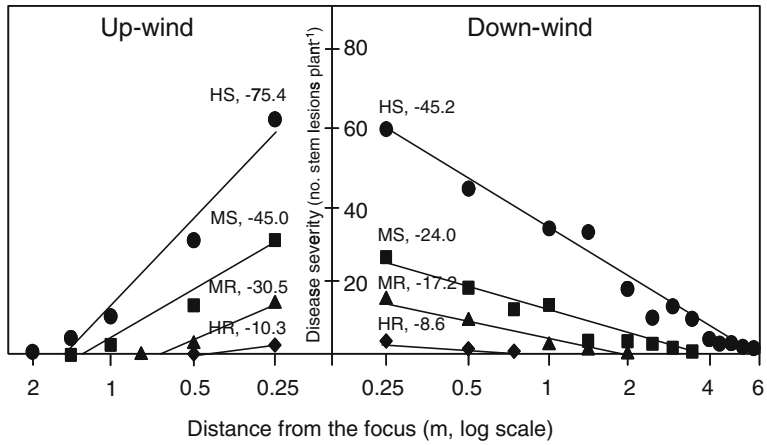

Fig. 5 Changes in ascochyta blight severity as a function of the distance from a disease focus, for four chickpea cultivars differing in their response to *D. rabiei*. The focus-plants were artificially inoculated 14 weeks prior to disease assessment. Cultivar response to *D. rabiei*: HS = highly susceptible (cv. Sfaradit); MS = moderately susceptible (cv. Ayala); MR = moderately resistant (cv. Hadas); and HR = highly resistant (cv. Bulgarit). Slopes of the regression lines (*b* values) are indicated for each cultivar. All regression equations are significant at $P \leq 0.05$

The role of seedling infection in subsequent ascochyta blight epiphytotics

In both experiments, the intensity of secondary infection was linearly related to the incidence of seedlings exhibiting ascochyta blight: the greater the incidence of primary infection, the more severe was the epiphytotic. In the first experiment, subsequent infections were assessed after completion of one disease cycle (rain event). The disease had intensified 4.5-fold (slope of the regression equation; Fig. 6A) and spread to a distance of 0.87 ± 0.07 m (mean ± SE) within rows. The greatest spread was 2 m (10 plants) within rows. In the second experiment, disease severity was assessed after the pathogen had completed four disease cycles. By that time, most plants in the experimental plots were diseased and, in some plots, ascochyta blight severity exceeded 50% (Fig. 6B). By the completion of the experiment, all plants in these plots were devastated by ascochyta blight.

A simulation model was used to explore the role of primary infection in subsequent ascochyta blight epiphytotics (Fig. 7). An increase in the incidence of primary infection resulted in an increase in the area affected by ascochyta blight. The extent of the increase was related to the susceptibility of the cultivar to the pathogen. For the highly susceptible and moderately susceptible cultivars, primary infection of as little as 0.5% resulted in an affected area >50% by the end of the season; primary infection of 1% resulted in an infected area >95%. Yield loss estimates were 69% and 35% for the highly and moderately susceptible cultivars, respectively. When moderately resistant or highly resistant cultivars were used, the final affected area was 23% and 4% for primary infection of 0.5% and 44 and 7% for primary infection of 1%, respectively. Yield loss estimates for moderately and highly resistant cultivars were <4% (Fig. 7).

Discussion

Analyses of the data enabled us to determine the fundamental factors governing the spread of *D. rabiei* from chickpea seedlings serving as the primary source of inoculum and then to quantify the role of primary infection in subsequent ascochyta blight epiphytotics. The temporal and spatial development of ascochyta blight from individual primary infections was governed by rain (or overhead irrigation), wind and cultivar response to the pathogen. As pycnidiospores of *D. rabiei* are dispersed by rain splash, the increase in disease incidence or ascochyta blight affected

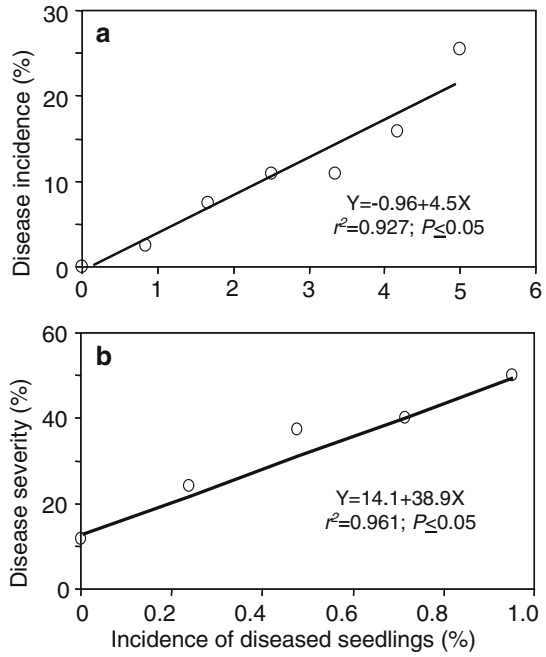

Fig. 6 Relationship between the incidence of chickpea seedlings with ascochyta blight and subsequent development of the disease in the susceptible cv. Ayala. Disease originated from naturally infected seeds and was assessed about 1 month after emergence. (**a**) Masuot Yitzhak, 2002; plants were exposed to one rain event (of 28 mm) between disease onset and assessment date. (**b**) Bet Dagan, 2003; plants were exposed to four rain events (of 30, 9, 31 and 88 mm) between disease onset and assessment date

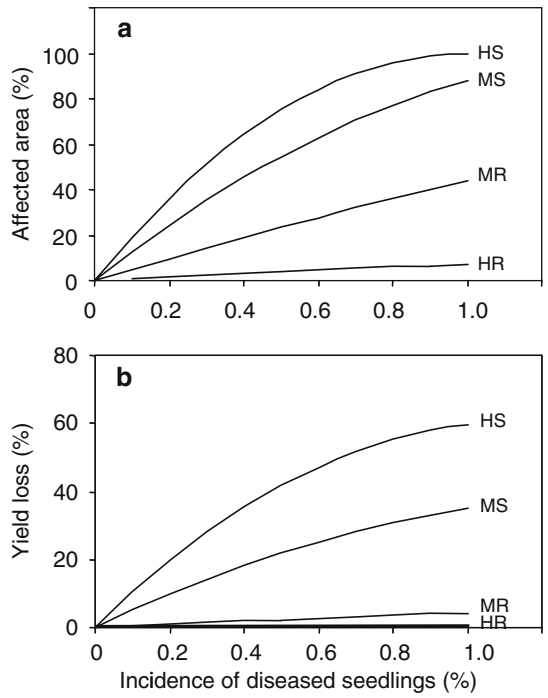

Fig. 7 Simulation of the relationship between the incidence of chickpea seedlings exhibiting ascochyta blight and the proportion of affected area (**a**) and yield loss (**b**), for cultivars with diverse response to *D. rabiei*. Procedures for simulation and values assigned for the parameters are given in the text. Cultivar response to *D. rabiei*: HS = highly susceptible; MS = moderately susceptible; MR = moderately resistant; and HR = highly resistant

area was associated with rain or overhead irrigation events (Figs. 1 and 3). Disease spread was in an outward direction from the primary infection and down-wind spread was five times greater than up-wind spread (Figs. 2 and 4), suggesting that wind-assisted droplet dispersal contributed to the spread of the disease. Late development of a primary focus and a slow rate of disease spread were observed in cv. Desavic (highly susceptible) plot 3 (DE-3, Figs. 1 and 2). The infected seedling within this plot was, by chance, within a small depression and surrounded by large aggregates of soil. Therefore, the seedling may have been overlooked at earlier ratings and the surrounding obstacles appeared to have reduced the opportunity of the pathogen to spread to surrounding plants.

Our findings support those of Kaiser (1992), who stated that wet and windy conditions are critical for rapid disease spread, and Zachos, Panagopoulos, and Makris (1963) who found that, in the presence of wind-driven rain, disease spread was in the direction of the wind. The season-long spread of the disease from the primary infected plants was limited to several metres in trials in both Australia and Israel (Figs. 2 and 4). Limited spread of disease from infected seedlings serving as the primary foci was also observed by Hewett (1973) for *Ascochyta fabae* (ascochyta blight of faba beans), such that disease spread up to 10 m from an infected seedling in an average season. Weltzein and Kaack (1984) described a similar distance of disease spread from chickpea seedlings infected with *D. rabiei* in studies of susceptible chickpea cultivars.

Cultivar response to *D. rabiei* markedly affected the distance of disease spread from the

source of primary infection, the area of the resulting foci and the intensity of the disease on infected plants within the foci (Figs. 3–5). As ASI values were comparable for all cultivars tested, regardless of their response (from highly susceptible to highly resistant) to *D. rabiei*, it is likely that differences in disease severity on the focus-plants, rather than factors related to the developmental characteristics of the cultivars (e.g., branching, sprouting) or canopy density, principally affected the spread of disease over time and space. Thus, even if cultivar resistance has no effect on the likelihood that harvested seeds would be infected by the pathogen or that diseased seedlings would emerge from infected seeds, using cultivars with resistance to the pathogen is advantageous. Resistant cultivars exhibit less intensity of disease on primary infected seedlings than do susceptible cultivars, which, in turn retards further development of the epiphytotic.

Next, the role of primary seedling infection on subsequent ascochyta blight epiphytotics at the population (crop) level was studied. The results from spatial distribution data in the individual foci experiments in Australia and Israel corroborated the finding that disease spread over short distances from primary infected seedlings. Nevertheless, data presented in Figs. 1 and 6B demonstrate that even if primary infection is rare (less that 1%), the consequences may be devastating when susceptible cultivars are used and environmental conditions (frequent rain events) favour disease development. This finding supports the observations made by Kaiser (1992) that very low levels of seed infection combined with cool, wet weather appeared to be responsible for outbreaks of ascochyta blight in commercial crops in northern Idaho, USA, in 1984.

Results of the simulation experiments (Fig. 7) were used to compare the significance of measures to reduce the incidence of primary infections. For susceptible cultivars, attempts to reduce the incidence of primary infection (for example, by seed dressing) may be inadequate. For example, reducing the incidence of primary infection by 80% (from 1% to 0.2%) may still result in substantial disease by the end of the season (32% of the area) and considerable reduction in yield (20%). Accordingly, collection of seeds from disease-free fields is critical if cultivars highly susceptible to *D. rabiei* are to be used. On the other hand, when moderately resistant cultivars are used, reducing the incidence of primary infection from 1% to 0.2% would reduce the diseased area to 10% and the yield loss to 1%. Application of control measures for highly resistant cultivars may be unnecessary or uneconomical as, even if the incidence of primary infection was 1%, only a small portion of the area would be affected (9%) and the yield loss would be negligible (0.7%). It should be noted that these results reflect the specific values assigned to each of the parameters in our simulation experiments and they do not represent actual experimental results. Nevertheless, similar trends and comparable conclusions were derived when other values were assigned to the model parameters (results not shown).

The epidemiological information and simulation model generated in this study provide an increased understanding of factors that influence the development of an epiphytotic of ascochyta blight from infected seed. It shows that for susceptible cultivars, seedlings exhibiting primary infection may provide an effective focus for rapid disease spread when conditions are favourable, such as frequent rain events in combination with wind. In 1998, all cultivars grown throughout southern Australia were highly susceptible. Surveys conducted in 1997 revealed that 30% of crops examined exhibited some degree of ascochyta blight (R.B.E. Kimber, The University of Adelaide, Australia, unpubl.). Infected seedlings originating from infected seed harvested in 1997 may have served as the source of primary infection and provided sufficient inoculum for the severe epiphytotic of 1998. This supports the statement made by Ramsey et al. (1999) and anecdotal evidence that the epiphytotic that occurred throughout southern Australia in 1998 was the result of a build up of seed-borne inoculum on highly susceptible cultivars.

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References

Dey, S. K., & Singh, G. (1994). Seedborne infection of *Ascochyta rabiei* in chickpea and its transmission to aerial plant parts. *Phytoparasitica, 22*, 31–37.

FAO (2005) From FAOSTAT on-line statistical database. Food and Agriculture Organisation of the United Nations. [http://faostat.fao.org/]: accessed April 2005.

Gossen, B. D., & Morrall, R. A. A. (1986). Transmission of *Ascochyta lentis* from infected lentil seed and plant residue. *Canadian Journal of Plant Pathology, 8*, 28–32.

Hewett, P. D. (1973). The field behaviour of seed-borne *Ascochyta fabae* and disease control in field beans. *Annals of Applied Biology, 74*, 287–295.

Kaiser, W. J., Okhovat, M., & Mossahebi, G. H. (1973). Effect of seed-treatment fungicides on control of *Ascochyta rabiei* in chickpea seed infected with the pathogen. *Plant Disease Reporter, 57*, 742–746.

Kaiser, W. J. (1992). Epidemiology of *Ascochyta rabiei*. In K. B. Singh & M.C. Saxena (Eds.), *Disease resistance breeding in chickpea* (pp. 117–143). Aleppo, Syria: ICARDA.

Khan, M. S. A. (1999). Epidemiology of ascochyta blight of chickpea in Australia. PhD thesis, Department of Applied and Molecular Ecology, The University of Adelaide.

Khan, M. S. A., Ramsey, M. D., Corbière, R., Infantino, A., Porta-Puglia, A., Bouznad, Z., & Scott, E. S. (1999). Ascochyta blight of chickpea in Australia: Identification, pathogenicity and mating type. *Plant Pathology, 48*, 230–234.

Kimber, R. B. E., Scott, E. S., & Ramsey, M. D. (2006). Factors influencing transmission of *Didymella rabiei* (ascochyta blight) from infected seed of chickpea. *European Journal of Plant Pathology, 114*, 175–184.

Kosman, E., & Cohen, Y. (1996). Procedures for calculating the differentiating synergism and antagonism in action of fungicide mixtures. *Phytopathology, 86*, 1263–1272.

Luthra, J. C., Sittar, A., & Bedi, K. S. (1935). Life-history of gram blight [*Ascochyta rabiei* (Pass.) Lab. = *Phyllosticta rabiei* (Pass.) Trot.] on gram (*Cicer arietinum* L.) and its control in the Punjab. *Agriculture and Livestock in India, 5*, 489–498.

Mitsueda, T., Hussain, S., Bashir, M., & Ahmad, Z. (1997). *Introduction to chickpea blight and its control. Plant Genetic Resources Institute*. Islamabad, Pakistan: National Agricultural Research Centre.

Nene, Y. L., & Reddy, M. V. (1987). Chickpea diseases and their control. In M. C. Saxena & K. B. Singh (Eds.), *The Chickpea* (pp. 233–270). Wallingford, UK: CAB International.

Nene, Y. L., Shiela, V. K., & Sharma, S. B. (1996). *A world list of chickpea diseases and pigeon pea pathogens* (5th ed.). Andhra Pradesh, India: ICRISAT, Patancheru.

Pande, S., Siddique, K. H. M., Kishore, G. K., Bayaa, B., Gaur, P. M., Gowda, C. L. L., Bretag, T. W., & Crouch, J. H. (2005). Ascochyta blight of chickpea (*Cicer arietinum* L.): A review of biology, pathogenicity, and disease management. *Australian Journal of Agricultural Research, 56*, 317–332.

Pulse Australia (2005) From Pulse Australia online resources: accessed May 2005 [http://www.pulseaus.com.au/statistics_and_market_overview/state_production].

Ramsey, M. D., Khan, S., & Scott, E. (1999). Ascochyta blight of chickpea – a lesson in plant disease. *Australasian Plant Pathology Society News, 12*, 9.

Reddy, M. V., & Singh, K. B. (1990). Relationship between temperature, relative humidity and Ascochyta blight development in winter-sown chickpea in Syria. *Phytopathologia Mediterranea, 29*, 159–162.

Trapero-Casas, A., & Kaiser, W. J. (1992). Development of *Didymella rabiei*, the teleomorph of *Ascochyta rabiei*, on chickpea straw. *Phytopathology, 82*, 1261–1266.

Weltzein, H. C., & Kaack, H. J. (1984). Epidemiological aspects of chickpea Ascochyta blight. In M. C. Saxena & K. B. Singh (Eds.), *Ascochyta blight and winter sowing of chickpeas* (pp. 35–44). The Hague, The Netherlands: Martinus Nijhoff/ Dr W. Junk.

Zachos, D. G., Panagopoulos, C. G., & Makris, S. A. (1963). Research on the biology, epidemiology and control of anthracnose of chickpea. *Annales de l'Institut Phytopathologique Benaki, 5*, 167–192.