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Factors influencing transmission of *Didymella rabiei* (ascochyta blight) from inoculated seed of chickpea under controlled conditions

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Abstract

Ascochyta blight of chickpea (*Cicer arietinum*), caused by the fungus *Didymella rabiei*, has the potential to cause 100% crop loss in severe epiphytotics. Management of this disease often involves reducing sources of inoculum. The influence of sowing depth, host resistance, seed infection level and soil temperature on disease transmission was investigated in a series of glasshouse and growth room trials using seed artificially inoculated with *D. rabiei*. A positive correlation ($R^2 = 0.9992$) was observed between rate of seed infection and the incidence of disease on seedlings. Disease transmission to seedlings was not significantly influenced by sowing depth (1, 3 and 6 cm) in separate trials on two cultivars. Susceptibility of the host showed no obvious influence on the frequency of disease transmission in two trials conducted using four cultivars ranging from highly susceptible to moderately susceptible/moderately resistant. Trials conducted in controlled conditions showed that there was no obvious relationship between soil temperature (5, 9, 14 and 19 °C) and the incidence of disease on seedlings.

Abbreviations: AI – artificially inoculated; MS – moderately susceptible; MR – moderately resistant; NI – non-inoculated; S – susceptible

Introduction

Ascochyta blight (caused by *Didymella rabiei* anamorph *Ascochyta rabiei)*) is one of the most important diseases of chickpea (*Cicer arietinum*) worldwide, particularly in West Asia, North Africa and the Mediterranean (Nene and Reddy, 1987; Haware, 1998). The pathogen was first reported in commercial crops in Australia in 1995 (Khan et al., 1999). Yield losses caused by ascochyta blight range from 5–100% in outbreaks around the world (Haware, 1998). Severe crop losses have been recorded throughout Australia since the first epiphytotic in 1998, the result of a build up of inoculum through infected seed harvested in 1997 and the adoption of highly susceptible cultivars (Ramsey et al., 1999). Losses in South Australia and Victoria in 1998 exceeded 50%, with many crops completely destroyed.

Didymella rabiei infects all foliar tissue of chickpea during the growing season and can survive on seed and chickpea debris (Nene, 1982). Butler (1918), the first to report seed infection by *D. rabiei*, also reported transmission of the pathogen from infected seed to seedlings during germination. The introduction of this disease to many countries has been attributed to infected seed (Kaiser, 1997). The pathogen is frequently seed-borne, surviving for extended periods internally or externally on chickpea seed (Maden et al.,

1975). The frequency of transmission from infected seed may influence the initial stages (or timing) in an epiphytotic. Therefore, the most important component of cultural management of this disease is to reduce or prevent the introduction of primary inoculum to the field.

Until recently, there was no evidence to suggest that the sexual stage was present in Australia and only one mating type (MAT1-1) had been identified (Khan et al., 1999). The teleomorph was reported in Western Australia in 2002 (Galloway and MacLeod, 2003) but has not been reported in other states within Australia. Consequently, in southeastern Australia, seed-borne inoculum plays a major role in dissemination of this disease.

Spread of ascochyta blight to seedlings from infected seed, referred to in this paper as transmission, is non-systemic, in that contact must be made between the emerging plant and lesions on the seed coat or cotyledons (Dey and Singh, 1994). In some instances, the frequency of seed transmission can be very low; less than one seed in a thousand (Gossen and Morrall, 1986). However, this is offset by the rapid spread of *D. rabiei* on infected tissues above ground when exposed to favourable environmental conditions. There is little published information about the factors that influence the efficiency of transmission of *D. rabiei* from infected seed. In general, disease development is influenced by the plant, the pathogen, the environment and human activity (Agrios, 1997). We examined the hypothesis that these components can also influence transmission of disease from infected seed. In a series of glasshouse and growth room trials, the role of incidence of seed infection (pathogen), cultivar resistance (host), soil temperature (environment) and seed sowing depth (human activity) in transmission was investigated. These experiments were conducted on desi-type chickpea, which is grown throughout the world.

Materials and methods

Cultivars of desi type chickpea were chosen based on the availability of clean seed and their susceptibility to ascochyta blight, according to disease screening trials conducted at SARDI (Waite Campus) in 1997–1999, based a quantitative 9-point rating scale (Reddy et al., 1981). Four commercially available cultivars; Desavic (highly susceptible – HS), Dooen (susceptible – S), Heera (S), Howzat (moderately susceptible – MS) and two advanced breeding lines; ICC 12004 and ICCV 96836 (both moderately susceptible / moderately resistant – MS/MR) were chosen. University of California (UC) potting mix (Baker, 1957) was used throughout.

To obtain the quantities of uniformly infected seed required for these experiments, artificially inoculated (AI) chickpea seed was used (Kaiser et al., 1973). Four isolates of *D. rabiei* were selected from different chickpea growing regions within South Australia to provide inoculum. They were cultured from storage in sterile distilled water by placing 100 μl aliquots of the suspension onto potato dextrose agar (PDA) and incubated at room temperature (approximately 22 °C) for 2–3 weeks under blacklight and fluorescent light to produce pycnidiospores. Plates were then flooded with 5 ml of sterile reverse osmosis (RO) water, the surface was gently scraped to suspend the pycnidiospores, then the suspensions were poured into a sterile beaker containing a magnetic stirrer, to give a combined inoculum of four isolates. The concentration of pycnidiospores was adjusted to approximately 2.5×10^7 spores ml^{-1} using a haemocytometer.

The following seed inoculation method was modified from that described by Kaiser et al. (1973). Seeds were surface-sterilised by submerging in 1.0% sodium hypochlorite for 5 min then rinsed three times with sterile RO water, placed on sterilised filter paper and allowed to dry for 3 h in a laminar flow cabinet. They were then submerged in the suspension of *D. rabiei* pycnidiospores and placed in an implosion-proof desiccation bowl (25 cm diameter). The bowl was sealed and placed under vacuum using −70 kpa suction. The vacuum was applied and released several times before being held for 3 h. Seeds were then drained through sterile muslin cloth, placed on sterilised, dry germination paper and air-dried for 24 h in the laminar flow cabinet. Non-inoculated (NI) control seed, used to monitor the effect of this process on seed health, was prepared as above except that surface-sterilised seeds were placed in sterile RO water instead of in a spore suspension.

Role of sowing depth in transmission

Two glasshouse trials were conducted to examine the influence of seeding depths of 1, 3 and 6 cm on transmission from AI seed of cvs Heera and Dooen, examined separately. Arborcell Easy-grower 50® trays, consisting of 50 moulded cells (0.14-l pots) per tray, were filled with UC potting mix. Holes for planting were prepared by pushing a 15 cm long × 2 cm diameter probe, limited with a rubber bung at the desired sowing depth, into slightly moistened potting mix. Fifty AI seeds (100% infection) and 50 NI seeds were placed at each depth, one seed per pot, and holes were then filled with potting mix.

The first experiment (cv. Heera) was arranged as a completely randomised block design with four replications. Immediately after sowing, the potting mix was brought to field capacity, then watered overhead daily. Emergence was counted 10 days after sowing. When seedlings in each treatment reached a mean height of approximately 3 cm, the trays were placed in a humidity chamber fitted with automatic overhead misters and misted for 1 s at 5 min intervals for 4 days, before being returned to the glasshouse. Disease incidence on emerging seedlings was assessed 14 days after removal from the humidity chambers and severity recorded on a 0–3 rating scale where 0 = no disease, 1 = small leaf/stem lesion, 2 = lesion(s) girdling stem and 3 = multiple stem lesions, seedling re-shooting.

The second experiment (cv. Dooen), was conducted 1 month later, also as a completely randomised block design with four replications as described above, with the exception that disease on emerging seedlings was assessed 5, 10 and 14 days after removal from the humidity chambers. Following disease ratings, plants were extracted from the pots and symptoms on below-ground parts were recorded. Representative samples of infected tissue from lesions occurring below the soil surface were surface-sterilised in 1.0% sodium hypochlorite for 30 s, rinsed in sterile RO water then drained and cultured on PDA.

Influence of incidence of seed infection on transmission

An experiment was conducted in the glasshouse to examine the effect of four incidences of seed infection, 0 (control), 2, 26 and 100% AI seeds, on disease transmission in cv. Dooen. Using 50 seeds for each treatment, seed infection levels were represented by planting known proportions of AI seed and healthy NI seed. The experiment was arranged as a completely randomised design with four replications. Arborcell Easy-grower 50® trays were used, filled with UC potting mix. AI seeds were randomly allocated to cells within a tray for each treatment and replicate and the position of the AI seed(s) in each tray was recorded to cross-reference with the incidence of disease transmission. NI seed were planted in the remaining cells. One seed was sown per cell at a depth of 3 cm. Immediately after sowing the potting mix was brought to field capacity, then watered overhead daily. Seedlings were subjected to high humidity as described above, then returned to the glasshouse. Disease on emerging seedlings was assessed 15 days after removal from the humidity chambers as described above.

Influence of host susceptibility on infection of seed

Seed that had been artificially inoculated with *D. rabiei* was tested to assess infection, using a method based on that described by Haware et al. (1986). A similar protocol is recommended by the International Seed Testing Association (1996). A sub-sample of 40 AI seeds of cvs Desavic, Dooen, Heera and Howzat and breeding lines ICC 12004 and ICCV 96836 were placed on PDA in four 9 cm Petri dishes, 10 seeds per dish. In addition, a sub-sample of 40 AI seed for each genotype was surfaced-sterilised in 2% sodium hypochlorite for 2 min before plating. The experiment was arranged as a randomised complete block design with four replicate plates and factorial treatment structure corresponding to seed genotype and treatment, with or without surface-sterilisation. Plates were incubated at 22 °C with a 12 h photoperiod of blacklight, cool-white and Tri-Phosphor® fluorescent lighting for 2 weeks. The proportion of seeds that germinated and that was infected with *D. rabiei* was recorded. *Didymella rabiei* was identified on the basis of colony morphology on PDA and microscopic examination of spore morphology at 400× with a compound microscope.

Influence of host susceptibility on disease transmission

The influence of host resistance on disease transmission was examined in the glasshouse using cvs Desavic (HS), Dooen (S), Howzat (MS) and breeding line ICCV 96836 (MS/MR) using AI and NI seeds prepared for each genotype as described above. The experiment was arranged as a randomised complete block design with four replications, with the exception of the controls (NI seed), which were included only in the first two replications due to limited space. Seeds were sown in Arborcell Easy-grower 50® trays filled with UC potting mix. Each treatment consisted of 50 seeds per cultivar, one seed per cell, sown at a depth of 3 cm. Immediately after sowing, the potting mix was brought to field capacity, then watered overhead daily. Seedlings were subjected to high humidity as described above, and then returned to the glasshouse benches. At this stage, plant height was recorded to determine vigour. Disease was assessed 5 and 15 days after removal from the humidity chambers as described above. The trial was repeated 1 month later. Data from the two trials were combined and subjected to analysis of variance allowing for effects of experiment and replication.

Effect of soil temperature on transmission

The incidence of transmission at soil temperatures of 5, 9, 14 and 19 °C was examined by manipulation of air temperature within an Environ Air controlled environment chamber (100×120×145 cm), utilising fan-forced refrigeration. Artificial lighting was provided by two cool white 115W fluorescent tubes (VHO Sylvania, F48T12) and four metal halide bulbs (Venture HIE 150W/C/U) controlled by a timer set for a 12 h photoperiod. Each trial was conducted at a desired constant air and soil temperature, monitored using a data logger (Tinytag plus, Hastings Data Loggers). Each treatment was repeated in time, constituting a second replicate. The treatments were conducted in a random order. The effect of soil temperature on transmission was examined for cvs Heera and Dooen, tested separately at each temperature setting. The experiments were conducted as a randomised complete block design with sub-sampling (trays). Each trial consisted of five trays (45×32×6 cm), each containing 20 punnets (180 ml) filled with UC potting mix. Four trays were sown with 20 AI seeds in each, and one tray with 20 NI control seeds, one seed per punnet at a depth of 2 cm. The trays were randomly allocated positions within the cabinet. When seedlings reached a mean height of approximately 3 cm, emergence was recorded and they were transferred to a humidity chamber fitted with automatic overhead misters for 4 days, as above, then placed on benches in the glasshouse. Seedlings were assessed for disease 18 days after removal from the humidity chambers. Plants were then extracted from the potting mix, root systems washed, and symptoms of infection by *D. rabiei* on below-ground parts of the plants recorded. Representative samples of infected plant tissue from lesions occurring below the soil surface were cultured onto PDA.

Statistical analysis

Data from each trial were analysed separately using a simple linear model – analysis of variance (ANOVA), 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. In addition, the R^2 value and corresponding intercept and slope were also reported for the strength of the relationship between rate of seed infection and incidence of diseased seedlings. This model was fitted using Microsoft Excel® for Windows 95®.

Results

Role of sowing depth in transmission

Emergence from AI seed of cv. Heera in trial 1 was poor, 40–50%, significantly ($P \leqslant 0.05$) less than that for NI seed (70–75%). Therefore, a second trial (trial 2) was conducted using cv. Dooen. Emergence of cv. Dooen was approximately 98% for both AI and NI seed across all sowing depths. Seedlings emerging from NI seed were healthy and the data were, therefore, omitted from the statistical analysis. Characteristic symptoms of infection by *D. rabiei*, comprising small lesions on leaves, or

dark, sunken lesions on stems with pycnidia, were visible on seedlings assessed 14 days after removal from humidity chambers.

There was no significant effect ($P > 0.05$) of sowing depth on the incidence of transmission of disease from infected seed to the resulting seedlings of cvs Heera or Dooen. Disease transmission to seedlings of cv. Heera was 10.2, 2.7 and 8.2% where sowing depth was 1, 3 and 6 cm, respectively. Disease transmission to seedlings of cv. Dooen (emergence approximately 60% more than of cv. Heera) was 20.6, 19.3 and 13.8% where sowing depth was 1, 3 and 6 cm, respectively. There were no significant differences in the incidence of disease transmission between sowing depths recorded 5, 10 and 14 days after removal from humidity chambers. However, the incidence of stem lesions (as opposed to leaf and petiole lesions) was less on plants sown at 6 cm than at 1 and 3 cm ($P \leq 0.05$) in cv. Dooen, when plants were assessed 14 days after removal from high humidity (data not shown).

When plants were extracted from pots 17 days after removal from humidity chambers, some showed dark or black sunken lesions, mainly on the epicotyl near the seed but below the soil surface. No lesions were recorded on controls. Between 3 and 6% of plants that developed from AI seed exhibited this symptom. Approximately 20% of tissue samples from lesions yielded *D. rabiei*, whereas the remainder yielded various soil-borne fungi such as *Penicillium* species. There was no significant effect ($P > 0.05$) of sowing depth on the incidence of these lesions below the soil surface.

Some plants with lesions below the soil surface did not exhibit symptoms either on the main stem or secondary shoots (Figure 1a). In some instances, plants with below-ground infection, and which formed secondary shoots, developed symptoms on the emerging secondary shoot (Figure 1b). Occasionally, this resulted in the spread of disease from the infected secondary shoot to the upper part(s) of the primary shoot (Figure 1c), perhaps as a result of splash during overhead watering.

Influence of incidence of seed infection on transmission

Approximately 18–20% of seedlings emerging from AI seed of cv. Dooen showed ascochyta blight symptoms 15 days after removal from high humidity. No disease was observed in the NI control treatment and a strong, positive relationship ($P < 0.001$) was observed between increasing incidence of seed infection and the incidence of

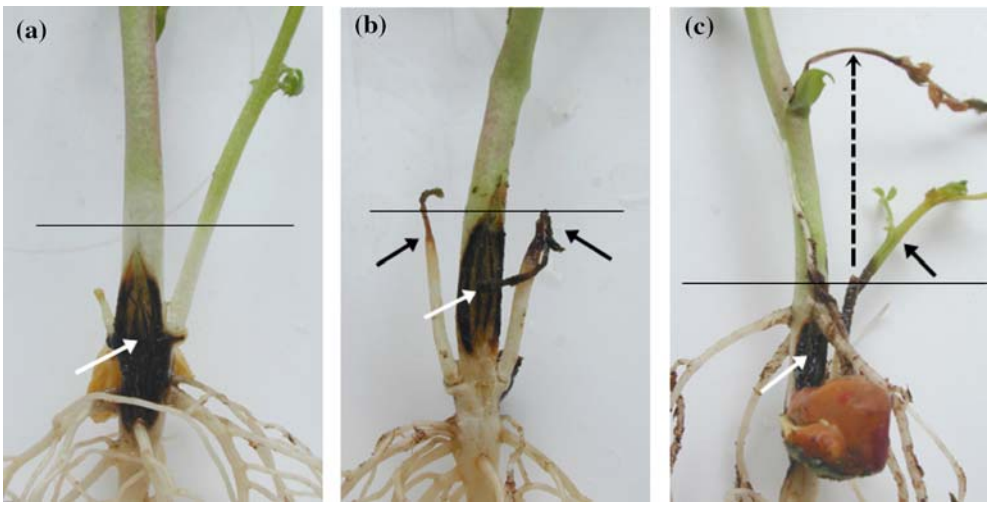

Figure 1. Lesions observed below the soil surface (represented by black line) on chickpea seedlings (cv. Dooen) from artificially inoculated seed, 17 days after removal from humidity chambers. (a) Above-ground plant parts without symptoms. (b) Secondary shoots infected with *D. rabiei*. (c) Infection on secondary shoot resulted in spread of disease to primary shoot. White arrows indicate below-ground lesions, black arrows indicate secondary shoots, broken arrow indicates disease spread to main shoot from infected secondary shoot.

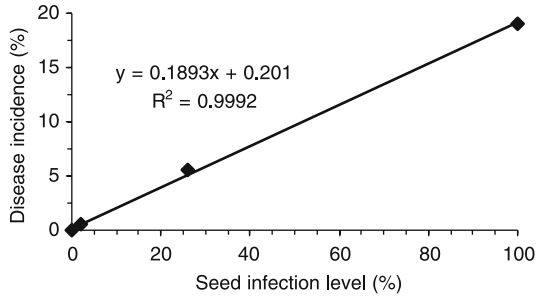

Figure 2. Positive relationship ($P < 0.001$) observed between seed infection level and disease transmission from artificially inoculated seed of chickpea cv. Dooen, 15 days after removal from high humidity. LSD(0.05) = 4.8.

diseased seedlings. A trend line fitted to the data ($R^2 = 0.9992$) shows that a 10% increase in the incidence of seed infection level resulted in a 1.9% increase in the number of seedlings infected with *D. rabiei* (Figure 2).

Influence of host susceptibility on infection of seed

Didymella rabiei was isolated from 100% of non-surface sterilised AI seeds of all cultivars plated on PDA. However, the pathogen was not isolated from some AI seeds that had been surface-sterilised before plating. There was a significant interaction ($P \leq 0.05$) between surface-sterilisation and cultivar/line. Only the highly susceptible cv. Desavic exhibited 100% seed infection after surface-sterilisation. A significant difference ($P \leq 0.05$) was observed in the number of seeds exhibiting infection following surface-sterilisation. *Didymella rabiei* was isolated from 58.5% of surface-sterilised seed of breeding line ICCV 96836, compared to 90% for cv. Dooen, 95% for cvs Heera and Howzat and 97.5% for breeding line ICC 12004.

Influence of host susceptibility on disease transmission

Cultivars Desavic, Howzat and the breeding line ICCV 96836 showed a significant reduction ($P \leq 0.05$) in emergence from AI seed compared to NI control seed, whereas cv. Dooen did not, which resulted in an interaction between cultivar and inoculation treatment ($P \leq 0.05$). There was a significant difference ($P \leq 0.05$) in plant emergence among cultivars regardless of treatment, with least to most emergence observed for cvs Desavic, Howzat, ICCV 96836 and Dooen, respectively.

Symptoms of ascochyta blight on seedlings of the four cultivars/lines were observed at 5 and 15 days after exposure to high humidity, as lesions on leaves or on stems, with pycnidia clearly visible. Using the combined data from both trials, a significant difference ($P \leq 0.05$) was observed in disease transmission between cultivars/lines that were HS to MS/MR to the disease. Disease transmission on seedlings emerging from infected seed was 12–18% (Figure 3). The MS/MR breeding line ICCV 96836 showed the highest percentage of plants infected with *D. rabiei* (Figure 3). Cultivar. Dooen showed considerable variation between trial 1 and trial 2, exhibiting disease transmission levels of 22% and 7%, respectively.

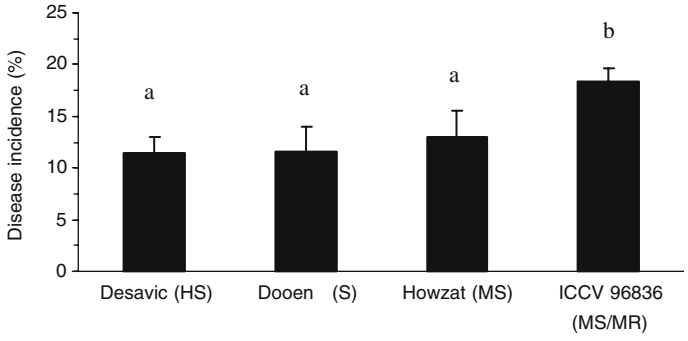

Figure 3. Disease transmission from artificially inoculated seed observed 15 days after removal from high humidity on three cultivars and one breeding line of chickpea with different degrees of susceptibility to ascochyta blight. Values are the means of duplicate experiments, each with four replicates / treatment, error bars represent the standard error. Values with the same letter are not significantly different from one another ($P > 0.05$).

There was no significant difference ($P > 0.05$) in disease severity between the cultivars/lines. However, on average, stem lesions occurred at twice the frequency of leaf lesions in all cultivars (data not shown).

Effect of soil temperature on transmission

The experiments investigating the effect of soil temperature on disease transmission were conducted over 15 months. The duration of each experiment varied as the temperature influenced the rate of plant growth and, consequently, so did the time when the trays were removed and placed into high humidity. The decision to include a second cultivar (Dooen) was made after initial experiments indicated poor emergence of cv. Heera, which reduced the number of seedlings available for assessment. The simple linear models used to analyse results for either emergence or disease transmission took into account the effect of differences between trays in the experimental design.

The effect of temperature on plant emergence was not significant ($P > 0.05$) for cvs Dooen or Heera. Cultivar Dooen showed better emergence than cv. Heera in all experiments. Additionally, neither cultivar showed a significant difference in emergence ($P > 0.05$) between AI and NI control seed.

There was a significant effect of soil temperature on disease transmission ($P \leq 0.05$) in cv. Heera, with transmission more frequent at soil temperatures of 5 and 14 °C and less frequent at 9 and 19 °C (Figure 4). The effect of soil temperature on disease transmission in cv. Dooen was similar (Figure 4), though differences were not significant ($P > 0.05$), and transmission rates were lower than for cv. Heera. Greater variation ($P \leq 0.05$) between trays in disease transmission was observed for cv. Heera as shown by standard error bars in Figure 4. No lesions were recorded on control plants at any soil temperature.

When plants were removed from pots 18 days after removal from humidity chambers, many showed lesions on the epicotyl below the soil surface level. Approximately 20% of infected tissue sampled resulted in cultures identified as *D. rabiei*, with the majority of samples yielding other fungi, such as *Penicillium* species. In some instances, plants with below-ground lesions formed secondary shoots which, on emergence, were infected with *D. rabiei* even though the disease was not evident on the primary shoot.

Data for below-ground lesions on cv. Heera were not amenable to statistical analysis. In cv. Dooen, there was a significant effect of soil temperature ($P \leq 0.05$) on the percentage of plants with below-ground lesions. Between 6% and 54% of plants exhibited lesions on the epicotyl. The incidence of below-ground lesions on cvs Heera and Dooen showed a trend similar to that observed for disease transmission above ground.

Discussion

The frequency of disease transmission and, consequently, the factors that affect it, can influence

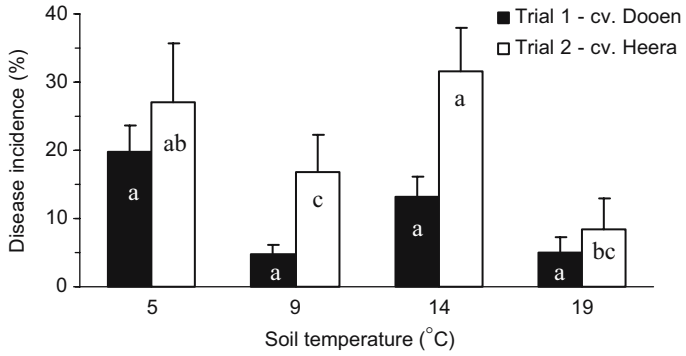

Figure 4. Influence of soil temperature on disease transmission from artificially inoculated seed of cv. Dooen (trial 1) and cv. Heera (trial 2) 18 days after removal from high humidity chambers. Values are the means of two replicates / treatment, error bars represent the standard error for disease transmission. In each trial, values with the same letter are not significantly different from one another ($P > 0.05$).

the initial stages (or timing) of development of an epiphytotic. An understanding of factors that influence transmission is important for diseases in which infected seed is the major source of inoculum. Transmission from infected seed provides an early onset for disease introduction, creating a substantial time, beginning soon after plant emergence, for development of an epiphytotic.

Seed to seedling transmission depends largely on the type of seed infection (Maden et al., 1975; Dey and Singh, 1994). Our experiments examining the role of host susceptibility on seed inoculation showed that 100% of AI seed was infected with *D. rabiei*, and the fungus had penetrated into the seed coat, as shown by high seed infection levels after surface-sterilisation. In MS cultivars, there were apparent differences in the level of seed infection expressed after surface-sterilisation, perhaps because penetration of the seeds by *D. rabiei* spores during inoculation was influenced by morphological barriers within the seed or seed quality, such as age (Neergaard, 1977). Surface-sterilisation was not used in subsequent disease transmission studies as commercial chickpea growers would not use it routinely. However, the reduction in seed infection observed in some cultivars after treatment does illustrate the role that seed treatments can have in reducing seed infection, particularly superficial contamination.

Reduced emergence was observed for chickpea seed infected with *D. rabiei* compared to healthy controls for the HS cv. Desavic or where seed quality was poor (cv. Heera). Disease transmission from AI seed ranged from 5% to 35%. Likewise, Halfon-Meiri (1970) reported reduced germination and emergence of naturally infected seeds, with disease transmission of approximately 25%. Studies conducted by Kaiser and Hannan (1988) using AI seeds showed disease transmission of approximately 35%. Disease transmission in field studies, using AI seed of cvs Howzat, Desavic and Dooen, was 5–14% (Kimber, unpublished data).

There was no significant effect of sowing depth, in the range of 1–6 cm, on the incidence of disease transmission. These sowing depths reflect those generally adopted in Australian farming systems to utilise soil moisture and promote seedling vigour in varying soil types. In comparison, previous studies have focussed on the effect of sowing seed deeper than 6 cm. Reddy (1983) stated that sowing at 15 cm and deeper rendered seed-borne inoculum ineffective as the disease could not be expressed above ground. Likewise, Singh et al. (1992) reported that when infected seeds were sown 10 cm deep, disease transmission was reduced compared to shallow sowing. Sowing chickpeas to depths of 10–15 cm would not typically be recommended in modern agricultural practices. However, our results did show that transmission observed on seedlings, 14 days after removal from humidity chambers, exhibited a lower proportion of stem lesions than leaf and petiole lesions when seeds were sown 6 cm deep, compared to more shallow sowing.

Reddy (1983) reported lesions below the soil surface on seedlings during investigation of the influence of sowing depth on disease transmission. However, there is very little literature on this aspect of disease transmission and further research is needed to elucidate its impact on plant development. Lesions below the soil surface were observed on plants from AI seed and not on the controls, although only 20% of the lesions sampled yielded *D. rabiei*, possibly due to competition from other fungi and bacteria. In some instances, secondary shoots that emerged from plants with lesions below the soil surface were infected with *D. rabiei*, perhaps as a result of contact with the infected seed. This form of infection could lead to a delayed introduction of the disease to above-ground parts of the plant.

The proportion of diseased seed required to pose a significant threat of an epiphytotic in optimal environmental conditions is often referred to as the 'inoculum threshold', and the importance of planting disease-free seed to reduce the incidence of the disease is well documented (Kaiser, 1984; Kaiser, 1992; Haware, 1998). Kaiser (1992) stated that very low levels of infection (0.01–0.1% infected seed) could cause epidemics when weather conditions are favourable. The glasshouse experiment on the influence of incidence of seed infection on transmission in this study showed a positive correlation between the proportion of infected seed and the incidence of transmission of the disease to above-ground plant parts. Although the levels of infected seed examined (2, 26 and 100%) were greater than those noted by Kaiser (1992), the relationship confirmed the importance of selecting seed-lots with low incidence of infection to reduce disease introduction. This was evident in the ascochyta blight epidemics in 1998 throughout

southeastern Australia, attributed in part to the planting of infected seed harvested in 1997 (Ramsey et al., 1999).

The influence of host resistance on transmission of disease from seed infected by fungi or bacteria has not been documented (McGee, 1995). In this study, there was no obvious relationship between host resistance and disease transmission from *D. rabiei*-infected seed. The MS/MR breeding line ICCV 96836, which is being considered for commercial release in Western Australia, showed the highest level of disease transmission. This line may be susceptible to the disease during the early stages of development, particularly during germination and emergence. There was a high level of variation in disease transmission for cv. Dooen, but the basis for this variation is not known. In seedlings exhibiting disease transmission, stem lesions were more common than leaf lesions for all cultivars tested. Chongo and Gossen (2001) reported similar responses. Further research is required to examine the influence of true 'resistance' on transmission of disease via infected seed, with particular focus on field experiments examining chickpea germplasm collections.

Disease transmission was greater in soil at 5 and 14 °C than at 9 and 19 °C, an unexpected response. The same trend was observed for the incidence of below-ground lesions, though this aspect of transmission was not considered in detail. Gossen and Morrall (1986) showed a significant negative relationship between soil temperature and disease transmission from lentil seed infected with *A. lentis*. Our results indicated that transmission of ascochyta blight from infected seed was variable, with no obvious relationship with soil temperature. However, the same trend was observed for both cultivars tested. Alternatively, it may reflect a complex interaction between either soil temperature and moisture or the interaction between optimal conditions required by the plant and pathogen. The most rapid disease development usually occurs when the temperature is optimum for the development of the pathogen but above or below the optimum for the development of the host (Agrios, 1997).

Disease transmission in glasshouse and growth-room trials was approximately 15%, even at low levels of seed infection. This study investigated certain factors that may influence the efficiency of disease transmission, chosen based on current knowledge of disease management and epidemiology of ascochyta blight of chickpea. There was no obvious influence of sowing depth or host resistance on transmission. The incidence of diseased seedlings from seed-borne infection was, however, influenced by soil temperature and incidence of seed infection, although further study is needed to clarify the relationship between soil temperature and disease transmission. Field experiments to investigate the interaction of host resistance and seed infection level, particularly with naturally infected seed, would elucidate transmission in field conditions and the impact of seed-borne inoculum on the early stages of a developing epiphytotic.

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