Didymella rabiei, the teleomorph of *Ascochyta rabiei*, found on chickpea stubble in Western Australia

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Abstract. Trapping *Didymella rabiei* ascospores identified chickpea stubble with naturally occurring pseudothecia. Ascospores produced typical *Ascochyta rabiei* cultures on PDA+G. Ascochyta blight developed on chickpea plants inoculated with conidia derived from single ascospore cultures. This is the first record of the teleomorph, *D. rabiei*, in Australia. Its presence has implications in the long distance dispersal and epidemiology of ascochyta blight.

Chickpea blight, caused by *Ascochyta rabiei*, was first introduced into Australia in 1973 on infected seed used in evaluation trials at the Waite Institute in South Australia. This outbreak was successfully eradicated and the country was considered to be free of chickpea blight until 1995 when the presence of *A. rabiei* was confirmed on commercial crops in South Australia (Kaiser 1997; Khan *et al.* 1999). Subsequent testing of seed lots indicated that *A. rabiei* had actually been present in South Australia since 1991 (Khan *et al.* 1999). From 1995 onwards chickpea blight occurred at low levels in South Australia and by 1997 40% of South Australian crops had some level of infection. During the 1998 season the disease reached epidemic proportions causing wide spread crop loss in South Australia and parts of Victoria.

Ascochyta blight was first detected in Western Australia in a seed crop, near Greenough, in 1998. The crop was destroyed and the paddock placed under quarantine restrictions in an effort to eradicate the disease. In 1999 the disease became widespread and severe in the northern agricultural region, in an area bound by Geraldton, Mullewa, Morawa and Dongara. Less severe outbreaks occurred in the central agricultural region near Merredin and an infected crop was positively identified as far south as Quindanning, near Williams. By the end of the 2000 growing-season the disease was identified in all the chickpea growing regions of Western Australia except for the Ord River production area, where it has not yet been recorded.

Stems from a chickpea crop naturally infected with *A. rabiei* during the 2001 growing-season were collected from near Dowerin, Western Australia in December 2001 after the crop was harvested. The chickpea stubble was stored dry in large sacks in an enclosed shed until April 2002 when sub-samples of stubble with visible ascochyta blight lesions were placed in nylon mesh pockets on the soil surface at Pingrup, near Katanning.

At fortnightly intervals from mid-May onwards the chickpea stubble was examined in the laboratory for the presence of *D. rabiei* ascospores by means of wind tunnels fitted with rotor-rod spore traps. A known weight of stubble (20 stubble pieces/pocket) was soaked in tap water for 5 min, placed in the wind tunnel and left to discharge ascospores for one hour. Ascospores were trapped onto adhesive tape on rotor-rods. Semi-permanent microscope slides were made by mounting the spore trap tapes in lactic-glycerol (1:1:2 lactic acid: glycerol: water). The tapes were inspected under the light microscope (400x) and the dimensions and number of ascospores trapped were recorded.

Spore trapping indicated the presence of low numbers (<50 ascospores/g of chickpea stubble) in May and June. On the 10 July the numbers of ascospores trapped increased significantly to an estimated 1396 ascospores/g of stubble. Inspection of the 20 pieces of stubble from the spore trap revealed the presence of dark brown-black, erumpent fruiting bodies with inconspicuous ostioles that contained eight-spored asci. Ascospores were hyaline, two-celled, with the upper cell broader than the lower cell, constricted at the septum and 16 (12.5-22) µm x 6 (5-7) µm in size. The length of the ascospores are larger than the 9.5-16 µm range described for *D. rabiei* by Trapero-Casas and Kaiser (1992).

Ascospore derived cultures were obtained by squashing individual pseudothecia in sterile distilled water on a microscope slide and examining the slide microscopically to confirm that ascospores conformed to the above description and to ensure that no conidia were present. The ascospores were washed off the slide onto potato-dextrose agar plus gentamicin (2 ml/L) (PDA+G) and spread evenly over the surface. The plates were incubated at 20°C with a 12 h photoperiod for 4 days. Individual, 2 mm diameter colonies (single ascospore isolates) were transferred onto PDA+G; 8 isolates were obtained in this way (WAC 10259 - 10266, Western Australian Department of Agriculture Culture Collection). After 8 days incubation single conidial isolates were obtained by streaking conidial ooze onto PDA+G.

On PDA+G typical *A. rabiei* cultures developed. These produced pycnidia containing hyaline, predominantly aseptate conidia 12 (10-15) µm x 4.5 (3.5-5) µm in size. The mean conidial width is wider than 3.5 µm given in the CMI description No 337 for *A. rabiei* (Punithalingam & Holliday 1972).

For inoculation of chickpea plants, week old sub-cultures on PDA+G were used to produce spread plates on chickpea meal-dextrose agar (CDA) of the 8 ascospore derived isolates and a conidial derived isolate (WAC 10258, Western Australian Department of Agriculture Culture Collection) from Nanson, near Geraldton, that had previously been shown to be pathogenic on chickpeas (Galloway, unpublished data). CDA plates, incubated for 5 days at 20°C with a 12 h photoperiod, were flooded with sterile distilled water and *A. rabiei* conidia harvested. Conidia suspensions ($5x10^5$ spores/mL) were sprayed onto 2 week old chickpea plants (cv. Norwin) and incubated in a humidity chamber at 20°C and >95% RH for 48 h. Plants were transferred to a glasshouse (20°C) for symptom development. Chickpea plants inoculated with conidia of the 8 ascospore derived isolates and the conidia derived isolate developed typical ascochyta blight lesions with pycnidia containing predominantly aseptate conidia measuring 12 (10-17.5) x 4.5 (3.5-5) µm.

In many of the chickpea producing areas of the Northern Hemisphere *Didymella rabiei*, the teleomorph of *A. rabiei*, has been found in chickpea residue. *Didymella rabiei* is heterothallic, requiring both mating types (MAT1-1 and MAT1-2) to be present for the development of fertile pseudothecia (Trapero-Casas and Kaiser 1992). In Australia only the MAT1-1 mating type has been identified by pairing conidial isolates obtained from South Australia and Victoria with the reference isolates (Kaiser *et al.* 1997; Khan *et al.* 1999; Nasir *et al.* 2000). That the MAT1-2 mating type has so far been undetected led Khan *et al.* (1999) to suggest that the teleomorph is not present in Australia.

The natural occurrence of *D. rabiei* on chickpea stubble implies that either both compatible mating types are present in Western Australia or that a low level of homothallic compatibility exists in *A. rabiei*. Determination of the mating types of the isolates obtained in this work is being pursued.

The teleomorph can play an important role in disease spread. Ascospores are wind dispersed and are considered to be the main means (other than seed infection) by which long-distance spread of the fungus occurs. Kaiser (1997) indicated that ascospores might initiate disease at a distance of several kilometres from the source of infection. On the basis of this preliminary investigation, it appears that under Western Australian conditions, pseudothecia of *D. rabiei* develop during winter on the previous season's stubble and ascospore release can occur from mid-winter onwards.

The presence of the sexual stage of *A. rabiei* may also lead to greater variability in the pathogen and thus reduce the stability or expression of resistance in the host.

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