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Alternative hosts and plant tissues for the survival, sporulation and spread of the Ascochyta blight pathogen of chickpea

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Abstract Various crop and weed species were infected naturally by *Didymella rabiei* (anamorph: *Ascochyta rabiei*) in blight-affected chickpea fields in the Palouse region of eastern Washington and northern Idaho, USA. The fungus was isolated from asymptomatic plants of 16 species commonly found in commercial crops in this region. Isolates of the pathogen from crop and weed species were pathogenic to chickpea and indistinguishable in cultural and morphological characteristics from isolates of *D. rabiei* from chickpea. Both mating types of *D. rabiei* were isolated from eight naturally infected plant species. Chickpeas were infected by *D. rabiei* when plants emerged through infested debris of seven crop and weed species. The teleomorph developed on overwintered tissues of seven plant species infected naturally by *D. rabiei* in a blight screening nursery and on debris of wheat, white sweet clover and pea inoculated with ascospores of *D. rabiei* or conidia of two compatible isolates of the pathogen. *Didymella rabiei* naturally infected 31 accessions of 12 *Cicer* spp. and the teleomorph developed on the overwintered debris of all accessions, including those of three highly resistant perennial species. The fungus developed on the stem and leaf pieces of ten plant species common to southern Spain inoculated with conidia of two compatible isolates of *D. rabiei,* and formed pseudothecia with asci and viable ascospores on six of ten species and pycnidia with conidia on all plant species.

Keywords *Ascochyta rabiei* · Asymptomatic hosts · *Cicer arietinum* · *Didymella rabiei*

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Introduction

Ascochyta blight of chickpea (*Cicer arietinum*) caused by *Didymella rabiei* (Kovachevski) v. Arx (syn. *Mycosphaerella rabiei*) [anamorph: *Ascochyta rabiei*] is one of the most important diseases of chickpea in many regions of the world (Nene and Reddy 1987). The fungus affects all aerial parts of the plant at any stage of development, causing necrotic lesions and blight that can result in death of the plant under favourable environmental conditions. Pycnidia develop profusely in the lesions and produce masses of conidia that serve as inoculum for secondary infections. Infected seed is important in the spread and survival of the pathogen (Nene and Reddy 1987;

Kaiser 1992). The fungus is heterothallic and requires the pairing of two compatible mating types for the teleomorph to develop (Tapero-Casas and Kaiser 1992a; Wilson and Kaiser 1995). To date, the teleomorph has only been found on chickpea debris that overwintered on the soil surface when both compatible mating types were present. The primary hosts of *D. rabiei* are species of the genus *Cicer*, particularly the cultivated chickpea, *C. arietinum.* Researchers have attempted to infect various plant species with *D. rabiei,* mostly under greenhouse conditions. Sprague (1930) was the first to report his failure to infect several plant species, including bean (*Phaseolus vulgaris*), lentil (*Lens culinaris*) and pea (*Pisum sativum*). Zachos et al. (1963) and Tripathi et al. (1987) also were unable to infect other plant species with *D. rabiei*. However, other investigators have demonstrated that the pathogen is capable of infecting different plant species, although without disease symptoms. Kaiser (1973), Nene and Reddy (1987), and Khan et al. (1999) were able to infect several leguminous and non-leguminous plant species. Kaiser (1990) reported that *D. rabiei* was isolated from 13 asymptomatic plant species collected from blighted chickpea fields or from plantings where debris from diseased chickpeas remained on the soil surface overwinter in the Palouse region of eastern Washington (WA) and northern Idaho (ID), USA. In Italy, Montorsi et al. (1992) isolated *D. rabiei* from naturally infected seeds of berseem clover (*Trifolium alexandrinum*). Hernandez-Bello et al. (2006) demonstrated that legume-associated *Ascochyta* spp. are host-specific, although they can infect other legumes species without causing any symptoms. In that study, *D. rabiei* was recovered consistently from inoculated tissues of pea. No information is available on the role played by the teleomorph in the infection and survival of the pathogen on alternative hosts or on its development on overwintered debris of these hosts and non-host plants. The objectives of this study were to (i) survey the plant species infected naturally by *D. rabiei* in blight-affected chickpea fields in the Palouse region; (ii) establish if the naturally-infested tissues were infective to chickpea; (iii) determine if the teleomorph developed on overwintered tissues of the alternative hosts; (iv) observe if the ascospores of *D. rabiei* from alternative hosts could infect chickpeas; and (v) quantify asexual and sexual reproduction of *D. rabiei* on infested debris of several plant species.

Materials and methods

Isolation of *Didymella rabiei* from naturally infected plant species

When these host range studies were conducted in the mid-1980s to early 1990s, Ascochyta blight was a serious problem in the Palouse region of eastern WA and northern ID (Trapero-Casas and Kaiser 1992a) and southern Spain (Trapero-Casas et al. 1996). Nineteen crop and weed species commonly found in and around chickpea fields were collected from three sites in the Palouse region during 1988–1992. These sites were: (a) a commercial chickpea field with severe Ascochyta blight near Dayton, WA; (b) a wheat field near Genesee, ID where the previous chickpea crop was severely damaged by the disease; and (c) a chickpea Ascochyta blight screening trial at Spillman Farm, Pullman, WA. The common names of weed species mentioned in the text are those often used in the western USA (Whitson 2006).

Asymptomatic plants of nineteen species (Table 1) at full bloom were selected at random to determine if they were infected by *D. rabiei*. Plant tissues were washed in running tap water and surface-disinfested in a domestic bleach solution (0.5% Na hypochlorite) for 5 min (Dhingra and Sinclair 1995), then dried on paper towels. Small pieces of tissue were plated on 2% water agar (WA) in plastic Petri dishes and incubated at 21 to 23°C under fluorescent lights (12-h photoperiod, 77 μmol $m^{-2}s^{-1}$). Hyphal tips of fungi emerging from the tissues were transferred to potato dextrose agar (PDA, Difco Laboratories, Detroit, MI) and incubated as described above. Fungi were identified as *D. rabiei* based on cultural and morphological characteristics (Punithalingham and Holliday 1972), and on pathogenicity tests.

Natural infection of *Cicer* spp.

Seeds of 31 accessions of 12 *Cicer* spp. were planted in a chickpea Ascochyta blight screening trial at Spillman Farm, Pullman, WA (Table 2). The experimental plot was a 1 m row with three to five plants of each accession. There were three replications in a complete block design. The *Cicer* spp., with the number of accessions tested in parenthesis, were three perennial species, *C. anatolicum* (2), *C. microphyllum* (1) and *C. oxyodon* (1), and nine annual species, *C. arietinum* (6),

Table 1 Isolation of *Didymella rabiei* from various plant species growing in three fields in the Palouse region of eastern Washington and northern Idaho, USA[a]

Plant species	Common name	No. pieces of tissue[b]	Total infected	Infection (%)
(a) Chickpea field				
Anthemis cotula	Dog fennel	48	1	2.0
Brassica nigra	Black mustard	72	1	1.4
Capsella bursa-pastoris	Shepherd's purse	24	0	0
Chenopodium album	Common lamb's quarters	24	0	0
Cicer arietinum	Chickpea	24	16	66.7
Lactuca serriola	Prickly lettuce	120	0	0
Lamium amplexicaule	Henbit	72	1	1.4
Pisum sativum	Pea	72	1	1.4
Solanum nigrum	Black nightshade	96	1	1.0
Triticum aestivum	Wheat	48	1	2.0
(b) Wheat field				
Amsinckia sp.	Fiddleneck	16	0	0
Anthemis cotula	Dog fennel	16	0	0
Brassica nigra	Black mustard	123	1	0.8
Chenopodium album	Common lamb's quarters	126	0	0
Cicer arietinum	Chickpea	16	2	6.2
Descurainia sophia	Flixweed	46	2	4.3
Galium aparine	Catchweed bedstraw	79	1	1.3
Lactuca serriola	Prickly lettuce	79	0	0
Lamium amplexicaule	Henbit	111	2	0.9
Sisymbrium altissimum	Jim Hill mustard	48	0	0
Thlaspi arvense	Fanweed	144	1	0.7
Triticum aestivum	Wheat	208	3	1.4
(c) Chickpea screening trial				
Amaranthus albus	Tumble pigweed	304	20	6.6
Brassica nigra	Black mustard	24	0	0
Capsella bursa-pastoris	Shepherd's purse	156	4	2.6
Chenopodium album	Common lamb's quarters	344	9	2.6
Cicer arietinum	Chickpea	100	65	65.0
Circium arvense	Canada thistle	32	0	0
Lactuca serriola	Prickly lettuce	220	11	5.0
Lamium amplexicaule	Henbit	340	14	4.1
Lens culinaris	Lentil	148	14	9.5
Medicago sativa	Alfalfa	196	5	2.6
Pisum sativum	Pea	188	12	6.4
Solanum nigrum	Black nightshade	460	7	1.5

[a] Asymptomatic plant tissues were sampled in three fields during 1988–1992: (a) a commercial chickpea field heavily infected with Ascochyta blight near Dayton, WA, (b) a wheat field near Genesee, ID where the previous chickpea crop was severely damaged by the disease, and (c) a chickpea Ascochyta blight screening trial at Spillman Farm, Pullman, WA.

[b] Isolations were made from 1 to 3 plants at each site.

Table 2 Ascochyta blight severity on 31 accessions of 12 *Cicer* spp. naturally infected in a screening trial at Spillman Farm, Pullman, Washington[a]

Cicer spp.	No. accessions	Linking letters y[b] (0–9)
Annual species		
C. arietinum	6	6–9[c]
C. bijugum	2	4–8
C. chorassanicum	2	7–9
C. cuneatum	1	8–9
C. echinospermum	3	7–9
C. judaicum	3	7–9
C. pinnatifidum	4	5–9
C. reticulatum	5	4–9
C. yamashitae	1	7–9
Perennial species		
C. anatolicum	2	0–1
C. microphylum	1	0–1
C. oxyodon	1	1–2

[a] The experiment was conducted 2 years (1989–1990) for the perennial species and 3 years (1988–1990) for the annual species.

[b] The severity of *Didymella rabiei* on plants in each plot was evaluated on a rating scale of 0–9, where 0 = no symptoms and 9 = plant dead.

[c] Range of mean values among accessions and years.

C. bijugum (2), *C. chorassanicum* (2), *C. cuneatum* (1), *C. echinospermum* (3), *C. judaicum* (3), *C. pinnatifidum* (4), *C. reticulatum* (5), *and C. yamashitae* (1). The experiment was conducted for two years (1989–1990) for the perennial species and three years (1988–1990) for the annual species. The disease severity of plants in each plot was evaluated 70 to 80 days after planting using a 0 to 9 rating scale, where 0 = no symptoms and 9 = plant dead (Trapero-Casas and Kaiser 1992b). Because mean variances were not homogeneous, disease severity data were analysed by the non-parametric test of Kruskal-Wallis using Statistix software (Analytical Software, Tallahassee, FL).

Mating type determination

Fifty-one isolates of *D. rabiei* from naturally infected plant species (Table 3) were selected to determine their mating type using the method of Wilson and Kaiser (1995). Conidial suspensions (10^6 spores ml^{-1}) of single-spore derived cultures of each isolate and compatible mating types [American Type Culture Collection (ATCC) 76501 (MAT1-1) and ATCC 76502 (MAT1-2) were prepared. Four to six previously autoclaved chickpea stem segments (6 to 8 cm long) were placed in sterile test tubes (2.2×17.5 cm) containing 20 ml of a conidial suspension of a single isolate, or a mixture of one isolate and ATCC 76501 or 76502 for 1 h, drained for 10 to 15 min, and placed on 10 sterile filter paper discs moistened with 15 ml of sterile water in 10-cm diam glass Petri dishes. The closed dishes were incubated for 24 h at 21 to 23° C in a laminar flow hood and then placed at 10°C in the dark. After 6 weeks, the chickpea stem pieces were air-dried at 21 to 23°C in a laminar flow hood. Discharge of ascospores was done by placing inoculated stem segments (2.5 cm long) on a 4 cm^2 WA block placed on the inner surface of a Petri dish lid inverted over a bottom dish containing WA. Ascospores were discharged down onto the surface of the WA. Dishes were incubated at 21 to 23°C for 18 to 24 h to observe germination.

Infection of chickpea from infested debris of alternative hosts

Seven crop and weed species were inoculated with a conidial suspension of *D. rabiei* isolate ATCC 58663 (Table 4). The fungus was cultured on PDA and chickpea seed meal-dextrose agar (CDA) (Wilson and Kaiser 1995), and incubated at 21 to 23°C under fluorescent lights (12-h photoperiod) in plastic Petri dishes. The foliage of seven plant species (3 to 5 weeks-old), grown in a greenhouse to avoid natural infection of the pathogen, was sprayed to run-off with a conidial suspension (10^6 conidia ml^{-1}) using a De Vilbiss atomiser (De Vilbiss Air Power Co., Jackson, TN, USA). Control plants were sprayed with sterile water. Inoculated and control plants were incubated in a moist chamber in the greenhouse at 18 to 20°C for 96 h and then maintained in the same greenhouse for 6 to 8 weeks. Plant tissues from the inoculated plants were harvested and dried 2 to 3 months after inoculation to allow for maximum plant growth. Fifteen disease-free chickpea seeds (PI 458870) were planted in pasteurised potting soil in 25-cm diam plastic pots in the greenhouse. Ten g of dried stem and leaf debris from each of the different inoculated plant species were placed on the surface of the soil. Control treatments were identical to the treated series except the tissues were autoclaved. There were two to five pots per species and the same number of control pots.

Table 3 Mating types of *Didymella rabiei* isolated from naturally infected plant species in the Palouse region of eastern Washington and northern Idaho, USA[a]

Plant species	Common name	Family	No. isolates		
			Total	MAT1-1	MAT1-2
Amaranthus albus	Tumble pigweed	Amaranthaceae	2	2	0
Anthemis cotula	Dog fennel	Asteraceae	1	0	1
Asperugo procumbens	Catchweed	Boraginaceae	2	1	1
Brassica nigra	Black mustard	Brassicaceae	1	1	0
Capsella bursa-pastoris	Shepherdspurse	Brassicaceae	1	0	1
Descaurainia sophia	Flixweed	Brassicaceae	1	0	1
Lamium amplexicaule	Henbit	Lamiaceae	4	2	2
Lens culinaris	Lentil	Fabaceae	24	13	11
Medicago sativa	Alfalfa	Fabaceae	4	0	4
Pisum sativum	Pea	Fabaceae	4	2	2
Solanum nigrum	Black nightshade	Solanaceae	2	1	1
Thlaspi arvense	Fanweed	Brassicaceae	2	1	1
Triticum aestivum	Wheat	Poaceae	3	1	2
			51	24	27

[a] Isolates of *D. rabiei* from the different hosts were paired with compatible mating types (MAT1-1 and MAT1-2) of the fungus.

Pots were separated from one another by at least 50 cm on greenhouse benches in a completely randomised design. The soil was maintained moist by placing the pots in individual trays of water. The plants were not watered from above. Twenty to 25 days after emergence, the number of diseased plants in each pot was counted, and isolations were made from necrotic lesions that developed on affected plants. Percentages of infected plants were transformed by the square root function $(Y = \sqrt{X + 0.5})$to stabilise the error variance. Analysis of variance (ANOVA) and means comparison were applied to the transformed data.

Development of the teleomorph on various plant species

Two experiments were used to determine if the teleomorph of *A. rabiei* developed on tissues of plant species infected naturally under field conditions or

Table 4 Spread of *Didymella rabiei* from debris of different plant species to chickpea plants in greenhouse pot tests[a]

Inoculum source	Common name	No. chickpea seedlings		Disease incidence (%)
		Exposed to deris	Infected	
Cicer arietinum	Chickpea	75	16	21 b[b]
Lactuca serriola	Prickly lettuce	30	1	3 c
Lamium amplexicaule	Henbit	45	1	2 c
Medicago sativa	Alfalfa	60	2	3 c
Melilotus alba	White sweet clover	60	6	10 bc
Pisum sativum	Pea	30	15	50 a
Thlaspi arvense	Fanweed	45	4	9 bc

[a] Infested debris from seven plant species inoculated with a conidial isolate (ATTC 58663) of *D. rabiei* were placed on the surface of plastic pots (10 g of dried debris per pot) filled with a pasteurised potting soil. Each pot was seeded with fifteen chickpea seeds and evaluations were done on chickpea plants that emerged through the debris.

[b] Percentage of infected chickpea plants. Mean values followed by the same letter are not significantly different according to Fisher's protected LSD test at P=0.05. No infection was detected on the control plants.

inoculated in the greenhouse. In the first experiment, 31 accessions of 12 *Cicer* spp. and seven other plant species growing in a chickpea Ascochyta blight screening trial at Spillman Farm, Pullman, WA were collected in September 1990 and air-dried for one week. Pieces of stem tissue were placed in nylon-net bags (11×13 cm) with a 1.6 mm mesh permeable to air. Three bags of each plant species were placed outdoors on the soil surface in October 1990 at a site in Pullman, WA >10 km from the nearest chickpea planting. They were collected the following April.

In the second experiment, plants were inoculated with a conidial suspension (10^6 conidia ml^{-1}) of the two compatible isolates of *D. rabiei*, ATCC 76501 and ATCC 76502, or with ascospores of the pathogen from naturally infested chickpea debris from Genesee, ID. Inoculation with conidia was done following the method of Wilson and Kaiser (1995). For inoculation with ascospores, pieces of naturally infested chickpea stem debris with abundant mature pseudothecia of *D. rabiei* were the source of ascospores. Stem pieces were soaked in deionised water for about 30 min, blotted dry with paper towels, and placed on a wire screen (10-mm mesh). Wet paper towels were placed over the stem pieces to keep them moist. Great care was taken to prevent formation of water droplets that could disperse conidia from pycnidia in the infested debris to the plants placed below. Ascospores were discharged directly onto the chickpea seedlings by placing the wire screen about 40 cm above healthy plants in plastic moist chambers. The plants were placed in metal trays partially filled with water in darkened controlled growth chambers (Controlled Environments, Winnipeg, Canada) at 20°C. Plants were exposed to inoculum for 48 h (Trapero-Casas and Kaiser, 2007). After inoculation, plants were incubated in a moist chamber in the greenhouse at 18 to 26°C for 96 h and then maintained in the same greenhouse for 6 to 8 weeks. Whole plants were harvested and air-dried at 21 to 23°C. Pieces of inoculated tissue were placed in nylon-net bags incubated outdoors on the soil surface as described above.

Two methods were used to test for the presence of the teleomorph of *A. rabiei.* In the first, pieces of tissue of each plant species were allowed to discharge over three Petri dishes containing WA following the procedure of Kaiser and Küsmenoglu (1997). Single ascospores were transferred to PDA for identification purposes. In the second method, tissue of each plant species was allowed to discharge over four 20 to 30 day-old chickpea plants (PI 458870) following the method described above. The chickpea plants were removed from the moist chamber after 96 h and placed in the greenhouse at 18 to 26°C. After 15 to 20 days, the disease was assessed and isolations were made from lesions that developed on the foliage of inoculated plants.

Colonisation and spore production on debris of various plant species

Two experiments were used to determine and quantify the formation of the teleomorph and spore production on detached stems or leaves of different plant species. In the first experiment, stem tissues of barley (*Hordeum vulgare*), chickpea, corn (*Zea mays*), faba bean (*Vicia faba*), lentil, pea, and wheat (*Triticum aestivum*) commonly cultivated in the U. S. Pacific Northwest were used. Disease-free plant parts were collected in the field in June 1993 and air-dried at 21 to 23°C. Tissues were sterilised with propylene oxide (Hansen and Snyder 1947). Pieces of sterilised tissues, 6 to 8 cm long, were inoculated with a conidial suspension of ATCC 76501 and 76502 following the procedure outlined earlier. Ascospores were discharged onto WA (Kaiser and Küsmenoglu 1997) and the number of ascospores mm^{-2} was counted.

In the second experiment, plant parts from ten cultivated or wild plant species commonly found in southern Spain were used, including chickpea, bean, faba bean, alfalfa (*Medicago sativa*), *Chrozophora tinctoria, Chenopodium album,* oats (*Avena sativa*), olive (*Olea europaea*), and *Papaver rhoeas.* Entire leaves were used for olive, stem and leaf pieces for oats, and stem pieces for the other species. The dried tissues were cut into pieces 6 to 8 cm long, sterilised with propylene oxide (Hansen and Snyder 1947), and inoculated with a conidial suspension (10^4 conidia ml^{-1}) of isolates ATCC 76501 and ATCC 76502. The sterile tissues were submerged in the conidial suspension for 20 min, drained for 30 min, and then dried on sterile filter paper. Once dry, the inoculated tissues were put on plastic screens placed 15 mm above 700 ml of water in transparent 2.2 litre plastic containers (21.5×15.5×9 cm), and incubated in the dark at 8°C and 100% relative humidity (RH) for the first two weeks of incubation. Thereafter, at weekly intervals for the next 6 weeks, the tissues were dried in an incubator at 8°C between pieces of filter paper

for 2 to 3 days, and then incubated again at 8°C and 100% RH. For each of the plant species, three pieces of tissue were examined at 14-day intervals and the following observations were made:

1. *Colonisation of plant tissues*. Saprophytic colonisation of the tissues by *D. rabiei* was evaluated on a scale of 0 to 5 that estimated visually the percentage of the surface area of the tissue with fungal fruiting bodies, where 0=0%, 1=1 to 25%, 2=26 to 50%, 3=51 to 75%, 4=76 to 99%, and 5=100%.
2. *Density of fruiting bodies*. Each tissue sample was examined under a stereoscopic microscope for fruiting bodies in 30 microscope fields (80×) selected at random. The area of each microscope field was 1.91 mm^2. One hundred fruiting bodies were removed and squashed in lactophenol-acid fuchsin and observed microscopically at 400× to determine the number of pycnidia and pseudothecia of *D. rabiei* on the tissues.
3. *Maturity of pseudothecia.* On each sample, at least 50 pseudothecia were selected at random and squashed in lactophenol-acid fuchsin on a slide and their stage of development determined microscopically using a scale of 1 to 7 (Trapero-Casas and Kaiser 1992a), where 1 = pseudothecial initials, 2 = paraphyses filling the lumen of pseudothecia, 3 = asci arising among pseudoparaphyses, 4 = immature asci, 5 = asci with ascospores, 6 = half-empty asci and released ascospores, 7 = empty pseudothecia. For each sample, a maturity index (MI) of pseudothecial development was calculated as a weighted average of the observed stages (Navas-Cortés et al. 1998a).
4. *Number of asci per pseudothecium.* The number of asci produced in each pseudothecium was calculated using 60 pseudothecia at development stage 5.
5. *Discharge of ascospores.* To estimate ascospore production and discharge at each sample date, the tissues were allowed to discharge over WA or sterile water. Four cm^2 of tissue strips colonised by *D rabiei* were placed on blocks of WA in Petri dishes as described above, and incubated in the dark at 20° C for 24 h. The number of ascospores mm^{-2} of WA or Petri dish surfaces was estimated using a grid.
6. *Production of conidia*. Sixty pycnidia present on infested tissue were selected at random, each placed separately in a drop of sterile water on a microscope slide and macerated to obtain homogeneous suspensions. Aliquots of these suspensions were distributed uniformly on the surface of acidified WA (AWA; 20 g agar and 0.25 ml lactic acid in 1,000 ml deionised water) in Petri dishes. The AWA dishes were incubated in the dark at 20°C for 8 to 12 days and the number of colonies of *D. rabiei* was used to calculate the number of conidia per pycnidium.

A randomised complete block design was used with three blocks (plastic containers) and three replications (tissue pieces) per block per plant species. Observations and counts were recorded every 2 weeks for each tissue piece and the average of the three pieces represented one block. ANOVA and Fisher's protected LSD test (P=0.05) were conducted to determine differences among plant tissues using Statistix software (Analytical Software, Tallahassee, FL). Based on the Bartlett's test for homogeneity of variance, data from counts (numbers of pycnidia, pseudothecia, fruiting bodies, discharged ascospores, asci and conidia) were transformed by the square-root ($Y = \sqrt{X + 0.5}$) to stabilise the error variance. ANOVA and means comparisons were applied to the transformed data. Tissue colonisation and pseudothecia maturity index variables showed homogenous variance and analysis was performed with the original data.

Results

Plant species naturally infected by *Didymella rabiei*

No disease symptoms were observed on any of the plant species other than chickpea. A species of the genus *Ascochyta*, morphologically similar to *A. rabiei*, was isolated from 16 of the 19 plant species collected from the three sites (Table 1). Infection was not detected on *Amsinckia* sp., *Circium arvense*, and *Sisymbrium altissimum*. Infection was rare in most weed species collected from the chickpea field near Dayton, WA and wheat field near Genesee, ID, but common in weed species in the chickpea Ascochyta blight screening trial at Spillman Farm Pullman, WA. The leguminous crop species from which the fungus was isolated included alfalfa, chickpea, lentil, and pea. All other plant species infected by the pathogen were weed species. Isolates of the fungus from

various crop and weed species were pathogenic to chickpea and indistinguishable in cultural and morphological characteristics from isolates of *D. rabiei* from chickpea (Punithalingham and Holliday 1972).

Single-conidial isolates of *D. rabiei* from *Capsella bursa-pastoris*, *Lamium amplexicaule*, lentil, *Thlaspi arvense*, and wheat were deposited in the fungal collection maintained by the U.S. Department of Agriculture, Western Regional Plant Introduction Station, Pullman, WA (Hernandez-Bello et al. 2006) and were identified as AR 33, 29, 32, 30, and 28, respectively.

Natural infection of *Cicer* spp.

Of the twelve *Cicer* spp. screened for resistance to Ascochyta blight (Table 2), only the perennial species, *C. anatolicum, C. microphyllum, and C. oxyodon,* were highly resistant, differing significantly from all accessions of annual *Cicer* spp. by the Kruskal-Wallis test. A few small lesions developed on the foliage 80 to 90 days after planting. Most of the annual *Cicer* spp. were susceptible or highly susceptible to the disease and there were extensive necroses of the foliage. Only three annual *Cicer* spp. had one or two accessions which were moderately resistant. These were *C. bijugum* (1 accession), *C. pinnatifidum* (2 accessions) and *C. reticulatum* (1 accession). Disease severity in these accessions ranged from 4 to 5 and differed significantly from those of other annual *Cicer* spp.

Mating types

Both mating types of *D. rabiei* were isolated from naturally-infected *Galium aparine*, *L. amplexicaule*, lentil, pea, *Solanum nigrum*, *T. arvense*, and wheat (Table 3). The most isolates tested were from lentil where about half of the isolates were of each mating type. Ascospore cultures from the alternative hosts were identical to isolates of the fungus from chickpea in cultural and morphological characteristics, and in their pathogenicity to chickpea.

Infection of chickpea from infested debris of alternative hosts

All inoculated plants were infected by *A. rabiei*, but blight symptoms and signs (pycnidia) of the pathogen only developed on chickpea. Leaves of *Lactuca serriola*, pea, *T. arvense*, and white sweet clover (*Melilotus alba*) showed small chlorotic to reddish brown lesions without any sign of the pathogen. Abundant pycnidia of *D. rabiei* developed on debris of chickpea and, to a lesser degree, on debris of alfalfa and white sweet clover. Chickpea plants that emerged through the infested debris of seven plant species were infected by *D. rabiei*. The percentage of infected plants significantly depended on the type of plant debris, ranging from 2% for alfalfa debris to 50% for pea debris (Table 4). No differences were observed in cultural characteristics among isolates of the pathogen from lesions on the foliage of chickpea plants infected from debris of the different plant species. These isolates were also highly pathogenic to chickpea. No disease was found in the control plants that emerged through the sterilised debris.

Development of the teleomorph on various plant species

The teleomorph of *A. rabiei* developed on the overwintered tissues of alfalfa, *Amaranthus albus,* chickpea, lentil, pea, wheat, and white sweet clover infected naturally in an Ascochyta blight of chickpea screening nursery at Spillman Farm, Pullman, WA (Table 5). The teleomorph also developed on the overwintered tissues of all *Cicer* accessions. Discharge of ascospores of *D. rabiei* was often sparse from the overwintered tissues of all plant species tested, except chickpea where ascospore discharge was usually high. No conidia were observed on the WA plates. The teleomorph of other ascomycetous fungi, such as *Mycosphaerella tassiana* (anamorph: *Cladosporium herbarum*), also developed on the tissues of these plant species and was similar to that of *D. rabiei*.

In the greenhouse inoculation studies, the teleomorph formed on the overwintered debris of chickpea and wheat inoculated with ascospores of *D. rabiei* from chickpea debris, and on overwintered tissues of chickpea, white sweet clover and pea inoculated with conidial suspensions of ATCC 76501 and 76502 (Table 5). Ascospores discharged from the tissues of these four plant species were able to infect chickpeas.

Colonisation of debris of various plants

The teleomorph of *A. rabiei* formed on the debris from infected plants of barley, chickpea, corn, faba

Table 5 Development of the teleomorph of *Didymella rabiei* on overwintered debris of different crop and weed species infected naturally or inoculated in the greenhouse[a]

Plant species	Common name	Ascospores discharged on water agar[b]	Disease severity on chickpeas[c]
Naturally infected			
Amaranthus albus	Tumble pigweed	−	1
Cicer arietinum	Chickpea	+++	9
Lens culinaris	Lentil	++	7
Medicago sativa	Alfalfa	−	3
Melilotus alba	White sweet clover	+	−
Pisum sativum	Pea	++	9
Triticum aestivum	Wheat	+	1
Inoculated			
Cicer arietinum	Chickpea	+++	9
Melilotus alba	White sweet clover	+	1
Pisum sativum	Pea	++	7
Triticum aestivum	Wheat	+	1

[a] Plants were exposed to high levels of natural inoculum in a chickpea Ascochyta blight screening nursery at Spillman Farm, Pullman, WA.

[b] Ascospores were discharged downwards onto the surface of water agar. Ascospore discharge was measured as follows: - = not tested, + = 1–100, ++ = 101–500, +++ = >500 ascospores mm^{-2}.

[c] Ascospores were discharged downwards onto the foliage of chickpea plants. Disease severity on chickpea plants was evaluated on a rating scale of 0–9, where 0 = no symptoms and 9 = plant dead (Trapero-Casas and Kaiser 1992b).

bean, lentil, pea, and wheat. Barley, wheat, and white sweet clover were the poorest substrates for production and release of ascospores (Table 6). Chickpea, corn, and pea debris favoured the development of mature pseudothecia of the pathogen. Ascospores from these different species were pathogenic to chickpea.

Tissues of 10 inoculated plant species were saprophytically colonised and bore pycnidia and/or pseudothecia of *D. rabiei* (Table 7). However, colonisation of the tissues by the fungus varied greatly among plant species and ranged from <25% to >70% of the surface area. There was no statistical

Table 6 Discharge of ascospores of *Didymella rabiei* from tissues of plant species inoculated with spore suspensions of two compatible isolates of the pathogen and incubated at 10°C for 6 weeks [a]

Plant species	Common name	Discharge of ascospores[b]
Cicer arietinum	Chickpea	+++
Hordeum vulgare	Barley	+
Lens culinaris	Lentil	++
Melilotus alba	White sweet clover	+
Pisum sativum	Pea	+++
Triticum aestivum	Wheat	+
Vicia faba	Faba bean	++
Zea mays	Corn	+++

[a] Results of two experiments with 10 to 15 stem pieces measuring 8 to 10 cm in length.

[b] Ascospores were discharged downwards from infested debris onto the surface of water agar. Density of ascospores mm^{-2} of water agar surface was estimated using four categories: - = no ascospores, + = 1–100, ++ = 101–500, +++ = >500 ascospores mm^{-2}.

Table 7 Amount of colonisation and number of fruiting bodies of *Didymella rabiei* on debris of various plant species[a]

Plant species	Common name	Colonisation (0–5)[b]	No. fruiting bodies mm^{-2}[c]		
			Total	Pycnidia	Pseudothecia
Avena sativa A (stems)	Oat	2.9 d[d]	2.8 de	2.4 cd	0.4 d
Avena sativa B (leaves)	Oat	1.9 ef	1.9 ef	1.4 de	NP[e]
Chenopodium album	Common lamb's quarters	2.3 e	4.8 cd	0.9 e	NP
Cicer arietinum	Chickpea	3.4 abc	6.1 bc	2.3 cd	3.8 a
Chrozophora tinctoria	Turnsole	3.3 bcd	6.0 bc	3.4 bc	2.5 abc
Medicago sativa	Alfalfa	3.8 a	10.0 a	10.0 a	NP
Olea europaea (leaves)	Olive	1.7 f	1.9 ef	1.0 e	NP
Papaver rhoeas	Poppy	3.0 cd	2.8 de	1.1 de	1.7 c
Phaseolus vulgaris	Bean	3.7 ab	7.1 ab	4.8 b	2.3 bc
Triticum aestivum	Wheat	1.8 f	1.3 f	0.9 e	NP
Vicia faba	Faba bean	3.4 abc	5.9 bc	2.4 cd	3.5 ab

[a] Dried and sterilised stem and leaf pieces were inoculated with two sexually compatible isolates of *Didymella rabiei* and incubated in the dark at 8°C and alternating relative humidity for 8 weeks.

[b] Evaluated on a scale of 0 to 5 according to the percentage of tissue colonised by pycnidia and/or pseudothecia (0=0%, 5=100% of the tissue colonised).

[c] The number of fruiting bodies was determined from 30 stereomicroscopic fields at 80x (1.91 mm^2 each).

[d] Each value is the mean of three blocks with 3 replications per block. In each column, values followed by the same letter are not significantly different according to Fisher's protected LSD test at P=0.05.

[e] NP = No pseudothecia.

difference in the amount of tissue colonised by the fungus on chickpea, bean, faba bean and alfalfa, where most colonisation occurred. Pseudothecia developed on the tissues of chickpea, bean, *C. tinctoria*, faba bean, oats, and *P. rhoeas*, but not on alfalfa, *C. album*, olive, and wheat. Pycnidial development mm^{-2} of stem tissue was highest on alfalfa and lowest on *C. album* and wheat.

Pseudothecia with mature asci developed on inoculated tissues of chickpea, *C. tinctoria*, bean, faba bean, oats, and *P. rhoeas*, and viable ascospores were discharged (Table 8). The teleomorph did not develop on the tissues of alfalfa, *C. album*, olive and wheat. The number of ascospores discharged mm^{-2} of tissue was highest in chickpea (324 mm^{-2}), but was not statistically different from that on bean and faba bean. Ascospore discharge was <8 ascospores mm^{-2} of tissue of *C. tinctoria*, oats, and *P. rhoeas*. The number of asci per pseudothecium was highest on chickpea stems, followed by that on bean, faba bean and *C. tinctoria* tissues, and was least on *P. rhoeas* stem pieces. The number of conidia per pycnidium varied greatly among tissues of plant species, with most on alfalfa stems (127.4 conidia per pycnidium) and least on olive leaves (0.3 conidia per pycnidium).

Discussion

Sixteen crop and weed species were infected naturally by *D. rabiei* in three widely separated areas of the Palouse region of eastern WA and northern ID. The pathogen infected chickpea, lentil, pea and wheat which are important commercial crops in the U.S. Pacific Northwest. The three leguminous crops are grown in rotation with wheat under rain-fed conditions. The 12 weed species naturally infected by *D. rabiei* commonly infest farmers' fields in the region. The percentage of plant tissues from which *D. rabiei* was isolated was low in the samples collected from the chickpea field in Dayton, WA and wheat field in Genesee, ID, but generally much higher in most plant species in the chickpea Ascochyta blight screening trial at Spillman Farm, Pullman, WA. This may be due to the larger amounts of inoculum of the pathogen present in the heavily infested chickpea debris that was spread

Table 8 Development of pseudothecia, discharge of ascospores and production of pycnidia and pseudothecia of *Didymella rabiei* on debris of various plant species[a]

Plant species	Maturity index (1–7)[b]	Number of ascospores mm^{-2}[c]	Reproductive potencial (no.)	
			Asci/pseudothecium[d]	Conidia/pycnidium[e]
Avena sativa A (stems)	5.56 a[f]	5.5 b	109.0 d	21.3 de
Avena sativa B (leaves)	NP[g]	–	–	30.3 d
Chenopodium album	NP	–	–	8.7 f
Cicer arietinum	5.09 b	323.8 a	172.8 a	80.3 bc
Chrozophora tinctoria	4.85 bc	7.8 b	136.0 c	69.9 c
Medicago sativa	NP	–	–	127.4 a
Olea europaea (leaves)	NP	–	–	0.3 g
Papaver rhoeas	4.66 c	5.0 b	74.3 e	5.3 f
Phaseolus vulgaris	5.06 bc	204.9 a	156.2 b	35.8 d
Triticum aestivum	NP	–	–	17.6 e
Vicia faba	4.93 bc	221.9 a	145.5 bc	94.9 b

[a] Dried and sterilised stem and leaf pieces were inoculated with two sexually compatible isolates of *Didymella rabiei* and incubated in the dark at 8°C and alternating relative humidity for 8 weeks.

[b] Maturity index of pseudothecia was calculated as the weighted average of frequency of the stages of maturity estimated in the pseudothecia (1 = pseudothecial initials, 7 = empty pseudothecia).

[c] Number of ascospores discharged over water agar or sterile water mm^{-2} of tissue. On each date, 4 mm^2 of tissue heavily infested by *D. rabiei* was used.

[d] The number of asci was determined in pseudothecia that had asci with differentiated ascospores (development stage 5).

[e] The number of viable conidia was determined by pouring the macerate of 60 pycnidia selected at random on acidified water agar.

[f] Each value is the mean of three blocks with 60 replications per block for the determination of the maturity index and number of asci per pseudothecium, and with three replications for the rest of the determinations. In each column, values followed by the same letter are not significantly different according to Fisher's protected LSD test at $P=0.05$.

[g] Experimental treatments in which there was no development of viable pseudothecia on the debris.

between the rows of chickpea lines being screened and to the chickpea spreader plots that became severely blighted. Although no visible symptoms were observed on the naturally infected hosts, the fungus was isolated from the tissues after surface-sterilisation of the leaves and stems in sodium hypochlorite, indicating that it had invaded the host tissues internally. Isolates of the pathogen from different alternative hosts were indistinguishable in cultural and morphological characteristics from isolates of the fungus from chickpea and these isolates were highly pathogenic to chickpea, indicating that *D. rabiei* retained its pathogenicity after colonising non-susceptible hosts. According to Milgroom and Peever (2003), plant pathogens exhibit different levels of host specificity. Some have very restricted host ranges (specialists), while others have broader host ranges (generalists). *Didymella rabiei* only causes severe disease on chickpea and other *Cicer* species. For that reason it is a specialist, even though it infects other crop and weed species in the Palouse region. However, naturally infected alternative hosts could serve as sources of inoculum for infection of chickpea crops.

Host specificity is a major factor in the speciation of *Ascochyta* species infecting wild and cultivated legumes (Peever, 2007). The species *D. rabiei* can be easily differentiated from other related *Ascochyta* spp. by morphological characters, host specificity in *Cicer* spp., and phylogenetic analyses (Peever et al. 2007). Our results confirmed the pathogenic specialisation of *D. rabiei* since *Cicer* spp. were the only susceptible hosts. However, the pathogen was able to infect other leguminous and non-leguminous hosts, which may increase its potential for survival and spread. Hernandez-Bello et al. (2006) and Peever et al. (2007) also demonstrated that interspecific crosses between closely related, but host-specific *Ascochyta* spp. such as *A. fabae* and *A. pisi*, were fertile but most

progeny from these crosses were unable to infect and induce disease on either parental host. In our studies, all isolates of *D. rabiei* from different hosts were highly pathogenic to chickpea, suggesting that hybrids between *D. rabiei* and other closely related *Ascochyta* spp., which are common in the same area, do not occur or are infrequent.

We isolated *D. rabiei* from several plant species in the Palouse region that are not legumes or related to *Cicer* spp. which are natural hosts of the pathogen. In a recent review article, Crous and Groenewald (2005) referred to a similar situation with species of *Mycosphaerella* that were thought previously to be host-specific. They report on *Mycosphaerella* spp. that infect the foliage of different plant species other than their usual host. The authors suggest that these *Mycosphaerella* spp. are able to infect dead tissues on the non-hosts, sporulate sparsely, and produce spores that may be dispersed back to their natural hosts. They call this phenomenon 'pogo stick' and define it as the ability of a pathogen to colonise non-host tissues, develop fruiting bodies, and disperse spores that may infect the natural host on which they are pathogenic. The 'pogo stick' phenomenon, similar to the 'green bridge' used by other authors (Cook and Veseth 2001), may apply to *D. rabiei* which also infects several non-susceptible hosts that may serve as secondary reservoirs of the fungus in the absence of the natural host, *Cicer* spp.

Most annual and perennial *Cicer* spp. are found in the Middle East and neighbouring countries in the former Soviet Union (van der Maesen 1987). There are nine annual and >30 perennial *Cicer* spp. (van der Maesen 1987; Kaiser et al. 1997). In our studies, most of the annual *Cicer* spp. were highly susceptible to Ascochyta blight. The annual *Cicer* spp. of particular interest to plant breeders regarding resistance to Ascochyta blight are *C. echinospermum* and *C. reticulatum*, because they can be readily crossed with the cultivated chickpea, *C. arietinum*. Only one of five accessions of *C. reticulatum* and none of three of *C. echinospermum* was moderately resistant to the blight pathogen in the screening trials at Pullman, WA. The three perennial species, *C. anatolicum*, *C. microphyllum* and *C. oxyodon* from Turkey, India and Turkey, respectively, were highly resistant to *D. rabiei*. The perennial species have yet to be crossed with *C. arietinum*, but this situation may change in the future as new biotechnology techniques becoming available to breeders may facilitate the transfer of disease resistance genes between annual and perennial *Cicer* spp. Even though the three perennial *Cicer* spp. were highly resistant to blight, the teleomorph of *A. rabiei* still developed on their overwintered infested debris, as it did on the overwintered debris of all annual *Cicer* spp. In nature, the junior author has observed blight on leaves, stems, and pods of two perennial species, *C. montbretii* in Bulgaria (Kaiser et al. 1998) and *C. ervoides* in the Republic of Georgia (W.J. Kaiser, *unpublished*), but disease incidence and severity were low. Both mating types of *D. rabiei* were isolated from infected tissues of *C. montbretii* (Kaiser et al. 1998). It is likely that the teleomorph of *A. rabiei* develops in native stands of *C. montbretii, C. ervoides*, and possibly other annual and perennial *Cicer* spp. wherever both mating types of *D. rabiei* occur. In Israel, Frenkel et al. (2007) reported on the sympatric distribution of *C. judaicum*, a wild annual species, with wild and domesticated legumes, including the cultivated chickpea. They found *C. judaicum* to be naturally infected by *D. rabiei*, suggesting that it may serve as an alternative host of the pathogen. A similar situation may occur in Turkey, where the anamorph and teleomorph of *D. rabiei* are widely distributed (Kaiser and Küsmenoglu 1997) and four wild annual *Cicer* spp., *C. bijugum*, *C. echinospermum*, *C. pinnatifidum*, and *C. reticulatum*, grow in sympatric distribution with cultivated chickpeas and lentils (W.J. Kaiser, *unpublished*). However, the sympatric distribution of *Cicer* spp. and chickpea crops do not occur in most chickpea growing countries where non-susceptible hosts may serve as an alternative source of inoculum for Ascochyta blight epidemics.

Didymella rabiei was isolated from the foliar tissues of many crop and weed species in several plant families infected naturally under field conditions in different areas of the Palouse region of the U.S. Pacific Northwest. Previous studies on the host range of the Ascochyta blight pathogen only demonstrated that other plant species could be alternative hosts of *D. rabiei* (Kaiser 1973, 1990; Nene and Reddy 1987; Khan et al. 1999). However, information is lacking on whether the teleomorph of *A. rabiei* develops on the debris of these asymptomatic hosts. Our study showed that pseudothecia formed on the senescent overwintered debris naturally infected or artificially inoculated with compatible isolates of the pathogen. Ascospores produced on these alternative hosts infected chickpea.

We demonstrated that the blight fungus spreads from artificially infested debris of chickpea and six alternative hosts to chickpea seedlings in a greenhouse study. In an earlier study, Trapero-Casas and Kaiser (1992a) observed that on moistened, overwintered chickpea debris, *D. rabiei* grew quickly from lesions and rapidly colonised senescent stem and pod tissues with pycnidia and pseudothecia. In a greenhouse study, debris of different inoculated plant species was placed on the surface of moist soil in pots into which chickpea seeds had been planted. On the foliage of several of the inoculated plant species small chlorotic to reddish-brown lesions were observed. *Didymella rabiei* may have grown from these small lesions and rapidly colonised the moist senescent debris, forming mycelia and pycnidia with viable conidia that infected the foliage of chickpea seedlings that emerged through the infested debris.

Our results demonstrated that *D. rabiei* naturally infected several hosts, without causing visible disease symptoms (non-susceptible hosts), although the pathogen survived in these infected plants and their residues. The potential of infested debris of several plant species in addition to chickpea as sources of inoculum for Ascochyta blight epidemics has been quantified using detached and sterilised tissues of ten different plant species under optimal conditions for sexual reproduction of the pathogen. The fungus colonised all plant tissues forming pycnidia and conidia, although the density of tissue colonisation and the production of conidia varied greatly among plant tissues, indicating some host preference for saprophytic growth and asexual reproduction. Higher colonisation and production of conidia, not significantly different from that on chickpea tissues, occurred not only on tissues of the legumes, but also on turnsole. Alfalfa tissues proved to be a more suitable substrate for asexual reproduction of the pathogen than chickpea tissues.

Didymella rabiei exhibited a higher host preference for sexual reproduction than for asexual reproduction. The teleomorph developed extensively (>200 ascospores mm^{-2}) on tissues of leguminous plants, except alfalfa, and scarcely (<10 ascospores mm^{-2}) on tissues of oat, turnsole and poppy. On oat debris, pseudothecia developed only on stems and not on leaves, suggesting some preference for the type of tissue besides plant species. The high incidence of asexual reproduction and lack of sexual reproduction on alfalfa tissues may be due to the origin of the plant residues used. The debris of all plant species, except alfalfa, came from senescent and dried stems or leaves. However, alfalfa stems were obtained from hay harvested green and then dried. The dried green alfalfa tissue may be more favourable for asexual reproduction of *D. rabiei* than senescent residues, as it is in chickpea where the teleomorph develops only on overwintered chickpea straw and the anamorph only on green living tissues (Trapero-Casas and Kaiser 1992a).

These results indicate the potential of residues of different plant species to support saprophytic growth and reproduction of *D. rabiei*. However, in nature, biotic and abiotic environments are not necessarily optimal for the pathogen (Navas-Cortés et al. 1998b). The presence of resident microorganisms in plant debris may alter greatly the growth and reproduction of the pathogen, as has been observed in chickpea debris (Dugan et al. 2005). Also, the teleomorph may form and mature under marginal environmental conditions (Gamliel-Atinsky et al. 2005) which would be limiting for the growth and reproduction of *D. rabiei* on residues of plant species other than chickpea. For these reasons, the importance of infested residues from other plant species, besides chickpea, as a source of inoculum under field conditions is difficult to determine. However, the high colonisation and reproduction observed on debris from leguminous plants, which did not differ significantly from chickpea debris, make them a suitable inoculum source and should be considered in the epidemics of the disease.

Reduced tillage systems that maintain most crop residues on the soil surface may increase Ascochyta blight epidemics of chickpea, as has been observed in the U.S. Pacific Northwest region (Kaiser 1992) and in Spain (Trapero-Casas 2004). Besides chickpea residues which are obviously the most important source of inoculum for *D. rabiei*, our results suggest that residues from other crops in the rotation and even from some weed species, should be taken into account when implementing a health management programme for chickpeas.

Our results indicate that alternative non-susceptible hosts of *D. rabiei* may contribute to the epidemics of the disease in the U.S. Pacific Northwest, Spain, and other countries where Ascochyta blight affects chickpea and ascospores are a major source of inoculum.

Ascospores produced in pseudothecia on overwintered debris of alternative hosts may serve as important sources of primary inoculum in the spring and/or inoculum necessary for secondary infections later in the growing season (Trapero-Casas et al. 1996). Infected alternative hosts also may aid in the pathogen's survival from one growing season to the next, as do chickpea debris and infected seeds (Kaiser 1990; 1992).

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