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Ascochyta Blight of Chickpeas

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Introduction

The chickpea (*Cicer arietinum* L.), also known as the garbanzo bean, is an annual grain legume crop that ranks among the world's three most important pulses (seed legumes used as food). It is an important source of protein in many parts of central Asia and Africa. It was one of the first grain legumes domesticated in the Old World and is thought to have originated in present-day southeastern Turkey and northern Syria due to the endemic presence of its progenitor (*Cicer reticulatum* Ladiz.) in this area (1,36).

Historically chickpea has been a minor crop in the United States, but interest in it as an alternative crop to spring cereals has increased in the Pacific Northwest and areas of the High Plains where rainfall is marginal. This is reflected in markedly increased production since the late 1980s (28) with 81,900 ha planted in 2008 (35). The majority of the 2008 crop was produced in Washington (36.6%), Idaho (32.6%), North Dakota (11.4%), and California (7.8%) (35).

Disease: Ascochyta blight.

Although chickpeas are reported to be susceptible to more than 25 well-documented pathogens, Ascochyta blight is among the most serious diseases of chickpea worldwide (12,25). The disease was first described in 1911 from the North-West Frontier Province of India, an area now part of Pakistan (4). However, the disease has apparently been known for centuries and has been postulated to be responsible for the shift of sowing dates from fall to spring by ancient Near East farmers, although the chickpea is agronomically better suited for fall planting (2). Ascochyta blight is problematic at cooler temperatures with disease development being most rapid at temperatures of 20°C with 17 h of leaf wetness (26,33). Little infection will occur at temperatures outside the range of 5-30°C or without leaf wetness, even when humidity is > 95% (21,26,37)

Pathogens

The pathogen occurs as both an anamorph (nonsexual state) and teleomorph (sexual state) (Fig. 1). It can overwinter in crop residues for several years before dissemination in spring via wind-blown ascospores which are produced by the teleomorph (5,9).

Anamorph: *Ascochyta rabiei* [Pass.] Labrousse, Teleomorph: *Didymella rabiei* (Kovacheski) var. Arx. (Syn. *Mycosphaerella rabiei* Kovacheski).

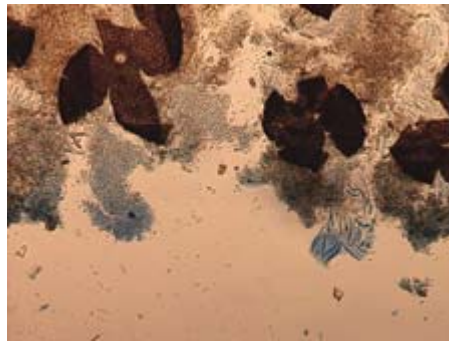


Fig. 1. Wet mount of fruiting structures from overwintered, infected chickpea residue exhibiting pycnidium and conidia (left) and pseudothecium with asci and ascospores (right).

Primary Host: chickpea, garbanzo bean (*Cicer arietinum* L.).

Symptoms and Signs

The pathogen attacks all aerial portions of the plant. Early in the growing season, individual plants infected as seedlings are found scattered in fields (Fig. 2). These plants, originally infected by windblown ascospores or conidia (spores from the anamorph) from adjacent infested debris, or in some instances from infected seed, may serve as foci for secondary spread within fields when favorable environmental conditions occur. Symptoms may be unnoticed initially until conditions at flowering become conducive for disease development.



Fig. 2. Isolated, early-infected chickpea plant (from seed infection) that may serve as a source of infection for later epidemics.

If the initial inoculum source is airborne ascospores, the first symptoms generally seen are small necrotic specks on newer leaves or stems. Under cool, moist conditions, the necrotic specks enlarge and coalesce to form large necrotic lesions (6-12 mm in diameter) on young leaves and buds. Lesions forming on pods (Fig. 3) and leaves (Fig. 4) are primarily circular to oval (up to 0.5 cm), containing concentric rings of pycnidia, the fruiting bodies of the anamorph (Fig. 5) which are visible with a 10× hand lens. Lesions that form on petioles and stems are usually elongate, but also will contain pycnidia arranged in circular patterns (Fig. 6). Stem lesions vary greatly in size, becoming 3 to 4 cm in length, and often girdling stems resulting in breakage (Fig. 7).



Fig. 3. Circular to oval lesions on chickpea pods containing pycnidia arranged in concentric rings.



Fig. 4. Circular to oval lesions with grayish-white centers with dark margins on chickpea leaves.



Fig. 5. Close-up of infected chickpea leaf showing the concentric rings of pycnidia in lesion centers.



Fig. 6. Elongate lesions on chickpea stems with concentric rings of pycnidia.



Fig. 7. Lesion that has girdled chickpea stem, resulting in stem breakage. Note also the circular-oval lesions on leaf in upper right portion of picture.

The fungus may also penetrate the pod wall and infect seeds. Infected seeds serve as a major mechanism for pathogen survival, long-distance dispersal, and initiation of new infections (12,26). Seed infections can be either internal or external on the seed surface, and both types of infections are equally capable of transmitting the pathogen to emerging seedlings (12,25). Infected seeds appear small and shriveled with brown discoloration (Fig. 8), but may also exhibit irregular cankers. The major signs of infection are pycnidia embedded in necrotic lesions on leaves, stems, pods, or seeds. Under conditions of high humidity or moisture, conidia are easily seen oozing from pycnidia in slimy, wet masses (Fig. 9).



Fig. 8. Effect of infection on seed size and quality. Seeds (top) harvested from pods (bottom). Healthy (left), and infected (right).

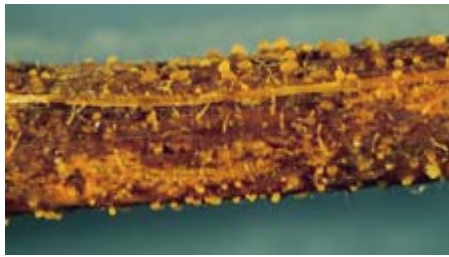


Fig. 9. Spore (conidia) masses of *Ascochyta rabiei* emerging from pycnidia embedded in chickpea tissue.

Host Range

A. rabiei has been shown to be pathogenic on lentil (*Lens culinaris* Medik), field pea (*Pisum sativum* L.), vetch (*Vicia* spp.), common bean (*Phaseolus vulgaris* L.), and cowpea (*Vigna unguiculata* L.) after artificial inoculation (16). The pathogen additionally infects prickly lettuce (*Lactuca serriola* L.) and field pennycress (*Thlapsi arvense* L.), while reproducing (producing pycnidia) on necrotic tissues of alfalfa (*Medicago sativa* L.) and white sweet clover [*Melilotus alba* (L.) Lam.] (16). *A. rabiei* has also been isolated from several plant species growing in fields containing infested chickpea residues from the previous year, including black mustard [*Brassica nigra* (L.) W.D.J. Koch], flaxweed tansymustard [*Descurainia sophia* (L.) Webb ex Prantl], stickyweed (*Galium aparine* L.), henbit deadnettle (*Lamium amplexicaule* L.), and common wheat (*Triticum aestivum* L.) (16).

Geographic Distribution

Since the first report in 1911 (4), the disease has been found in at least 34 countries on 6 continents (26), and is generally considered to be the most limiting production factor wherever chickpeas are grown. It is relatively new to North America and Australia, and was apparently introduced when the chickpea crop was first brought into these areas (15,18,24,26). In the United States, the disease was first reported from eastern Washington in 1984 (15), and has been further identified from California (10), Idaho (7), Montana and North Dakota (23), and Nebraska (11).

Pathogen Isolation

The pathogen is slow-growing, but is still easily isolated from infected tissues. Any infected tissues (e.g., leaves, pods, etc.) incubated in humidity chambers for 24 h at room temperature will yield mucilaginous masses of conidia that can be transferred to various growth media. One method is to blot the oozing pycnidia on the media surface, followed by streaking on plates with a glass "hockey stick," or bacterial inoculating loop. Another is to incubate the piece of infected tissue in a sterile water blank (10 ml), shake, make a series of dilutions and either streak the dilutions or decant them onto surfaces of plates, pouring off excess liquid. After 24 h incubation, these methods yield numerous germinating spores that can be transferred to new plates with the aid of a dissecting scope to obtain single-spored, pure cultures (34).

Media reported to successfully propagate the pathogen include oatmeal agar and 4-8% chickpea seed meal agar (13,25,29). Chickpea dextrose broth (40 g chickpeas, 20 g dextrose per liter) has provided a good medium for large scale increase of the pathogen (29). Other media that have been used successfully include potato dextrose agar (PDA) and V8 juice agar (clarified), either full or half strength. Optimal growth occurs at 22-24°C and 12 h light with relative humidity between 70 and 90% (3,12,13,22,26). It was also reported that the best conditions for pycnidial development include Richard's medium at a pH of 7.6 to 8.0 at 20°C (3).

Pathogen Taxonomy

Phylum Ascomycota; Class Loculoascomycetes; Order Dothideales; Family Dothideaceae; Genus *Didymella*.

Pathogen Identification

Pathogen presence may be easily detected on a field scale by transplanting greenhouse-grown chickpea seedlings in field perimeters as trap crops. Isolation can then be accomplished from resulting infected plants as previously described.

Morphological identification. *A. rabiei* isolates may vary in colony color, morphology and growth rates, but the pathogen is generally slow-growing and may take 14 to 21 days to cover a standard 9-cm Petri plate (4-6 mm/day) (Fig. 10). Hyphae are septate, and the asexual or imperfect state (anamorph) of the pathogen is characterized by the formation of pycnidia (fruiting bodies) which produce the infective spores, known as conidia (or, in some literature, pycnidiospores) (Fig. 11). The pycnidia are recognized as small black dots (up to 245 μm) embedded within lesions on the host. Each pycnidium is spherical or pear-shaped with a single opening (Fig. 12) called an ostiole. The pycnidia contain numerous hyaline spores embedded in a mucilaginous matrix. In the presence of free moisture, the material within the pycnidia absorbs water, becomes wet and swollen, causing conidia to ooze out the ostiole in a slimy mass (Fig. 10). Conidia are oval to oblong, and straight or slightly bent at the ends. They usually are single-celled or two-celled measuring 8-10 \times 4.0-4.5 μm , although precise dimensions can vary (Fig. 13). Two-celled conidia tend to be more frequent when pycnidia are recovered from living plant material, and one-celled conidia tend to predominate when the fungus is grown on agar media.

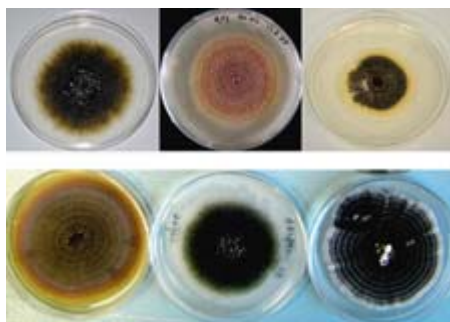


Fig. 10. Variation in colony characteristics among *Ascochyta rabiei* isolates growing in culture.

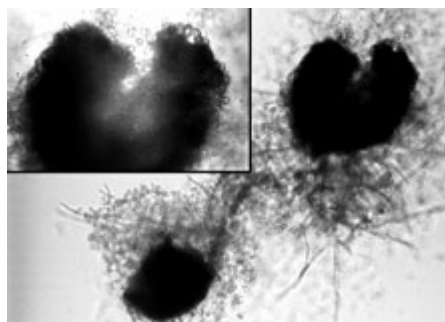


Fig. 11. Wet mount of a mature pycnidium breaking open and releasing conidia.

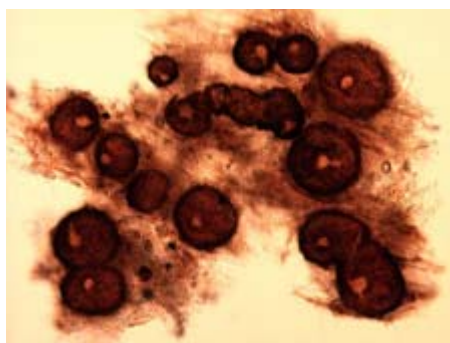


Fig. 12. Pycnidia viewed from above, displaying ostioles.

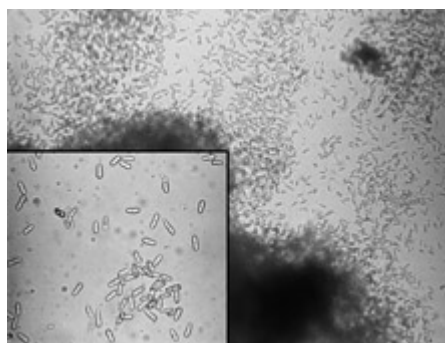


Fig. 13. Conidia of *Ascochyta rabiei*, showing morphological variation of spores.

The first observation of the sexual stage of the fungus was in Bulgaria (19). The pseudothecia (sexual fruiting bodies) were found overwintering on chickpea residue. Pseudothecia are dark brown or black and globose with a very small beak and ostiole, ranging from 76 to 152 μm in height \times 112 to 250 μm in width (19). Asci are cylindrical to clavate and slightly curved (Fig. 14), measuring 48 to 70 \times 9 to 14 μm . Eight ascospores are arranged in a single row per ascus and ascospores are ovoid and divided into two unequal cells (Fig. 15). The ascospores are constricted at the septum and measure 12.5 to 19 \times 6.5 to 7 μm

(19). The teleomorph requires the pairing of two compatible mating types (MAT1-1 and MAT1-2) under cool (4 to 8°C) conditions for successful sexual reproduction (5,17,18,33). Thus, the sexual stage develops only during winter months. The first report of the sexual stage from the western Hemisphere was in 1987 from Washington and Idaho (14), but it is unknown how widespread the two mating types are throughout the United States.

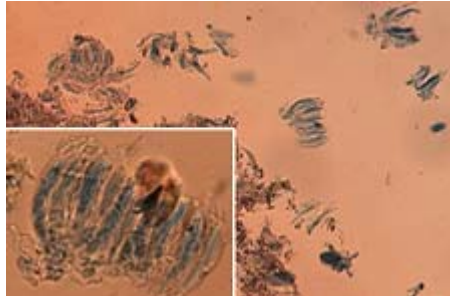


Fig. 14. Wet mount of crushed pseudothecium releasing cylindrical, slightly curved asci containing two-celled ascospores.

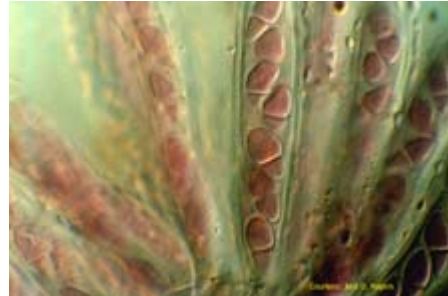


Fig. 15. Close-up view of asci with diagnostic unequally-divided, two-celled ascospores.

Molecular identification. Identification of *A. rabiei* is largely based on morphological characteristics. However, the slow growth rate of some *A. rabiei* isolates predisposes them to being over-grown in culture. Therefore, molecular detection methods are an area of interest and have been reviewed (32). Quantitative PCR based methods are currently being developed (Chilvers Per. Comm.). A PCR-RFLP based diagnostic test has been reported from Australia where it was found to be effective in detecting *A. rabiei* from infected leaves and seeds of chickpea (27). The test is based on the use of ITS 4 and ITS 5 primers designed for conserved sequences of the 18-25S ribosomal genes. The primers amplify the internal transcribed spacer (ITS) regions of *A. rabiei* and other closely related *Ascochyta* species commonly found in pulses (*A. lentis*, *A. pinodes*, and *A. fabae*). The amplicon from this PCR reaction, when digested with the restriction enzymes *Nla*IV and *Sau*96I, gives a distinct banding pattern that can be used to differentiate *A. rabiei* from the other *Ascochyta* species. Certain laboratories also use direct sequencing of the PCR amplicon from the ITS region using the ITS 4 and ITS 5 primers followed by BLASTN searches against the GenBank non-redundant database for confirmation of *A. rabiei* based on matches with sequences deposited by other researchers. This approach, when used in conjunction with evaluation of morphological characteristics, can prove to be a fairly reliable method. However, in certain cases a phylogenetic analysis may have to be conducted for further verification.

Pathogen Storage

For short-term storage, isolates can be kept on agar slants in the refrigerator. Alternatively, conidia collected from PDA or V8 juice agar can be stored in sterile water at 4°C. For long-term storage, the traditional method is to suspend conidia in 15% glycerol and store at -40 or -80°C.

A more economical method is to store isolates on sterile filter papers (e.g., Whatman No 1 filter paper) (8). Filter papers are cut into 7 to 9 pieces, wrapped with aluminum foil, and sterilized by autoclaving with dry cycle (gravity cycle). The filter paper pieces (3 to 4 pieces) are placed on a fresh centrally inoculated PDA plate around the inoculation point. Plates are incubated for 10 to 14 days at room temperature or until the filter paper is covered by the colony. The colonized filter papers are carefully removed with sterile forceps and placed into sterile coin envelopes without gummed flaps, which can serve as nutrients for potential contaminants. The coin envelopes containing the colonized filter pieces are placed in a desiccator connected to a vacuum source to dry the inoculum under vacuum overnight. The dried filter pieces in the coin envelopes can be stored at 4°C in a plastic food container containing dry desiccant. The

desiccant should be replaced with dried desiccant regularly as needed. Isolates can be retrieved by aseptically cutting a tiny piece from the filter paper and placing it onto a suitable medium (either PDA or V8 juice agar) (8).

Pathogenicity Tests

There have been numerous reports describing field screening techniques, although most involve some form of infested residues placed in proximity to plants (Fig. 16) (20,21,30). High humidity and moisture such as from sprinkler irrigation or rainfall are also critical for disease development, following inoculation. Other reports have additionally included spraying plants with spore suspensions if needed (6,29,31). Two week-old seedlings were sprayed with an aqueous spore suspension of approximately 20,000 spores per ml, covered with plastic bags to maintain high humidity, and incubated in the greenhouse (29).



Fig. 16. Chickpea variety evaluations for *Ascochyta* blight resistance: susceptible (foreground) and resistant (background) entries.

This method was also used for confirming results in the field (29). Spraying a spore suspension has also been used successfully on detached leaflets incubated in Petri dishes for approximately 2 weeks at 20°C with a 12-h photoperiod (6,31).

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