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Ascochyta Blight of Peas

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Introduction

Field pea (*Pisum sativum* L.) is an annual, cool-season legume native to northwest to southwest Asia (16,28). It was among the first crops cultivated by man. Wild pea can still be found in Afghanistan, India, Iran, and Ethiopia (18). In the United States, field pea or "dry pea" is marketed as a dry, shelled product for either human or livestock food (1).

Diseases are a constraint to production; peas are affected by more than 25 fungi, bacteria, nematodes, and viruses (13). Perhaps the most important disease in terms of acreage affected is Ascochyta blight. Yield losses of 5 to 15% are common in Saskatchewan when wet conditions persist. Yield losses up to 50% have occurred in research fields but losses of this magnitude are rare in commercial fields (2). Yields have increased 15 to 75% when fungicides are applied (13). Seed contamination over 5 or 10% is considered high in North Dakota and Saskatchewan, respectively, and seed treatment is recommended (2,17).

For the 2010 growing season, total acres planted and total estimated production of dry pea nationally were 756,000 acres (306,000 ha) and 14.2 million cwt (0.65 million MT), down 12% and 17%, respectively, from 2009. Average yield per acre decreased by 2%. In the top three U.S. dry pea producing states of North Dakota, Montana, and Washington, 435,000, 220,000, and 70,000 acres (176,000, 89,000, and 28,300 ha), respectively, were planted in 2010, representing a decrease in dry pea production in these states of approximately 13, 8, and 18% from the previous year (27). Variable production between years is due primarily to economic concerns and government programs that encourage or discourage growers from planting peas (14).

Host: Pea (*Pisum sativum* L.)

Disease: Ascochyta blight

Ascochyta blight is a serious disease of pea worldwide. It was first described in Europe by Libert who named the pathogen *Ascochyta pisi* in 1830 (15). Descriptions of other fungi causing very similar disease symptoms (below) were later made by Berkeley and Bloxam in 1861 and Jones in 1927 (12). Jones reported that isolates of *Mycosphaerella pinodes* and *A. pisi* could be distinguished by their appearance in culture (12).

More than one fungal species can cause this disease. All above ground portions and growth stages of pea plants are susceptible (Fig. 1). Symptoms include the development of purplish black to brown spots or lesions on stems, leaves, tendrils, and pods. Black spore-producing structures may form in these lesions. Pod lesions may become sunken. The fungi can overwinter in seed, infected crop residue, and in the soil. The disease was a constant threat in the United States until about 1915, when it declined with the shifting of the seed industry to areas in the West where the low rainfall, before and during harvest reduced the amount of infected seed. However, in years with unseasonal rainfall contaminated seed may still be found, and seed contamination remains a periodic problem (24). Management strategies include crop rotation, even though it has minimal impact reducing *M. pinodes* or *P. pinodella* (13), and using pathogen-free seed. Seed infection can negatively affect emergence and vigor (13), but seed-to-seedling transmission in the field is low (2).

Fig. 1. Field of pea with severe seedling infection of Ascochyta blight. (Courtesy W. Chen).

Ascochyta blight disease development is favored by temperatures between 20 to 21°C and high relative humidity. Disease normally will not develop at temperatures below 4°C and above 35°C or when periods of leaf wetness are less than 6 h. The pathogens vary in length of incubation period; *M. pinodes* and *A. pinodella* are 2 to 4 days, compared with 6 to 8 days for *A. pisi* (24).

Pathogens

Stem, crown, pod, and foliar diseases of pea are caused by a complex of *Ascochyta pisi* Lib. (teleom. *Didymella pisi* M.I. Chilvers, J.D. Rogers & T.L. Peever) (6), *Mycosphaerella pinodes* (Berk. & A. Bloxam) Vestergr. (ana. *Ascochyta pinodes* L.K. Jones) (3), and *Phoma pinodella* (L.K. Jones) Morgan-Jones & K.B. Burch [syns. *Ascochyta pinodella* L.K. Jones, and *Phoma medicaginis* var. *pinodella* (L.K. Jones) Boerema] (3). The three organisms resemble each other closely, but are distinct species (9) and difficult to distinguish based on symptoms. Each pathogen can overwinter in seed, infested crop residue, and in the soil.

Taxonomy

Ascochyta pisi Lib. is the type species of the genus *Ascochyta*. The *Didymella* sexual state is typically assigned to class Dothideomycetes (Ascomycota).

Didymella pinodes (Berk. & A. Bloxam) Petr. is the most taxonomically correct name for the sexual state of *Ascochyta pinodes*. The name most commonly favored by plant pathologists is the synonym *Mycosphaerella pinodes* (10). In Chilvers et al. (6), isolates of *D. pinodes* clustered with *D. exigua* (Niessl) Sacc., the type species for *Didymella*, and were distant from *Mycosphaerella punctiformis* (Pers.) Starbäck, the type species for *Mycosphaerella*. It appears that *Didymella* is a more correct generic assignment for "*Mycosphaerella*" *pinodes*. The fungus, *D. pinodes*, has recently been placed in a new combination as *Peyronellaea pinodes* (Berk. & A. Bloxam) Aveskamp, Gruyter & Verkley (3).

Phoma pinodella is the third species in this pathogen complex. A sexual state for this fungus would most likely be in Class Dothideomycetes, since molecular-genetic analysis grouped representative isolates with *D. pinodes* (6). Although the sexual state has been observed, the putative teleomorph was not named (5). The asexual state also has a new name, *Peyronellaea pinodella* (L.K. Jones) Aveskamp, Gruyter & Verkley (3).

Symptoms and Signs

All parts of the pea plant can become infected by any of the three fungi causing Ascochyta blight (Fig. 2) but the pathogens are very difficult to distinguish based on symptoms in the field. Symptoms on pea include the development of purplish black to brown spots or lesions on stems, leaves, and pods. Black spore-producing structures may form on these lesions. Pod lesions may become sunken. Early symptoms (purple-brown irregular flecks) are first observed under the plant canopy on lower leaves, stems, and tendrils, where conditions are more humid. These flecks enlarge and coalesce, resulting in the lower leaves becoming completely blighted and falling off. Severe infections on the stem may lead to girdling near the soil line, which is known as foot rot. Typical foot rot lesions are purplish-black in color and may extend above and below the soil line. Girdling lesions weaken the stem and can lead to lodging and yield loss (2).

Fig. 2. Leaf and stem lesions with concentric ring pattern of Ascochyta blight on pea collected in the field. (Courtesy S. Markell).

In addition to girdling foot lesions, black to purplish streaks may develop on stems. These are most conspicuous at the nodes, and may enlarge into brown or purplish irregular-shaped areas anywhere between the root zone and 10 inches (25.4 cm) up the stem.

Lesions may develop on pods under prolonged moist conditions or if the crop has lodged. Pod lesions are initially small and dark, but may become extensive and lead to early pod senescence. Pod spots are gray to purplish, lack concentric rings, and are sunken (Fig. 3). Severe pod infection may result in small, shrunken or discolored seed, or alternatively, seed may show no symptoms.

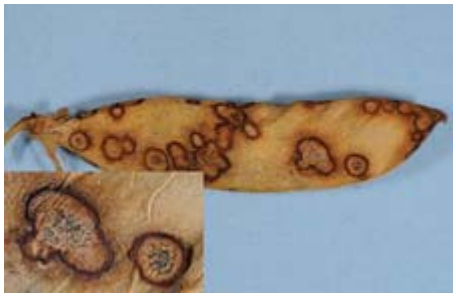

Fig. 3. Pea pod infected with Ascochyta blight. Enlargement shows pycnidia in the lesions. (Courtesy S. Markell).

Lesions caused by *A. pisi* differ from *M. pinodes*. *A. pisi* lesions are typically tan or brown in color with a distinct dark brown margin and visible pycnidia within the lesion. Pycnidia will also develop in lesions caused by *M. pinodes*, but will be less obvious as they blend into the already dark lesion.

Host Range

Ascochyta pisi is reported to infect 20 genera of plants and more than 50 plant species including soybean (*Glycine max*), sweet pea (*Lathyrus odoratus*), lentil (*Lens culinaris*), alfalfa (*Medicago sativa*), common bean (*Phaseolus vulgaris*), clover (*Trifolium* spp.), black-eyed-pea (*Vigna unguiculata*), and broad bean (*Vicia faba*) (10). *Mycosphaerella pinodes* is reported to infect 13 genera of plants and can infect more than 30 species including sweet pea (*Lathyrus odoratus*), common bean (*Phaseolus vulgaris*), black-eyed-pea (*Vigna unguiculata*), and vetch (*Vicia* spp.) (10). *Phoma pinodella* is reported to infect 11 genera of plants and at least 18 species including: alfalfa (*Medicago sativa*), barley (*Hordeum vulgare*), red clover (*Trifolium pretense*), lentil (*Lens culinaris*), palm (*Trachycarpus fortunei*), parsley (*Petroselinum crispum*), sweet pea (*Lathyrus odoratus*), soybean (*Glycine max*), and vetch (*Vicia sativa*) (10).

Geographic Distribution

Ascochyta pisi and *Phoma pinodella* are considered to have a cosmopolitan distribution throughout the world; however, *Mycosphaerella pinodes* is mostly confined to temperate and subtropical regions (10). Recently, another fungus similar to these species has been reported in Australia: *Phoma koolunga* J.A. Davidson, D. Hartley, M. Priest, M. Krysinska-Kaczmarek, Herdina, A. McKay, and E.S. Scott (8).

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Pathogen Isolation

The pathogens causing Ascochyta blight are easily isolated from infected pea tissues via surface sterilization with 96% ethanol for 30 s and plating the tissue on malt or water agar (7,19) (Fig. 4). When isolating these pathogens from seed, they will typically grow out of infected seeds in 10 days, at which time pycnidia form. Isolates typically grow approximately 3 to 5 mm per day in pure culture on malt or V8 juice media at 20°C. Media reported to successfully propagate the pathogen include potato dextrose, V8 juice, Czapek's, malt and oatmeal agars (6,13). *M. pinodes* produces pycnidia on many media and perithecia on PDA (13) and oatmeal agar (19) in pure cultures incubated at 24°C under constant light (13) and 20°C under 12-h photoperiod (19) (Fig. 5). *A. pisi* produces pycnidia on oatmeal agar under conditions of high intensity light and temperatures of 20 to 24°C (6,13). *P. pinodella* forms pycnidia on PDA at a slightly lower temperature range (18 to 24°C) and constant light (13) and on oatmeal agar at 20°C under 12-h photoperiod (19).

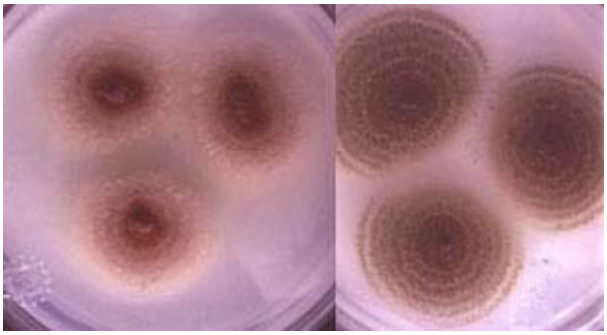

Fig. 4. Cultures of *Ascochyta pisi* (left) *and Phoma pinodella* (right) grown on PDA. (Courtesy F. Dugan).

Fig. 5. Variation in appearance of isolates of *Mycosphaerella pinodes* grown on oatmeal agar. (Courtesy F. Dugan).

Infected tissues (e.g., leaves, pods) incubated in humidity chambers for 24 h at room temperature will yield mucilaginous masses of spores that can be transferred to various growth media. One method for fungal isolation is to blot the oozing pycnidia on the surface of the medium, followed by streaking spores on the plate with a bacterial inoculating loop. Another is to place the piece of incubated tissue in a sterile water blank (10 ml), shake and decant onto surface of an agar plate, and pour off the excess solution. After 24 h of incubation, both methods yield numerous germinating spores that can be transferred to new plates with the aid of a dissecting scope to obtain single-spore, pure cultures (26).

Seed of field pea is routinely tested for infection with *Ascochyta* spp. For each test, 500 seeds are surface disinfested with 2% sodium hypochlorite solution for 2 min, rinsed six to seven times with deionized water, air dried, and plated on PDA. Plates are incubated at room temperature and ambient light conditions for 10 days, after which they are examined for typical colonies and pycnidia formation (Skoglund, *personal communication*) (Fig. 6).

Fig. 6. Pycnidia of *Ascochyta* spp. growing out of a pea seed (10×). Cultures on PDA were incubated at room temperature and ambient light for 10 days. (Courtesy L.G. Skoglund).

Pathogen Identification

Morphological identification. The asexual or imperfect state of each species is characterized by the formation of pycnidia (fruiting bodies) (Fig. 7) that produce the infective spores known as pycnidiospores or conidia (Fig. 8). The pycnidia are small black dots (100-200 µm for *A. pisi* and *M. pinodes* and 200-300 µm for *P. pinodella*) embedded within lesions on the host. They are spherical or pear-shaped with an opening called an ostiole. Each pycnidium contains numerous hyaline spores embedded in a mucilaginous matrix. During periods of high moisture, the material within the pycnidia absorbs the water, becomes wet and swells, causing the spores to ooze out in a slimy mass. In pure culture on artificial media, the light-colored mycelium and the abundant pinkish to carrot-red spores of *A. pisi* readily distinguish it from the darker-colored mycelium and relatively scarce light-buff spore exudate of *M. pinodes* and *P. pinodella*. Spores of *A. pisi* are hyaline, straight or slightly curved, 1-septate, slightly constricted at the septum, have rounded ends, and are 10-16 × 3-4.5 µm. *M. pinodes* conidia are hyaline, slightly constricted at septa, ellipsoid, guttulate, with one (rarely more) septum, and 8-16 × 3-4.5 µm. Spores of *P. pinodella* are hyaline, unicellular, and are much smaller than *A. pisi* and *M. pinodes* (4.5-8 × 2-3 µm). *M. pinodes* is the only species for which the perfect state is routinely observed and frequently ascospores (hyaline, 1-septate, approximately 12-18 × 4-8 µm) are produced in culture. *P. pinodella* and *M. pinodes* often produce dark chlamydospores in culture (Fig. 9), but such structures are rare or absent in *A. pisi* (21,22,23).

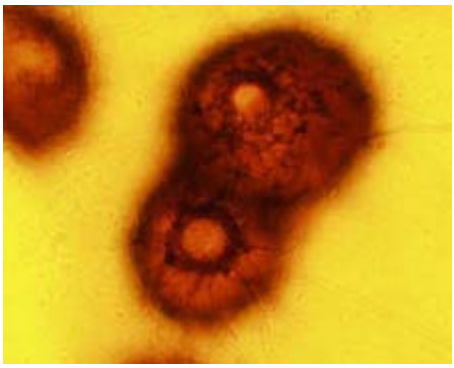

Fig. 7. Pycnidia of *Phoma pinodella*. (Courtesy F. Dugan).

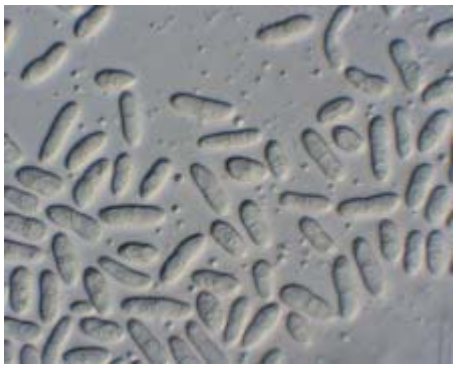

Fig. 8. Conidia of *Mycosphaerella pinodes* grown on oatmeal agar. (Courtesy F. Dugan).

Fig. 9. Chlamydospores of *Phoma pinodella*. (Courtesy F. Dugan).

Molecular identification. There is a growing body of research on molecular identification of *Ascochyta* species and similar fungi, including methods that distinguish between the species of the *Ascochyta* complex on pea. Chilvers et al. (6) used nucleotide sequences of RNA polymerase II (RPB2) to separate *D.* ("*Mycosphaerella*") *pinodes* and *A. pisi*. Aveskamp et al. (3) used sequences for large and small ribosomal subunits, the internal transcribed spacer region (ITS), and beta-tubulin for a thorough revision of these and other, closely related, fungi. Tadja et al. (25) could discriminate between *A. pisi* and *A. pinodes* or *A. pinodella* using ITS1 and ITS4, but the latter two species could not be distinguished this way. Peever et al. (20) separated *A. pisi*, *P. pinodella* and *M. pinodes* on the basis of glyceraldehyde-3-phosphate-dehydrogenase (G3PD) sequence data. Such methods are an improvement over previous studies using RFLPs (9) or RAPDs (4), and it is probable that kits will eventually be available for rapid molecular detection and differentiation of these pathogens.

Pathogen Storage

For short term storage, isolates can be kept on agar slants in the refrigerator at 4°C or stored in sterile water at the same temperature as conidia collected from PDA or V8 juice agar. 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) (11). Filter papers (11-cm diameter) are cut into seven to nine pieces, wrapped with aluminum foil, and autoclaved. Three to four sterile filter paper pieces are placed on a fresh, centrally inoculated PDA plate around the inoculation point. The plates are incubated for 10 to 14 days at room temperature or until the filter paper is covered by the fungal mycelia. The colonized filter papers are carefully removed with sterile forceps and placed into sterile coin envelopes without gummed flaps (gum can serve as a nutrient for potential contaminants). The coin envelopes containing the colonized filter pieces are then 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. To retrieve the isolates, aseptically cut a tiny piece from the filter paper and place it onto a suitable medium (either PDA or V8 juice agar) (11).

Pathogenicity Tests

In pathogenicity tests carried out under greenhouse and growth chamber conditions, 3- to 5-week-old plants are spray-inoculated with a spore suspension of 1×10^5 conidia/ml prepared from 2-week-old V8 juice agar cultures. To insure high humidity, plants are covered with plastic covers or incubated in a humid chamber for 24 h. They are then incubated at 20°C under 12 to 14 h of natural light. Disease is scored on the first four leaves and first four

internodes 10 and 20 days after inoculation (6,19). Disease severity is assessed on a visual scale of 0 to 5: 0 = no lesions; 1 = a few scattered flecks; 2 = numerous flecks; 3 = 10 to 15% of leaf area necrotic and appearance of coalesced necrosis; 4 = 50% of the leaf areas dehydrated or covered by lesions; 5 = 75 to 100% of the leaf area dehydrated or necrotic (19). Davidson et al. (8) assessed disease development expressed as percent leaf area (%LAD) and internodes (%IAD) on the three lowest leaves after 7 days (8).

It has been noted that lesions develop within 3 days with *M. pinodes* and *P. pinodella*, whereas lesions of *A. pisi* take 11 days to develop. Fewer lesions are formed with *A. pisi* than the other species. Also, lesions caused by *A. pisi* are circular and tan with dark margins, as opposed to *M. pinodes* and *P. pinodella* which are indistinct (6).

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