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Phytophthora Blight on Pumpkin

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Disease: Phytophthora blight
Primary Economic Host: Processing pumpkin (*Cucurbita moschata*) (Fig. 1)
Pathogen: *Phytophthora capsici* Leonian

Fig. 1. A processing pumpkin field at harvest.

Taxonomy

Phytophthora capsici was first described by Leonian on pepper in 1922 (11). In 1931, Tucker (19) classified species of the genus *Phytophthora* and considered *P. capsici* as a host-specific fungus, pathogenic on pepper. Subsequently, taxonomists (12,17,18) studied Phytophthora isolates from various hosts in the world and re-described the taxonomy of *P. capsici*. A detailed description of *P. capsici* taxonomy is provided by Erwin and Ribeiro (5).

Symptoms and Signs

P. capsici can strike cucurbit plants at any stage of growth. The infection usually appears first in low areas of the fields where soil remains wet longer. The pathogen infects seedlings, vines, leaves, and fruit.

Damping-off. *P. capsici* causes pre- and post-emergence damping-off in processing pumpkin plants in wet and warm (20 to 30°C) soil conditions (6) (Fig. 2). In seedlings, a watery rot develops in the hypocotyls at or near the soil line, resulting in plant death. Post-emergence seedling death is preceded by plant wilting (Fig. 3). Mature plants show symptoms of crown rot. Initial symptoms include a sudden, permanent wilt of infected plants without a change in color (20). The wilt of leaves progresses from the base to the extremities of the vines. Often plants die within a few days of the first symptoms or after soil is saturated by excessive rain or irrigation. The stem near the soil line turns light to dark brown and becomes soft and water-soaked. Infected stems collapse and die. Tap and lateral roots of infected processing pumpkin plants usually do not exhibit any symptoms. Following death of the foliage, roots may give rise to new vines if environmental conditions become less conducive for development of disease. Phytophthora damping-off may result in partial to total loss of the crop.

Fig. 2. Post-emergence damping-off of a processing pumpkin seedling.

Fig. 3. Plant wilt as a result of crown infection with *P. capsici*.

Vine blight. Vines can be affected at any time during the growing season (2). Water-soaked lesions develop on vines (Fig. 4). The lesions are dark olive in the beginning (Fig. 4A) and become dark brown in a few days (Fig. 4B). The lesions girdle the stem, resulting in rapid collapse and death of foliage above the lesion site (Figs. 4C and D). Unaffected parts of the vine continue to grow if no girdling lesion develops along the vine.

Fig. 4. Vine symptoms: (A) lesions on newly infected vine; (B) a fully-developed lesion; (C) a girdling lesion affecting a part of a vine; (D) crown infection.

Leaf symptoms. *P. capsici* infects both the petiole and blade (2). Dark brown, water-soaked lesions develop on petioles (similar to lesions on vines), resulting in rapid collapse and death of leaves. Infection of the leaf blade with *P. capsici* results in development of leaf spots ranging from 5 mm to more than 5 cm in diameter (Fig. 5). Infected areas are chlorotic at first and then become necrotic with chlorotic to olive-green borders in a few days. Under wet and warm conditions, leaf spots expand rapidly, coalesce, and may cover the entire leaf. Under dry conditions, expansion of leaf spots may cease.

Fig. 5. Phytophthora spots on a processing pumpkin leaf. Note chlorosis and necrosis.

Fruit rot. Fruit rot can occur from the time of fruit set until harvest (9,20) (Figs. 6 and 7). Fruit rot generally starts on the site of the fruit that is in contact with the ground. However, when an infected leaf or vine comes in contact with a fruit, fruit rot will start at the point of contact. Also, symptoms on the upper surface of the fruit develop following rain or overhead irrigation, which provides splashing water for pathogen dispersal. Fruit rot can also develop after harvest, during transit, or in storage. Fruit rot typically begins as a water-soaked lesion, expands, and becomes covered with white mold (9,20) (Fig. 6). The pathogen produces numerous sporangia on infected fruit (Figs. 6 and 7). Fruit infection progresses rapidly, resulting in complete collapse of the fruit (Fig. 7). Phytophthora foliar blight and fruit rot may result in total loss of the crop (2). *P. capsici* also causes fruit rot on other cucurbit crops (Fig. 8).

Fig. 6. Lesions on an immature processing pumpkin fruit, note yeasty white mold (mycelium and sporangia of *P. capsici*) on the lesions.

Fig. 7. Severely infected and collapsed processing pumpkin fruit.

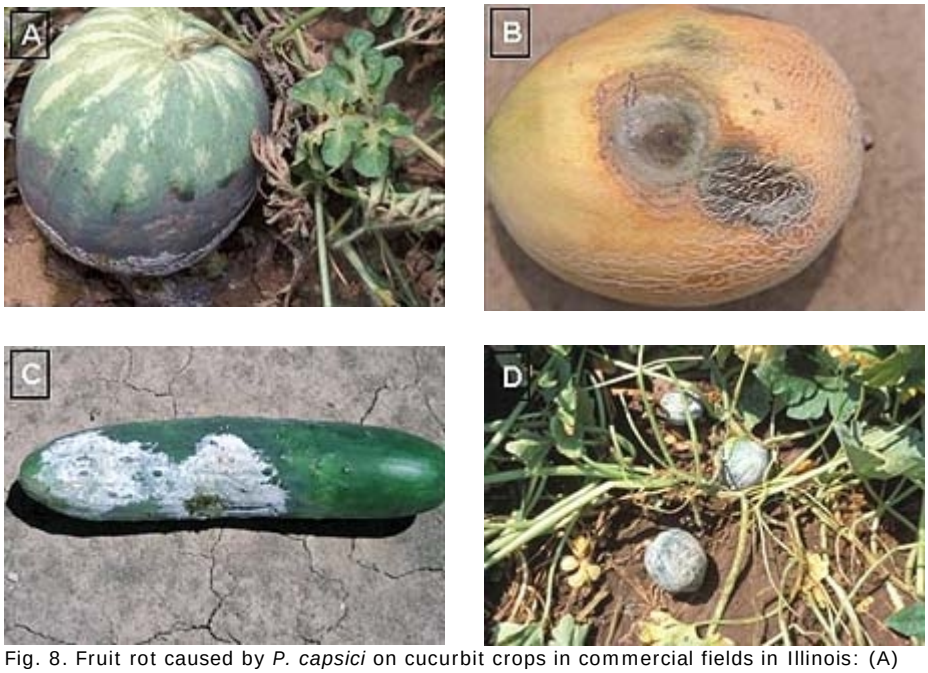

Fig. 8. Fruit rot caused by *P. capsici* on cucurbit crops in commercial fields in Illinois: (A) watermelon; (B) muskmelon; (C) cucumber; (D) acorn squash.

Host Range

More than 40 plant species have been reported to be infected with *P. capsici* (5). Among the major hosts of *P. capsici* are red and green peppers (*Capsicum annuum*), watermelon (*Citrullus lanatus*), cantaloupe (*Cucumis melo*), honeydew melon (*Cucumis melo*), cucumber (*Cucumis sativus*), pumpkin (*Cucurbita pepo*), gourd (*Cucurbita pepo*), acorn squash (*Cucurbita pepo*), yellow squash (*Cucurbita pepo*), zucchini squash (*Cucurbita pepo*), processing pumpkin (*Cucurbita moschata*), blue Hubbard squash (*Cucurbita maxima*), tomato (*Lycopersicon esculentum*), black pepper (*Piper nigrum*), and eggplant (*Solanum melongena*). Interspecific pathogenicity tests of *P. capsici* isolates from cucurbit and pepper fields have shown variation among the isolates (7,10,14).

Geographical Distribution

P. capsici was first described by Leonian in 1922 as the causal agent of a blight of chili pepper in New Mexico (11). *P. capsici* on cucurbits was first reported in 1937 in Colorado and California (8,16). A widespread foliar blight and fruit rot in processing pumpkin fields, caused by *P. capsici*, occurred in Illinois in 1999 (2). Since then, Phytophthora blight has been observed in cucurbit and pepper fields in Illinois every year. *P. capsici* infection commonly occurs in temperate, subtropical, and tropical environments in the world (4,5,20).

Pathogen Isolation

P. capsici can be easily isolated from newly infected tissues (7). Firm tissue near the margin of the lesion on leaf or vine should be selected. The tissue should be washed in tap water to remove adhering soil, immersed in 70% ethanol for 3 min, rinsed in sterile distilled water (SDW), and blotted dry. Tissue segments then are placed on cornmeal agar amended with pimaricin, ampicillin, rifampicin, pentachloronitrobenzene, and hymexazol (PARPH) (6,13). This is a semi-selective culture medium for *P. capsici*. The plates are incubated at 24°C under 12 h of fluorescent light and 12h of darkness for 2 to 3 days. Mycelial tips from the edge of the growing colony of *P. capsici* are transferred onto potato

dextrose agar (PDA) in Petri plates and the plates are incubated at 24°C for 3 to 5 days.

For isolation of *P. capsici* from fruit, rind tissue is disinfested by rubbing the fruit surface with a paper towel moistened with 70% ethanol. Pieces of rind from the margin of the lesion are cut and placed on PARPH in Petri plates. The plates are incubated at 24°C as described above.

To prepare pure cultures, 10 ml SDW is added to a 5-day-old *P. capsici* culture plate and sporangia are dislodged. The suspension is incubated at 20°C for 1h to allow the sporangia to release their zoospores. The zoospore suspension is diluted to approximately 100 spores/ml, 200 µl of the suspension is transferred onto the surface of an agar plate and spread over the surface, and the plate is incubated at 24°C for 8 to 12 h. The plate is then examined (X200) and colonies are marked that are developing from single zoospores. The germinating zoospore and a small portion of surrounding agar is transferred onto PDA.

Pure cultures of *P. capsici* also can be obtained by transferring a diluted zoospore suspension onto the surface of a thick layer of agar. The entire agar disk then is turned with a spatula. The next day, the bottom of the plate is examined and colonies are marked that are developing from single zoospores. Within 48 to 96 h, mycelium that has grown to the agar surface is transferred to fresh plates of PDA (15).

Pathogen Identification

Identification of *P. capsici* is mainly based on the morphology of sporangia (5). Sporangia of *P. capsici* are variable in shape and are papillate (Fig. 9A and B). Sporangial shapes are influenced by light and other cultural conditions, and are subspherical, ovoid, obovoid, ellipsoid, fusiform, and pyriform. Sporangia are tapered at the base and are caducous with long pedicels.

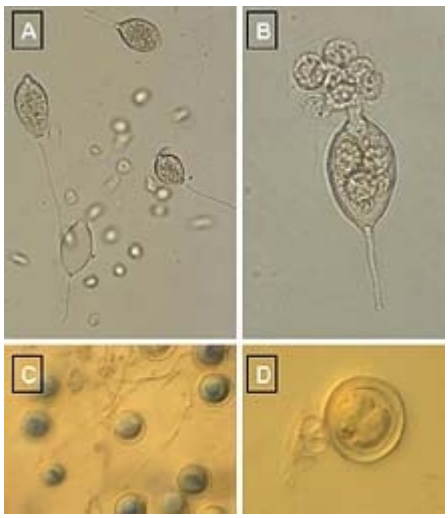

Fig. 9. Reproductive structures of *P. capsici* isolates from processing pumpkin fields: (A) sporangia and zoospores in a culture plate; (B) a sporangium releasing zoospores; (C) oospores in a culture plate; (D) an oospore with amphygynous antheridium.

Results of studies on 56 isolates of *P. capsici* from processing pumpkin fields showed that the length and width of sporangia ranged from 35 to 68 and 20 to 46 µm, respectively (7). Length/width ratios of sporangia ranged from 1.2 to 2.0. Pedicel lengths were highly variable among the isolates, ranging from 34 to 101 µm.

Other characteristics of *P. capsici* isolates from processing pumpkin plants are: (i) colonies grow from 10 to 36°C, with optimum temperatures of 24 to 32° C; (ii) growth pattern of colonies are cottony, petaloid, rosaceous, and stellate (Fig. 10); (iii) oospore diameter ranges from 20 to 38 µm; and (iv) antheridia are amphigynous (Figs. 9C and D). The pathogen is heterothallic and both A_1 and A_2 types exist in processing pumpkin fields in Illinois.

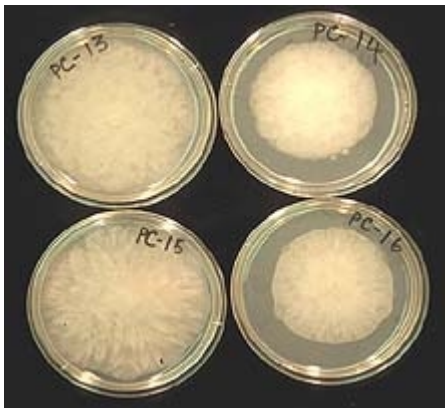

Fig. 10. Growth pattern of *P. capsici* colonies from processing pumpkin fields on lima bean agar, 5-day-old cultures.

Pathogen Storage

P. capsici can be maintained on lima bean agar (LBA) slants at room temperature or in a refrigerator for several months (6). Frequent transferring is necessary to keep the cultures viable.

Cultures also can be stored in SDW (1,3). Small blocks of agar media on which the pathogen is growing are transferred to autoclaved, screw-capped bottles or tubes containing SDW. The cap is tightened for storage. The agar blocks are removed when necessary and transferred to culture medium.

Other storage methods include techniques of cryopreservation (5). Cultures can be preserved using cryoprotective solutions such as 8.5% sterile skim milk, 10% glycerol, or 15% dimethylsulfoxide in vials. The vials are cooled to -40°C (at the rate of 1°C per min) and stored under liquid nitrogen.

Pathogenicity Tests

Zoospores and mycelia can be used as inoculum for pathogenicity tests. Inoculation of plants with zoospores is the fastest and easiest way of conducting pathogenicity tests (6).

Zoospore inoculation. For zoospore production, 5-mm-diameter disks are transferred from the margin of an advancing culture of *P. capsici* onto LBA in 9-cm-diameter Petri plates. The plates are incubated at 24°C under continuous white fluorescent light for 5 to 6 days. A sporangial suspension is prepared by adding 10 ml of SDW to each plate and the sporangia are dislodged using a soft brush. The suspension is incubated at 20°C for 1 h to allow the sporangia to release their zoospores. If culture plates are maintained more than 10 days at 24°C, very few zoospores will be released from sporangia. Zoospores are separated from the empty sporangia by passing the suspension through a 4-layered facial tissue. An aliquot of the suspension is vigorously mixed using a vortex mixer for 1 to 2 minutes to allow zoospores to encyst prior to counting with a hemacytometer. The concentration of zoospores is adjusted to 2×10^5 zoospores/ml water and used for inoculation of plants.

Seeds of processing pumpkin, or any other host, are sown in 10-cm diameter pots containing steamed soil mix (soil:sand:peat; 1:1:1) and are grown in a greenhouse. Three- or four-week-old seedlings are inoculated by adding 5 ml of a *P. capsici* zoospore suspension (2×10^5 spores/ml) over the surface of the soil around each seedling. The pots are watered before inoculation to keep the soil wet. After inoculation, the pots are watered twice daily. Beginning the second day after inoculation, seedlings are examined for disease development. Brown lesions develop on the stem base within a week and plants collapse and die quickly (Fig. 11).

Fig. 11. Wilting and damping-off of 4-week-old seedlings of processing pumpkin following inoculation of plants in the greenhouse. Seven pots in the front were inoculated and four pots in the back were not inoculated. Wilt symptoms develop in 4 days.

Foliage of pumpkin plants can be inoculated at any stage of growth by spraying with a zoospore suspension in water (≥104 spores/ml) in the greenhouse or field. Overhead irrigation for at least 2 days is necessary to provide moisture for infection. Within 5 days, lesions develop on leaves, vines, and fruit.

Mycelium inoculation. *P. capsici* is grown on oatmeal-V8 juice broth (V8JB) substrate in 1-liter flasks. Five-mm-diameter plugs are transferred to the flasks from the margin of a young colony of *P. capsici* onto LBA. The flasks are incubated at 24°C in darkness. After 6 week, the colonized oatmeal is added to steamed soil mix and mixed thoroughly. The inoculum potential of the soil mix should be more than 100 (preferably 500 or higher) colony-forming units (cfu) per gram of soil. *P. capsici* inoculum density in soil is determined using the dilution-plate-count method (6,13). The infested soil is placed into 10-cm-diameter pots and seeds of pumpkin or any other host plant are sown in the pots. The pots are kept in a greenhouse at 18 to 25°C. Pre- and post-emergence damping-off occur within 3 weeks from sowing seeds.

Pumpkin fruit or fruit of other cucurbits can be inoculated with *P. capsici* mycelia and sporangia. Fruit is washed under tap water, rinsed with distilled water, and dried in a sterile hood. A 10-mm-diameter plug is transferred from 5-day-old *P. capsici* culture grown on LBA onto the fruit. The inoculated fruit is kept in a sealed plastic bag on a laboratory bench or in an incubator at 20 to 25° C. Fruit rot develops within 7 days.

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Appendix

PARPH medium (6,13). For isolation of *P. capsici* from plant tissue and soil.

- 17 g Difco cornmeal agar
- 1000 ml distilled water

Autoclave the above ingredients and add the following when agar cools to 45 to 50°C:

- 10 mg pimaricin
- 250 mg ampicillin
- 10 mg rifampicin
- 100 mg pentachloronitrobenzene
- 50 mg hymexazol

Oatmeal-V8 Juice-Broth (V8JB). For production of mycelium inoculum to be added to soil:

- 200 g oatmeal
- 120 ml V8 juice
- 1000 ml distilled water