Diagnostic Guide for Gummy Stem Blight and Black Rot on Cucurbits

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Disease
Gummy stem blight. The fruit rot phase is often called black rot.

Hosts
All commonly cultivated cucurbits (members of the botanical family Cucurbitaceae) are susceptible to gummy stem blight, although the degree of susceptibility differs among species and horticultural types. Rankings of hosts also differ depending on whether susceptibility to foliar blight, crown, stem and vine cankers, or fruit rot is considered.

The following list is arranged in an approximate order of susceptibility to both the foliar blight and canker phases of gummy stem blight, starting with the species that are the most susceptible: muskmelon, honeydew melon, and other melons (Cucumis melo subsp. melo); watermelon (Citrullus lanatus var. lanatus); cucumber, slicing and pickling (Cucumis sativus); Hubbard squash, giant pumpkin, buttercup squash, turban squash (Cucurbita maxima); jack-o-lantern pumpkin, ornamental gourds, acorn squash, summer squash, and zucchini (Cucurbita pepo); and butternut squash and tropical pumpkin (Cucurbita moschata) (14). Within C. pepo, jack-o-lantern pumpkin and ornamental gourds tend to be more susceptible to foliar blight than squashes. C. moschata is resistant to crown cankers.

Susceptibility of cucurbit crops to black rot differs from susceptibility to foliar blight and crown cankers. There is considerable variation in fruit susceptibility to black rot within C. pepo and Cucumis melo. The fruit of butternut squash, jack-o-lantern pumpkin, ornamental gourds, muskmelon, and greenhouse cucumber are the most susceptible (3,23,26,27). Black rot also affects watermelon when foliar blight is severe (27).

Pathogen
Gummy stem blight and black rot are caused by the ascomycete fungus Didymella bryoniae (Auersw.) Rehm (19), which is the name of the teleomorph. The anamorph recently was renamed Stagonosporopsis cucurbitacearum (Fr.) Aveskamp, Gruyter & Verkley; Phoma cucurbitacearum (Fr.:Fr.) Sacc. was the name used previously (1). In the older literature, Mycosphaerella melonis (Pass.) Chiu & J.C. Walker and Ascochyta cucumis Fautrey & Roum. were used for the teleomorph and anamorph names, respectively.

Taxonomy

As of 1 January 2013, changes to the International Code of Botanical Nomenclature specify that each fungus is to be known by only one name, even if it has teleomorphic (sexually reproducing) and anamorphic (asexual) states. D. bryoniae (Auersw.) Rehm published in 1881 is the oldest name for this pathogen. Note that some taxonomists use the author citation D. bryoniae (Fuckel) Rehm, since this is the author citation published by Saccardo in 1882. However, it is clear from Rehm’s original note that he based his name on
Auerswald’s species name, published in 1869, and considered Fuckel’s identical species name for this fungus, published in 1870, a synonym (21). The taxonomic treatment of the genus *Didymella* by Corlett includes a useful summary of primary literature (6).

*Phoma cucurbitacearum* (Fr.:Fr.) Sacc. recently was renamed *Stagonosporopsis cucurbitacearum* (Fr.) Aveskamp, Gruyter & Verkley based on DNA sequence data that divided *Phoma* species into a number of genetically distinct clades (1). Current taxonomic information is available at Fungal Databases at the Systematic Mycology and Microbiology Laboratory, USDA-ARS (http://nt.ars-grin.gov/fungaldatabases) and Mycobank (www.mycobank.org).

**Symptoms and Signs**

Gummy stem blight symptoms affect all above-ground vegetative and reproductive parts of cucurbits, including leaves, petioles, vines, stems, tendrils, pedicels, flowers, peduncles, fruit, and seed (5,11). Leaf spots generally are the symptom noticed first and are the symptom most often used to diagnose the disease. On most hosts, more than half of the spots start at the margins of leaves or extend to the margins (Fig. 1). As leaf spots expand, they coalesce, which results in leaf blighting (Fig. 2). Actively expanding lesions on leaves, petioles, and pedicels often are water-soaked, as the pathogen produces cell wall-degrading enzymes, in particular polygalacturonase (27) (Fig. 3). Water-soaking may also be visible on the leaf underside on main veins that pass through lesions. Constricting lesions at the apex or the base of a petiole or on the midrib of a leaf may cause the entire leaf to collapse (Fig. 4).

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**Fig. 1.** Leaf spots of gummy stem blight at the leaf margins on watermelon (*Citrullus lanatus* ‘Mickeylee’).

**Fig. 2.** Leaf blight phase of gummy stem blight on watermelon (*Citrullus lanatus* ‘Royal Star’) resulting from expansion and aggregation of individual leaf spots.

**Fig. 3.** Actively expanding leaf spots of gummy stem blight on a bottle gourd (*Lagenaria siceraria*) leaf with water-soaked margins due to cell wall-degrading enzymes produced by *Didymella bryoniae*.

**Fig. 4.** Gummy stem blight lesion with fruiting bodies of *Didymella bryoniae* on petiole of squash (*Cucurbita argyrosperma* ‘Green Striped Cushaw’).
Color and shape of leaf spots vary somewhat with host, but the following characteristics are common. Leaf lesions are round or triangular, particularly those at the leaf margins, or sometimes rhomboid (Figs. 3 and 5). Leaf spots are tan on cucumber; brown on melon, squash, and pumpkin; and dark brown on loofah and watermelon. The center of spots often is a lighter shade of brown than the surrounding portion (Figs. 3 and 5C). Leaf spots frequently display alternating rings of dark and light brown necrosis, which correspond to diurnal periods of pathogen growth when leaves are wet with dew (Fig. 1 and 5A). Old leaf spots have a defined margin (Figs. 5B and C). On squash and pumpkin, old leaf spots may shred or tear (Fig. 5B and C).

Cankers may form on crowns, main stems, or vines of melons and other hosts (14). Cankers are light brown to beige to off-white, noticeably lighter in color than foliar leaf spots (Fig. 6). On some hosts in the early stage of development, cankers are water-soaked; this is most common on C. melo (Fig. 7). As cankers expand, they become dry and rough, and the surface cracks. Drops of gummy, amber plant sap may appear on some cankers under certain, undefined conditions (Fig. 8). Although this exudate gives the disease its common name, the presence of this symptom is not diagnostic; stem injuries can provoke a similar host response.
Gummy stem blight can be distinguished from cucurbit anthracnose by the larger leaf spots, larger lesions that encircle petioles, and crown cankers. On watermelon leaves, the shape of anthracnose spots is distinctly angular and irregular, while leaf spots of gummy stem blight generally are round. The centers of anthracnose leaf spots are more likely to dissolve than centers of leaf spots of gummy stem blight.

Gummy stem blight can occur on seedlings of watermelon, muskmelon, and other cucurbits grown in greenhouses for use as transplants (11,15). A large, water-soaked lesion at the point on the hypocotyl where the cotyledons attach is a typical initial symptom. If these plants are transplanted in the field and survive, cankers may form on crowns or vines. Spots on cotyledons or true leaves also occur in the greenhouse. After secondary spread, it may be possible to identify the seedling initially infected, because it will have died and will be surrounded by symptomatic seedlings (Fig. 9).
Symptoms of black rot vary with host species and horticultural type. The most distinctive symptom of black rot is found on butternut squash, which display amber- to bronze-colored lesions with a curlicue pattern of darker, slightly raised lines within the lesions (27). On jack-o-lantern pumpkin, black rot appears as numerous small or several large, sunken lesions (3). The small spots may be confused with anthracnose fruit rot on this host; signs of the pathogen should be used to confirm the diagnosis. On muskmelon, both external and internal rot may occur; black mycelial growth of the pathogen may be present in the cavity of some affected fruit. Black rot on watermelon appears as a large, expanding rot that starts at the blossom end of the fruit (Fig. 10). Fruiting bodies of the pathogen in the affected tissue can be used to distinguish black rot from blossom end rot on watermelon.

Diagnostic signs are the pycnidia and pseudothecia of the pathogen that form readily in leaf spots and in lesions on other above-ground plant parts, including petioles, vines, stems, tendrils, and pedicels of flowers and fruit (Figs. 6 and 11). Because *D. bryoniae* is a necrotrophic pathogen, fruiting bodies form first in the center of lesions (Figs. 4 and 5C). Fruiting bodies form readily when diseased tissue is placed in a simple humidity chamber made from a moist paper towel and a self-sealing polyethylene bag and held overnight at ambient temperature and lighting.

Pseudothecia are dark brown to black and usually are slightly immersed in the host tissue (Fig. 11). Hyaline pseudoparaphyses are present in pseudothecia, and in humidity chambers, pseudoparaphyses sometimes can be found, with a dissecting microscope, protruding from the ostiole (opening) in the top of pseudothecia. Pycnidia are tan when young and become brown as they age (25). They usually are slightly larger than pseudothecia.
Host Range

In addition to the six commonly cultivated cucurbit species listed above, foliar or crown lesions and black rot have been reported on bitter melon (also known as balsam pear) (*Momordica charantia*), bottle gourd (*Lagenaria siceraria*), smooth loofah (*Luffa cylindrica*), and chayote (also known as vegetable pear) (*Sechium edule*) (2,14,22,25). Citron (*Citrullus lanatus* var. *citroides*) is susceptible to foliar blight and crown cankers, but the hard-rind fruit are resistant to black rot (14). Wild hop (also known as white bryony) (*Bryonia alba*) is susceptible to gummy stem blight; *B. cretica* was the host on which *D. bryoniae* was first discovered (6). Fruits of wax gourd (also known as winter melon) (*Benincasa hispida*) are susceptible to black rot (25), but whether vines or foliage are susceptible to gummy stem blight is unknown. Host susceptibility is summarized in Keinath (11), Punithalingam and Holliday (18), and the Fungal Databases at the Systematic Mycology and Microbiology Laboratory, USDA-ARS (http://nt.ars-grin.gov/fungaldatabases).

Geographic Distribution

*D. bryoniae* is found on all six inhabited continents, although the number of confirmed reports from South America and Africa is limited (7,9,11). In general, the pathogen is found most commonly in humid, temperate, semi-tropical, and tropical regions (2,20). In the United States, *D. bryoniae* is found throughout the eastern half of the country, as far west as southeastern Texas, and occasionally in California (15,16,26). The Fungal Databases at the Systematic Mycology and Microbiology Laboratory, USDA, ARS include geographic distribution by country.

Pathogen Isolation

Pure cultures of the pathogen often can be obtained from discrete leaf or petiole lesions. Pieces approximately 3 x 3 mm are cut from the margins of lesions so that they include diseased and healthy tissue. (If the tissue is not turgid, the pieces should be submerged in tap water to rehydrate for several minutes before proceeding.) The pieces are disinfested in 0.6% sodium hypochlorite for 1 min, rinsed in sterile water, blotted on clean paper towels, and placed onto one-quarter-strength potato dextrose agar amended after autoclaving with 100 mg chloramphenicol, 100 mg streptomycin sulfate, and 60.5 mg mefenoxam (0.25 ml Ridomil Gold EC) per liter (10). An incubation temperature between 20 and 25°C is adequate. A photoperiod of 16 h of light is required to induce rapid production of fruiting bodies (10,18). Under these temperature and light conditions, pycnidia and conidia typical of *D. bryoniae* are produced as
soon as 3 days after culturing, on either the tissue or the surrounding agar (Fig. 12). Immature pseudothecia also may form.

To obtain a pure culture from a single conidium, a 3-mm-square piece is cut from an area of the colony with pycnidia, taking care to avoid other microorganisms if an isolation plate is used as the source. The piece is put into 2 to 4 ml sterile water, which is agitated for 20 to 30 sec to release conidia. A 0.1-ml aliquot of this spore suspension is spread onto water agar amended with 0.1 g/liter streptomycin sulfate. After 20 to 24 h, conidia will be seen germinating at 30× magnification. While viewing the plate through a dissecting microscope with subsurface illumination, a tiny piece of agar that includes only an individual conidium and its germ tube is cut out and placed onto one-quarter-strength potato dextrose agar.

**Pathogen Identification**

Fruiting bodies of *D. bryoniae* form in the center of the colony in a random pattern, whereas pycnidia of *Phoma* species non-pathogenic on cucurbits form in regular, circular patterns when grown under 16-h photoperiod. Young pycnidia are tan with a dark ring of cells around the ostiole (Fig. 13). Although this coloration is not specific to *D. bryoniae*, it distinguishes pycnidia of *D. bryoniae* from the uniformly dark brown pycnidia of other *Phoma* species isolated from cucurbits that are non-pathogenic or weakly pathogenic that may be present in the same leaf spots as the pathogen (13). Conidia of *D. bryoniae* measure approximately 8.0 × 3.3 μm, which is greater than dimensions of *Phoma* conidia, 6.4 × 2.7 (± 0.21) μm, on one-quarter-strength potato dextrose agar (13). Another useful characteristic is that a variable proportion of *D. bryoniae* conidia are two-celled, whereas all conidia of other *Phoma* species commonly isolated from cucurbit leaves are one-celled (Fig 14) (13, 25).

Pseudothecia form in cultures grown in unsealed, plastic Petri dishes on one-quarter strength potato dextrose agar incubated at a constant temperature of 22-24°C, 16-h photoperiod produced from cool white fluorescent bulbs (12). One-quarter-strength potato dextrose agar is preferred over full strength medium, because *D. bryoniae* produces less aerial mycelium and more fruiting bodies on the lower nutrient version. It takes 7 to 10 days for pseudothecia to produce mature ascospores in culture. Note that production of pseudothecia is variable
among isolates and between laboratories. Ascospores measure 13 × 5.5 μm (13,25). The bicellular ascospores have a diagnostic shape: they appear as two unequally sized triangles joined at the bases, pointing in opposite directions, and the base of the apical cell is wider than the base of the inverted basal cell (6,18).

Several PCR primer sets have been developed to identify D. bryoniae. The most recently developed set of real-time PCR primers reacts with two distinct genotypes, RAPD Group (RG) I and II, whereas the previous set of real-time PCR primers only detected RG I (8,17). A set of three conventional PCR primers is available to identify D. bryoniae RG I and RG II isolates as well as other Phoma isolates from cucurbits (21).

**Pathogen Storage**

*D. bryoniae* can be stored as dried cultures on sterile filter paper. With standard sterile technique, a sterile filter paper circle 75 mm in diameter is centered on a 100-mm-diameter Petri dish containing one-quarter-strength potato dextrose agar. The filter paper circle should not completely cover the agar—there should be a narrow border of agar around the edge of the dish. Two pieces cut from an established culture are placed around the edge of the filter paper on the agar, not on the filter paper. The culture is incubated 7 to 14 days as described previously.

As soon as the colony has covered the filter paper circle, the filter paper is removed with sterile forceps, placed into a sterile, covered Petri dish, and left in an operating laminar flow hood for 3 to 5 days. With sterile forceps and sterile scissors, the dry filter paper is cut into 0.5- to 1-cm-square pieces that are stored in sterile glass vials at 5°C. Cultures on filter paper will remain viable up to 10 years. To start a fresh culture, one or two filter paper squares are placed onto agar and incubated as described previously.

**Pathogenicity Tests**

For greenhouse inoculations, seedlings of muskmelon cultivars Classic and Athena are readily susceptible, more susceptible than watermelon seedlings (16). The standard inoculum concentration is 1 to 2 × 10⁶ spores/ml when plants are incubated for 3 days; watermelon may require 4 days of incubation. Alternative inoculation conditions are 1 × 10⁵ spores/ml and 6 days incubation. Inoculum concentrations less than 1 × 10⁵ spores/ml will yield inconsistent infection. Spore suspensions can be comprised of conidia or a mixture of conidia and ascospores (12). (Suspensions of only ascospores are difficult to prepare but presumably would also be effective.)

To produce inoculum, isolates of *D. bryoniae* are grown on one-quarter strength potato dextrose agar in unsealed Petri dishes held at 23 to 25°C with a 16-h photoperiod for 10 to 14 days. Cultures are flooded with 10 ml sterile 0.1% sucrose-0.05% hydrolyzed casein solution and scraped gently to release spores. [The dilute nutrient solution is essential to promote spore germination (4).] The spore suspension is filtered through cheesecloth, and the spore concentration is adjusted with sterile sucrose-casein solution.

Seedlings with one or two true leaves are sprayed with approximately 1 ml spore suspension per seedling using a hand-held pump sprayer until all surfaces of the leaves and cotyledons are thoroughly wet. Non-inoculated seedlings are sprayed with sterile sucrose-casein solution. Seedlings are placed immediately into 1 × 2 × 1 m chambers in which cool-mist atomizing humidifiers (Trion Herrmidifier, Sanford, NC), one per chamber, are used to maintain 100% relative humidity. Ideally, chambers should be shaded or protected from direct sunlight for several hours after inoculation.

To increase the number of plants that can be inoculated in a minimum of space, three seedlings per 10-cm-diameter pot may be used. Disease incidence is determined for each pot, although normally all seedlings are infected (Fig. 15). Disease severity is rated visually as the percentage of symptomatic area on each seedling on a 0 to 100 percent scale with 5% increments plus an additional increment of 1% for plants with one tiny leaf spot. If the cotyledons on
inoculated plants are senescent or necrotic, the non-inoculated plants are examined as a reference to distinguish necrosis due to gummy stem blight from natural senescence. Severities are averaged across the three seedlings (subsamples) per pot. If only one seedling in a pot is rated as having 1\% severity, the single leaf spot may be cultured as described previously to verify infection by *D. bryoniae* (12).

In an alternative inoculation procedure, a muskmelon leaf is removed by cutting through the petiole, leaving a 1 cm portion of the petiole attached to the stem. The cut surface is inoculated with 10 μl of a suspension of 10^5 to 10^7 conidia/ml or an agar plug. Plants are incubated at 100\% relative humidity at 25°C for up to 2 weeks (24).

![Fig. 15. Symptoms of gummy stem blight on muskmelon ('Cucumis melo 'Athena') seedlings 4 days after inoculation with *Didymella bryoniae*.](image)

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**Literature Cited**