

PHOMA SPECIES ON BEET: MORE CAUSE DISEASE THAN JUST *PHOMA BETAE*

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Phoma can cause damage to sugar beet (*Beta vulgaris*) at multiple growth stages. It has historically been an important seedling disease, but this is largely managed by ensuring clean seed for planting. The pathogen also can cause a root rot, a leaf spot, and rotting of beets during storage. In the United States, the only pathogenic *Phoma* associated with beets has been *Phoma betae*. In Europe, other species of *Phoma* have been reported to cause symptoms on sugar beet. *Phoma* isolates from sugar beet in the United States were examined to determine whether there might be more species causing symptoms on beet. *Phoma* isolates had been collected as part of ongoing surveys for seedling diseases and root rot, and additional samples were collected from leaf spots.

Methods: Samples were taken from seedlings or root rot by cutting tissue (approximately 2 mm) from the edge of lesions. Tissue pieces were surface disinfested for 60 sec. in 10% bleach and plated on PDA. Fungi were transferred to water agar and single hyphal tips transferred to PDA to obtain pure cultures. For identification, isolates were plated on malt extract agar and oatmeal agar. Plates were incubated 7 days with no light at 22-24 C for initial characterization followed by 7 days at 24-26 C with light for characterization of sporulation. DNA was extracted from isolates with a phenol:chloroform extraction and a portion of the ITS sequenced and compared to known fungal sequences.

For pathogenicity tests on leaves and seedlings, a spore suspension of each isolate was prepared by pouring 5 ml of sterile water on agar plates and gently rubbing with a sterile plastic cell spreader. The water was collected and spore concentration estimated by counting with a hemacytometer. The spore concentration for leaf inoculations was adjusted to approximately 1×10^4 spores/ml. 0.1% Tween 20 was added as a sticking agent. For seedling inoculations, the spore concentration was adjusted to 1×10^6 spores/ml.

For leaf inoculations, plants at the 8-10 leaf stage were watered until soil runoff, sprayed with a spore suspension (above) or with sterile water with 0.1% Tween 20 as a control until runoff and covered with a clear plastic bag to maintain high humidity. Bags were loosened after 48 hours and removed after 72 hours. Plants were maintained in the greenhouse under standard conditions and leaves were observed for symptoms. An isolate of *Phoma betae* was included as a positive control.

For seedling inoculations, plants at the cotyledon stage were drizzled with a spore suspension (above). Plants were maintained with standard conditions and observed for damping-off symptoms. After 3 weeks, all plants were removed from the soil and rated for damping-off with a 0-5 rating scale where 0=no symptoms and 5=plant dead. 15 plants were inoculated with each isolate. A *Phoma betae* isolate was included as a positive control and sterile water was used as a negative control.

For postharvest rot inoculations, beets (cultivar USH20) were sliced into 2 cm thick sections of at least 4 cm diameter. Four slices were used per beet. A 6 mm plug of media and hyphae was cut from a 7 day old culture and placed, hyphal side down, on the top of a 2 cm thick

slice of beet root tissue. Each beet was inoculated (one treatment per slice) with a media negative control, a *Phoma betae* positive control and two of the *Phoma* isolates representing other species. Four replicates per isolate were tested. Slices were placed into metal pans with lids lined with moistened paper towels. Pans were incubated at 22-24 C for 7 days and the diameter and depth of rot visible was measured with a ruler. Samples were taken from beets as done for initial isolation to confirm presence of the isolate used in inoculations.

Results: Of 16 isolates identified as *Phoma* by both morphological and molecular testing, eight were identified as *P. betae* while the remaining isolates showed highest identity with *Phoma* species other than *P. betae*, particularly in *Phoma* section Peyronellaea. Symptoms, including storage rot and leaf spot, were produced on beets inoculated with different *Phoma* species. To date, only *P. betae* has produced seedling damping-off in greenhouse tests.