Phoma glomerata as a Mycoparasite of Powdery Mildew

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Ampelomyces and Phoma species are frequently confused with each other. Isolates previously attributed to the genus Ampelomyces were shown to be Phoma isolates through studies of their morphology and life cycle and ribosomal DNA internal transcribed spacer region 1 sequence analysis. Phoma glomerata can colonize and suppress development of powdery mildew on oak and may have utility as a mycoparasitic agent.

Powdery mildews are widespread plant pathogens that are conspicuous by their white mycelia and powder-like conidia (20). The fungus Ampelomyces quisqualis Ces. is the only fungus that has been demonstrated to be generally effective as a biocontrol agent of powdery mildew (4, 9, 16). Many morphologically similar species may be confused with A. quisqualis (10). To evaluate this possibility, we examined and identified Ampelomyces-like fungi isolated from powdery mildew and compared these cultures with isolates identified as Ampelomyces in culture collections.

Isolation and growth. Leaves of sycamore trees (Platanus occidentalis L.) bearing infections of powdery mildew (Mycosphaera penicillata Wallr.:Fr.) Lév.) were located in South River, New Jersey, in July 1998. Microscopic examination of the leaves revealed two types of pycnidia: stipitate pycnidia, typical of A. quisqualis, and sessile pycnidia, typical of the genus Phoma (Fig. 1) (17). Both types of pycnidia were removed from leaves with fine needles and placed on potato dextrose agar (Difco, Inc., Detroit, Mich.) containing the antibiotics gentamicin (40 mg/liter), streptomycin (40 mg/liter), and penicillin (20 mg/liter) (PDA + 3). Two different fungi were consistently recovered. The stipitate pycnidia developed into slow-growing colonies whose characteristics corresponded to those expected for A. quisqualis (5, 11). The sessile pycnidia developed into rapidly growing colonies whose characteristics corresponded to those of Phoma glomerata (Cda) Wollenw. (2, 19).

Agar plugs (6 mm in diameter) of mycelia cut from the margins of rapidly growing colonies of both the South River Ampelomyces and South River P. glomerata isolates were transferred to five plates each of PDA + 3 and incubated at room temperature (21 to 22°C) for 3 weeks to measure growth rates. We measured an average growth of 8 ± 1 mm/day for the P. glomerata isolates and an average growth of 0.8 ± 0.1 mm/day for the Ampelomyces isolates. With age, cultures of P.
glomerata produced alternarioid dictyochlamydospores measuring 41 ± 6.7 ± 12 ± 1.4 μm.

Inoculation experiments. Koch's postulates (1, 3) were used to establish the pathogenicity of P. glomerata to powdery mildew. A suspension of P. glomerata conidia from cultures grown on PDA+3 was made in sterile water (∼8 × 10⁶ conidia/ml). The conidial suspension was used to inoculate epiphyllous mycelia of the powdery mildew Phyllactinia guttata (Wallr.:Fr.) Lév. on intact (left on the tree) leaves of oak (Quercus coccinea Münch.) by moistening an approximately 15-mm² region on the upper surface of the leaves. Controls were repeats of this process with sterile water. Ten replicates of both the treatment and control were made, and the sites of inoculation were marked by placing white tape on the reverse of the leaves at the inoculation sites. The leaves were monitored for 30 days. During this time, control leaves developed powdery mildew cleistothecia while all leaves treated with P. glomerata conidia developed abundant pycnidia in and around the inoculation sites but did not produce powdery mildew cleistothecia. None of the control leaves showed development of P. glomerata pycnidia, and cleistothecia developed normally. To fulfill Koch's postulates, pycnidia were removed from treated leaves with fine needles and plated on PDA+3 medium to recover P. glomerata. Colonies that developed were confirmed to be P. glomerata by observation of dictyochlamydospores, pycnidia, and subsequent sequence analysis.

Phylogenetic analysis. The nuclear ribosomal DNA internal transcribed spacer region 1 (ITS1) from P. glomerata, several Ampelomyces spp., and several Phoma spp. were sequenced. The South River P. glomerata and A. quisqualis, as well as American Type Culture Collection (ATCC) cultures of Ampelomyces heraclei (Dejeva) Rudakov (ATCC 36804) and A. quis-
significantly reduced conidial germination of an apple scab ([Venturia inaequalis](Cooke) Wint.) (13), and [Phoma etheridgei](Hutch. & Hirat. produced antifungal compounds inhibitory to the tree pathogen [Phellinus tremulae](Bond) Bond et Borisov (8).

Our results suggest that [P. glomerata] is frequently misidentified as [A. quisqualis] or other species of [Ampelomyces]. Additionally, [P. glomerata] often may inhabit powdery mildew infections and may be an important component of a hyperparasitic guild of fungi that naturally infect powdery mildews. Further study is warranted to evaluate the effectiveness of [P. glomerata] in the hyperparasitic control of fungal plant pathogens.

**Nucleotide sequence accession numbers.** The following sequences were deposited in GenBank: [A. quisqualis ATCC 200245 and South River, P. glomerata South River, A. heraclei ATCC 36804. Their accession numbers are listed in the legend for Fig. 2.**

**REFERENCES**