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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 (*Microsphaera penicillata* (Wallr.:Fr.) Lèv.) 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.*

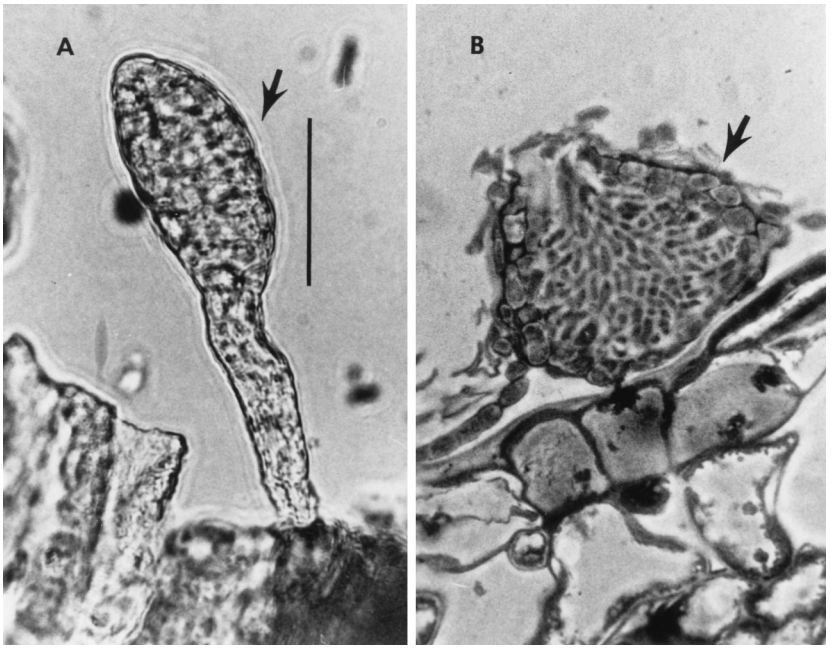

FIG. 1. (A) Stipitate pycnidium of *A. quisqualis* (arrow). (B) Section of sessile pycnidium of *P. glomerata* on oak leaf (arrow). Scale bar = 20 μm.

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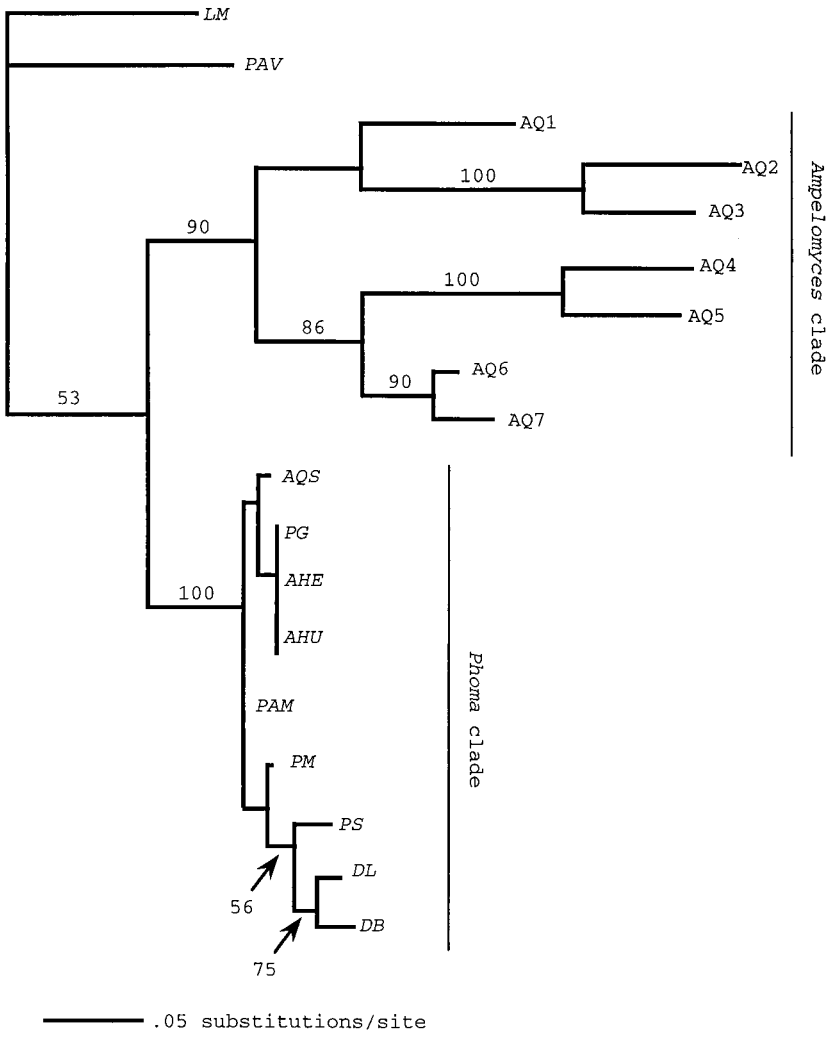

FIG. 2. Maximum likelihood phylogram based on ITS region sequences between the 18S and 5.8S ribosomal DNA (ITS1). Bootstrap confidence levels (percent) of branches are shown. The scale bar is based on a total tree length of 204. Taxa included in the analysis, GenBank numbers (if known), and their sources are as follows: *LM*, *L. microscopica* (LMU04234); *PAV*, *P. avenaria* (PAU77357); AQ1, *A. quisqualis* (AQU82451, DSM [Deutsche Sammlung von Mikrooganismen und Zellkulturen GmbH, Braunschweig, Germany] 2223); AQ2, *A. quisqualis* (AF126818, South River); AQ3, *A. quisqualis* (AF126817, ATCC 200245); AQ4, *A. quisqualis* (AF035782); AQ5, *A. quisqualis* (AQU82449, CBS [Centraalbureau voor Schimmelcultures, Baarn, The Netherlands] 130.79); AQ6, *A. quisqualis* (AF035783, Ecogen AQ10); AQ7, *A. quisqualis* (AF035781, CBS 131.31); *AQS*, *A. quercinus* (AF035778, ATCC 36786); *PG*, *P. glomerata* (AF126816, South River); *AHE*, *A. heraclei* (AF126819, ATCC 36804); *AHU*, *A. humuli* (AF035779, ATCC 38616); *PAM*, *P. americana* Morgan-Jones et White (AF046016); *PM*, *P. macrostoma* Mont. (AF046020); *PS*, *P. sorghina* (Sacc.) Boerema (AF046022); *DL*, *D. lycopersici* Klebahn (AF046015); and *DB*, *D. bryoniae* (Auersw.) Rehm (AF046014).

glomerata produced alternarioid dictyochlamydospores measuring 41 ± 7.5 × 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 (~8×10^6 conidia/ml). The conidial suspension was used to inoculate epiphyllous mycelia of the powdery mildew *Phyllactinia guttata* (Wallr.:Fr.) Lèv. on intact (left on the tree) leaves of oak (*Quercus coccinea* Münch.) by moistening an approximately 15-mm^2 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-*

qualis (ATCC 200245), were grown on PDA+3. DNA extraction, amplification, and sequencing were accomplished as described previously (14).

Several additional ITS1 sequences identified as *Ampelomyces* spp., including *Ampelomyces humuli* (Fautr.) Rudakov, *Ampelomyces quercinus* (Syd.) Rudakov, *Phaeosphaeria avenaria* (Weber) Eriksson, and *Leptosphaeria microscopica* P. Karst. were obtained from GenBank (Fig. 2).

The SeqLab interface for the Wisconsin Package Version 9.1 (Genetics Computer Group, Madison, Wis.) was used to generate alignments and make manual adjustments. PAUP version 4.0b2 for Macintosh (17) was used for phylogenetic analysis. Heuristic searches were performed by using maximum parsimony (14). Bootstrapping, using the same criteria with 400 replicates, was performed to determine the confidence levels of the inferred phylogenies. Trees found by maximum parsimony were subjected to heuristic searches by using maximum likelihood criteria by the Hasegawa-Kishino-Yano model (6) to find the most likely tree (See Treebase [http://herbaria.harvard.edu/treebase] submission no. SN145 for alignment and tree construction details).

Maximum parsimony analysis resulted in ten trees. Maximum likelihood analysis of these trees resulted in one tree ($-\ln L = 1200$, tree length = 204, consistency index = 0.78, homoplasy index = 0.22, retention index = 0.79). The South River *P. glomerata* is identical to *A. heraclei* and *A. humuli*, and they group together with *A. quercinus* in the *Didymella*/*Phoma* clade (Fig. 2). Our South River *A. quisqualis* isolate grouped in the *Ampelomyces* clade with Ecogen's *A. quisqualis* AQ10. There was strong bootstrap support for the *Ampelomyces* (90%) and *Phoma* (100%) clades.

Distinguishing *Phoma* from *Ampelomyces*. Stipitate pycnidia developing into slow-growing colonies characterize *A. quisqualis* (5, 11). Sessile pycnidia developing into rapidly growing colonies characterize *P. glomerata* (2, 19). The cultures identified as *A. heraclei*, *A. humuli*, and *A. quercinus* are typical representatives of their species.

The process of pycnidium formation in association with the powdery mildew is also different between the two genera. *Ampelomyces* spp. infect conidiogenous cells of powdery mildew, internally colonizing and forming pycnidia within the conidiophore; the pycnidia appear stipitate (Fig. 1). *Phoma* sp. does not appear to internally infect conidiogenous cells, and pycnidia are formed directly on the leaf surface; they are sessile (Fig. 1). While many species of *Phoma* are plant pathogens (17), *P. glomerata* is not. However, *Phoma* can grow saprophytically in tissues of plants and is known to be a secondary invader of diseased tissues, perhaps feeding on fungal saprophytes or pathogens of diseased tissues (12, 17, 19). *P. glomerata* has been isolated from species of the powdery mildew genus *Microsphaera* in the United States (this study) and Russia (as *A. quercinus*) and from species of the genus *Sphaerotheca* (as *A. humuli*) in Russia (15). It also has been isolated from the downy mildew of grapes (*Plasmopara viticola* (Berk. et Curt.) Berl. et De Toni) in Russia (as *A. heraclei*) (15). It is apparent that *P. glomerata* has a widespread distribution.

Potential new mycoparasitic agent. Currently a single strain of fungus, *A. quisqualis* AQ10 Biofungicide, is in commercial use for biocontrol of powdery mildew on grapes and other crops (7). This strain grouped within the divergent *Ampelomyces* clade and appears to correctly represent a species of that genus. A few reports have identified *Phoma* species that are antagonistic to fungal plant pathogens. A *Phoma* sp. (P66A) 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.

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