Petri and esca disease fungi in declining grapevines in Pennsylvania and New York

E. L. Stewart, N. G. Wenner, L. A. Long, and B. E. Overton
The Pennsylvania State University, Department of Plant Pathology, University Park, PA 16802

Introduction

A survey was conducted in 2001 and 2002 to evaluate grapevine decline across Pennsylvania and New York due to fungi, viruses, and nematodes. Symptoms of Petri and Esca disease were found at most of the 27 locations surveyed. Isolations were made from symptomatic vines and a culture collection was established.

Based on RFLP analysis of the ITS rDNA region, Dupont et al (2000) synonymized *Phaeoacremonium angustius* with *P. aleophilum* and described *P. viticola* as a new species. ITS sequences from the ex-type cultures of *P. angustius* and *P. aleophilum* are deposited in GenBank. Dupont et al (2000) submitted an ITS sequence for *P. angustius*, (CBS 249.9, GenBank accession number AF118138) that differs from the ex-type ITS sequence for *P. angustius* (CBS 249.95, AF197974) submitted by Crous et al (1996, 2001). Clearly, the culture sequenced by Dupont is different from that sequenced by Crous and the synonymy proposed by Dupont et al (2000) is questionable. *Phaeoacremonium angustius* is described from North America and therefore should be recollected and sequenced comparing molecular data against that published by Crous and Dupont.

Objectives

Isolation and identification of fungi from declining grapevines in Pennsylvania and New York using PCR and ITS rDNA.
Screen isolates from PA and NY against GenBank data from ex-type cultures of *Phaeoacremonium* and *Phaeomoniella*.
Determine if *P. angustius* and *P. aleophilum* are synonymous based on ITS sequence data and determine their relationship to *P. viticola*.

Methods

The ITS gene regions of thirty randomly selected isolates from PA and NY, which were preliminarily identified based on morphology as *Phaeoacremonium* and *Phaeomoniella*, were amplified using PCR. The ITS rDNA gene region was sequenced and compared to GenBank ITS submissions from ex-type cultures for the purpose of identification.

Healthy grapevines

Declining grapevines

Cross-section of declining grapevine showing the dark spotting and exudate symptomatic of *Phaeomoniella* and *Phaeoacremonium* infection.

The yellow stars indicate the locations of the 27 sites that were surveyed during 2001 and 2002.

Conclusions

Isolates 344 ,338, 316, and 254 had ITS sequences identical to the ex-type GenBank sequence of *P. angustius* (AF197974) deposited by Crous, but these isolates produce pink colonies on malt extract agar, a morphological character inconsistent with the protolog of *P. angustius* which cites colony color as honey to isabelline (brownish yellow). Concomitantly, the production of the pink pigment in 344, 338, 316, and 254 is consistent with the protolog of *P. viticola*.

The GenBank sequence (AF118138) deposited by Dupont under the name *P. angustius*, putatively from the culture CBS 249.95, is phylogenetically close to *P. aleophilum.*

The GenBank sequence (AF197974) deposited by Crous under the name *P. angustius*, putatively from the culture CBS 249.95, is phylogenetically close to *P. viticola.*

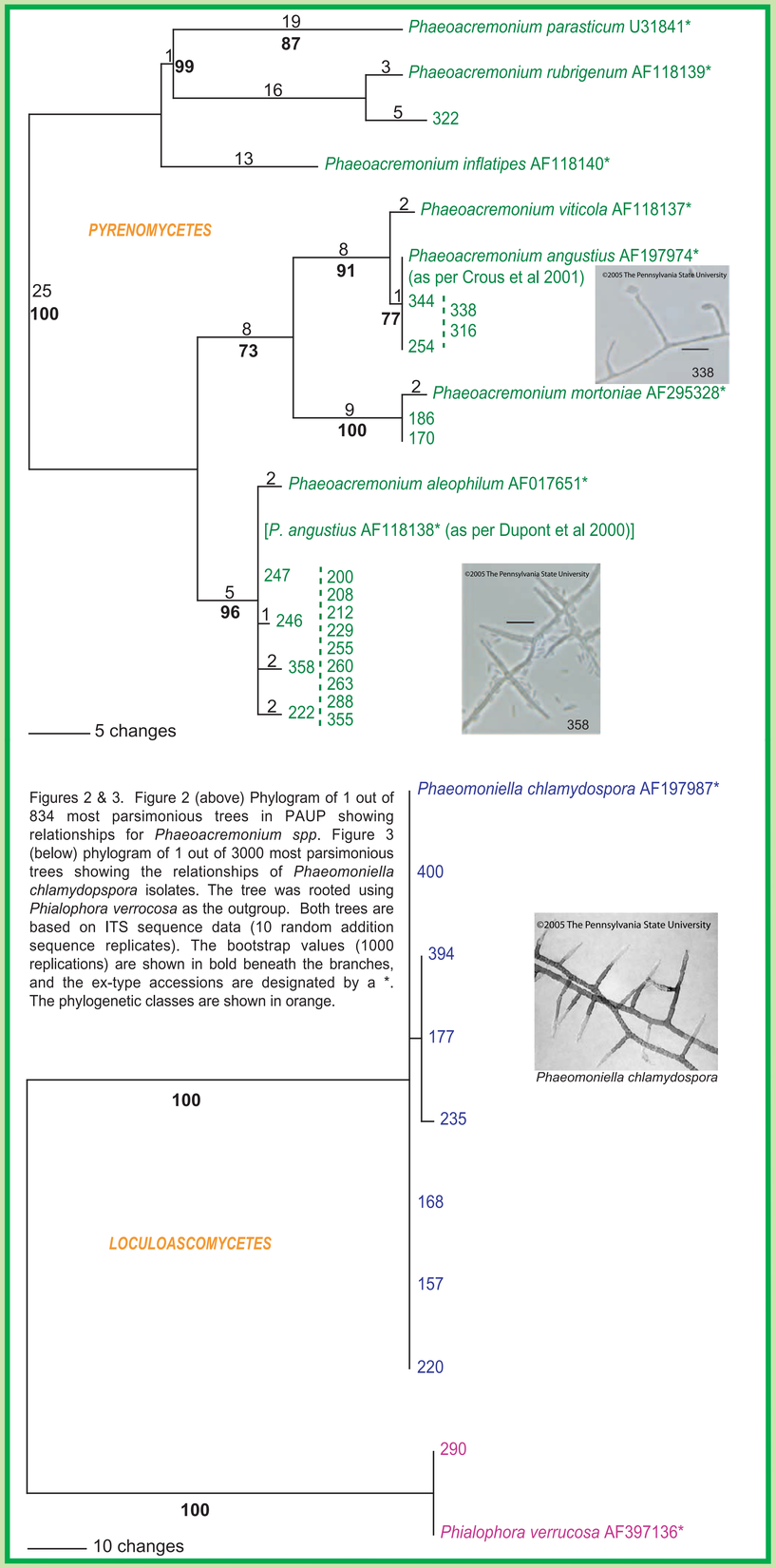
It is unclear which GenBank isolate AF118138 or AF197974 is the ex-type ITS rDNA sequence. It is possible that Crous sequenced an undescribed species closely related to *P. viticola*. It is also possible that Dupont sequenced an isolate close to *P. aleophilum*. Colonies of isolates 344, 338, 316 and 254 produced a pink pigmentation on malt extract agar. Based on this colony characteristic, it appears likely that Crous submitted a sequence to GenBank for a previously undescribed taxon related to *P. viticola* (also pink). *Phaeoacremonium angustius* (*sensu* the ITS rDNA sequence deposited by Crous), including isolates 344, 338, 316 and 254, may represent an undescribed species from North America.

Fourteen out of the thirty isolates screened were *P. aleophilum*, four were *P. angustius* (*sensu* Crous), and two were *P. mortoniae*.

Seven out of the 30 isolates screened were *Phaeomoniella chlamydospora*, and one isolate was an unidentified *Phialophora* spp. (not shown in trees).

Two human pathogenic species, *Phialophora verrucosa* (isolate 290) and *Phaeoacremonium rubrigenum* (isolate 322), were isolated from declining vines.

Based on the PCR screen, *Phaeomoniella chlamydospora* and three species of *Phaeoacremonium* [*P. aleophilum*, *P. angustius* (*sensu* Crous), and *P. mortoniae*] are the predominate species isolated from declining grapevines in PA and NY.



Figures 2 & 3. Figure 2 (above) Phylogram of 1 out of 834 most parsimonious trees in PAUP showing relationships for *Phaeoacremonium spp*. Figure 3 (below) phylogram of 1 out of 3000 most parsimonious trees showing the relationships of *Phaeomoniella chlamydopspora* isolates. The tree was rooted using *Phialophora verrocosa* as the outgroup. Both trees are based on ITS sequence data (10 random addition sequence replicates). The bootstrap values (1000 replications) are shown in bold beneath the branches, and the ex-type accessions are designated by a *. The phylogenetic classes are shown in orange.

Phaeomoniella chlamydospora

This project was supported in part by grants from The Pennsylvania Department of Agriculture, Viticulture Consortium East, and The Pennsylvania State University College of Agricultural Sciences. We would like to thank Christopher Burkhart for his assistance in the field surveys and fungal isolations. References: (1) Crous, P. W., Gams, W., Wingfield, M. J., and Wyk, P. S. van. 1996. *Phaeoacremonium* gen. nov. associated with wilt and decline diseases of woody hosts and human infections. Mycologia. 88. no. 5: 786 - 796. (2) Dupont, J, Laloui, W., Magnin, S., Larignon, P., and Roquebert, M. 1999. *Phaeoacremonium viticola*, a new species associated with Esca disease of grapevine in France. Mycologia 92: 499-504. (3) Groenewald, M., Kang, J., Crous, P. W., and Gams, W. 2001. ITS and beta-tublin phylogeny of *Phaeoacremonium* and *Phaeomoniella* species. Mycologial Research 105: 651-657.