Illustration of Key Morphological Characteristics of *Phytophthora* Species Identified in Virginia Nursery Irrigation Water

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Introduction

Phytophthora diseases are commonly diagnosed on ornamental plants, but taxonomic identification to species can be intimidating. This illustrative guide is designed to aid diagnosticians in morphological identification of certain *Phytophthora* spp. that have been reported in irrigation and effluent water in nurseries (3,10,11,16). Using both morphological and molecular methods to examine numerous isolates recovered during survey work and isolates received from colleagues in the United States and overseas, we have identified a set of diagnostic characteristics that are consistent and easy to use for morphological identification of species recovered in our survey work (3).

A range of *Phytophthora* spp. have been documented in diverse locations in the United States in association with effluent and recycled irrigation water at nurseries (3,10,11,16). *Phytophthora citrophthora*, *P. citricola*, *P. cinnamomi*, *P. cryptogea*, *P. nicotianae* (= *P. parasitica*), *P. megasperma*, and *P. syringae* were isolated from nursery irrigation effluent in California (11); *P. cinnamomi*, *P. cryptogea*, and *P. nicotianae* were isolated from nursery effluent retention basins in North Carolina (10); and *P. cinnamomi, P. citricola, P. citrophthora*, *P. cryptogea*, and *P. nicotianae* were isolated from nursery irrigation runoff and recycled irrigation water in Oklahoma (16). During a statewide Virginia survey for *Phytophthora* spp. in nursery water recycling systems, seven species of *Phytophthora* were recovered from one nursery (3) and a total of nine species were recovered from all locations surveyed (Hong, *unpublished data*). In contrast to previous surveys in other states (10,11,16), *P. cinnamomi* and *P. cryptogea* were not isolated during the Virginia survey, but four other species (*Phytophthora cactorum, Phytophthora drechsleri, Phytophthora palmivora*, *Phytophthora tropicalis*) were recovered.

Pathogens

P. cactorum, P. citricola, P. citrophthora, P. drechsleri, P. megasperma, P. nicotianae (= *P. parasitica*), *P. palmivora, P. syringae*, and *P. tropicalis*

Taxonomy

P. tropicalis, formerly considered conspecific with *P. capsici*, is a recently described taxon (1). For additional taxonomic information on the other *Phytophthora* spp. illustrated in this publication, see CABI Sciences Databases (4) or Erwin and Ribeiro (5).

Hosts

Phytophthora spp. are destructive plant pathogens that cause disease on numerous plants worldwide, including many herbaceous and woody ornamentals. Most of the species illustrated in this guide are commonly associated with root rot and/or dieback on ornamental plants (2,5,6).

Disease

Phytophthora root rot is the most common problem caused by *Phytophthora* spp. in nurseries and on ornamental plants; however, foliar blight can occur when *Phytophthora* inoculum is airborne or carried by splashing water to susceptible foliage (2).

Symptoms

Aboveground symptoms of Phytophthora root rot include chlorosis, stunting, and wilt. Stem tissue of affected plants may be discolored. Roots are often discolored or appear water-soaked and deficient in feeder roots. Symptoms of foliar dieback include water-soaked lesions that may progress rapidly downward in susceptible hosts, causing foliar dieback or, in severe cases, death. Woody stem tissue colonized by *Phytophthora* spp. often appears water-soaked and blackened (2).

Isolation, Culture and Long-term Storage of *Phytophthora* species

Isolates recovered in the Virginia nursery water survey were recovered on P_5ARP medium (see Appendix), and P_5ARP amended with 50 ppm hymexazol (Tachigaren, 70% a.i.; Sankyo Co., Tokyo) (8) to suppress faster growing members of the Pythiaceae (5). It is a good practice to include non-hymexazol-amended media when attempting isolation of hymexazol-sensitive *Phytophthora* spp. (5) or when culturing from zoospore suspensions, since zoospore cysts are sensitive to hymexazol (5,14). Additionally, we found 10 ppm benomyl (E. I. DuPont DeNemours and Co.; Wilmington, DE) effective in suppressing growth of non-target zygomycetes (12).

Production of asexual and sexual structures is necessary for identification of *Phytophthora* spp. Most species require an aqueous environment to produce sporangia. In our work, isolated putative *Phytophthora* spp. were transferred to media amended with 5% clarified V8 (see Appendix), which provides sterols necessary for sporulation (5). A simple and reliable method to induce sporangia production that was often used in the Virginia survey is to transfer mycelial plugs from the leading edge of cultures to Petri dishes containing soil extract (see Appendix). Cultures were then incubated at room temperature under continuous 40-watt fluorescent illumination for 1 to 4 days and observed for sporangia. Pure cultures of isolates producing sexual structures in V8-amended media after incubating in the dark were presumed homothallic. (For more information and references on production of asexual and sexual structures see Erwin and Ribeiro [5]).

Sporangia were observed with a compound microscope to determine caducity, papillation, pedicel length, etc. To determine caducity, sporulating agar plugs of mycelium were removed from aqueous culture and with the sporulating portion of the plug in contact with a microscope slide, moved briskly to dislodge sporangia. If pedicel lengths of dislodged sporangia are homogenous, this allows confidence that one is truly observing a caducous species (Fig. 1). Branching habits were observed on mycelial plugs in aqueous culture (Fig. 2).

Fig. 1. Caducous sporangia and long pedicels of *P. tropicalis*. Bar = 10 µm.

Fig. 2. Umbellate branching habit and globule (arrow) in empty sporangium of *P. tropicalis*. Bar = 10 µm.

For long-term storage of *Phytophthora* cultures, liquid nitrogen (15) is the preferred method, but many laboratories do not have this option. In our work *Phytophthora* isolates were stored as follows. Glass vials containing approximately 5 ml distilled H_2O and two hemp seeds were sterilized. Several 3- to 5-mm agar plugs taken from the leading edge of a pure culture growing on V8 medium were transferred to the vials and after active mycelial growth appeared (1 to 2 days), vials were capped and sealed with Parafilm and stored in the dark at room temperature. Cultures were recovered by placing colonized agar plugs or hemp seeds on V8 culture medium. Storage in V8 medium slants covered with sterile mineral oil also works well for *Phytophthora* species. Isolates stored by the latter two methods typically retain viability for at least two years.

Potential Pitfalls Associated with a Taxonomic Approach and Identification of *Phytophthora* species Using Single-strand Conformation Polymorphism (SSCP)

The *P. megasperma* isolates recovered in the Virginia survey failed to produce sexual structures both in single and dual culture (paired with both A1 and A2 mating types). Therefore, they were initially misidentified as *P. cryptogea*. SSCP analysis (9) of these isolates showed banding patterns consistent with type isolates of *P. megasperma* (*data not shown*).

Identification using taxonomic keys (5,7,13,17,18) is prone to error, due to sometimes variable and overlapping morphological characteristics among species. Molecular methods of identification minimize uncertainty associated with morphological identification, in addition to expediting identification. However, many diagnostic laboratories are not equipped for this type of analysis and must rely on taxonomic methods. In the Virginia survey, SSCP analysis (*data not shown*) (9) was used in addition to taxonomic methods and results were compared. Molecular methods may be necessary for initial identification of difficult or sterile species; however, when a positive molecular identification is made one gains reasonable confidence in subsequent identifications of the same species using morphological characteristics.

Phytophthora cactorum

P. cactorum is one of four homothallic species recovered in the survey (Table 1). *P. cactorum* has conspicuously papillate (i.e., apical thickening ≥ 4 µm) and caducous sporangia (Figs. 3, 4, and 5). Isolates of this species produced abundant sporangia, oogonia, and paragynous antheridia in culture media (Fig. 5). Confirming the presence of these three structures in culture media and the caducity and papillation of sporangia (i.e., apical thickening ≥ 4 µm) allows accurate identification of *P. cactorum* isolates.

Fig. 3. Papillate sporangium of *P. cactorum*. Bar = 10 µm.

Fig. 4. Short pedicel of *P. cactorum*. Bar = 10 µm.

Fig. 5. Papillate sporangium, paragynous antheridium and oogonium of *P. cactorum*. Bar = 10 µm.

Table 1. Diagnostic characteristics of *Phytophthora* spp. recovered from Virginia nursery water

	Sporangial characteristics			Useful characteristics	Water-house group (17)
	Papillation	Caducity	Pedicel length		
Homothallic species					
P. cactorum	Papillate[a]	Caducous	Short[b]	Abundant production of sexual and asexual structures in culture media	I
P. citricola	Semi-papillate[c]	Non-caducous	–	Sporangia may have > 1 apex.	III
P. megasperma[d]	Non-papillate[e]	Non-caducous	–	No growth ≥ 35°C	V
P. syringae	Semi-papillate	Non-caducous	–	Catenulate hyphal swellings	III
Heterothallic species					
P. citrophthora	Papillate	Non-caducous	–	Sporangia often irregular and/or with > 1 apex	II
P. nicotianae	Papillate	Non-caducous	–	Arachnoid mycelium branching habit	II
P. palmivora	Papillate	Caducous	Short		II
P. tropicalis	Papillate	Caducous	Long[f]	None to little growth at ≥ 35°C	II
P. drechsleri	Non-papillate	Non-caducous	–	Growth ≥ 35°C	VI

[a] Apical thickening ≥ 4 μm.

[b] < 5 μm.

[c] Apical thickening < 4 μm.

[d] *P. megasperma* isolates recovered in our work failed to produce sexual structures in single or dual culture (paired with both A1 and A2 mating types).

[e] Apical thickening not apparent.

[f] 16 to 120 μm.

Phytophthora citricola

P. citricola is a homothallic species and sporangia, oogonia, and antheridia typically develop in culture media (Table 1, Figs. 6 and 7). *P. citricola* was readily distinguished from the other two homothallic species (*P. cactorum, P. megasperma*) recovered in the Virginia survey by comparing papillation and caducity of sporangia. Sporangia of *P. citricola* are semi-papillate (i.e., apical thickening < 4 μm) (Fig. 6) and non-caducous, whereas sporangia of *P. cactorum* are conspicuously papillate and caducous (Fig. 4). Sporangia of *P. megasperma* are non-papillate (i.e., apical thickening not apparent) (Fig. 8). *P. citricola* can be easily differentiated from the homothallic *P. syringae* by the absence of hyphal swellings in cultures of *P. citricola*.

Fig. 6. Semi-papillate sporangium of *P. citricola*. Bar = 10 μm.

Fig. 7. Oogonium and paragynous antheridium of *P. citricola*. Bar = 10 μm.

Fig. 8. Non-papillate sporangium of *P. megasperma*. Bar = 10 μm.

Phytophthora citrophthora

P. citrophthora was commonly recovered during the Virginia survey. The heterothallic *P. citrophthora* can be easily differentiated from the homothallic species by the absence of sexual structures in single cultures. *P. citrophthora* produces papillate, non-caducous sporangia (Table 1), which are often asymmetrically shaped and may possess more than one apex (Figs. 9 and 10). *P. citrophthora* is easily distinguished from the heterothallic, papillate species *P. palmivora* and *P. tropicalis* by comparing caducity of sporangia. Sporangia of *P. citrophthora* are non-caducous, whereas sporangia of *P. palmivora* (Fig. 11) and *P. tropicalis* are caducous (Fig. 1). Sporangia of the heterothallic *P. nicotianae*, like *P. citrophthora*, are non-caducous. *P. nicotianae* can be distinguished from *P. citrophthora* by: (i) an arachnoid mycelial habit (Fig. 12); and (ii) more

regular, symmetrical sporangia with a single apex (Fig. 13), in contrast to the asymmetrical sporangia or sporangia with more than one apex of *P. citrophthora*.

Fig. 9. Papillate, asymmetrical sporangium of *P. citrophthora*. Bar = 10 µm.

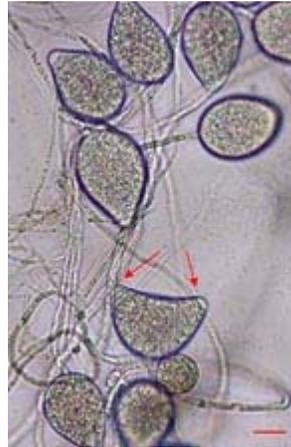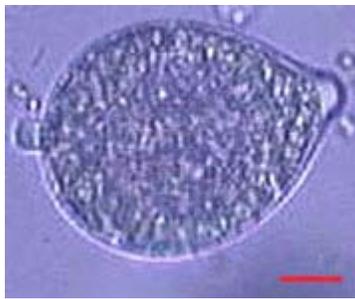Fig. 10. Papillate sporangia and sporangium with two apices (arrows) of *P. citrophthora*. Bar = 10 µm.

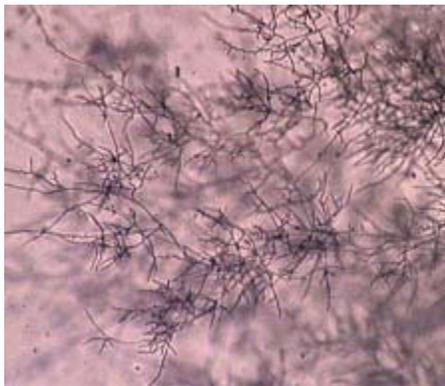Fig. 11. Papillate sporangium and short pedicel of *P. palmivora*. Bar = 10 µm.

Fig. 12. Mycelium of *P. nicotianae* with an arachnoid branching habit.

Fig. 13. Papillate sporangia of *P. nicotianae*. Bar = 10 µm.
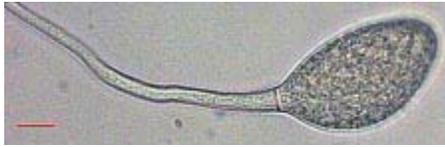
Phytophthora drechsleri

Fig. 14. Non-papillate sporangium of *P. drechsleri*. Bar = 10 µm.

P. drechsleri was frequently recovered from nursery water. Like *P. megasperma*, *P. drechsleri* produces non-papillate sporangia (Table 1, Fig. 14). *P. drechsleri* is morphologically identical to *P. cryptogea*, which was recovered from nursery recycling irrigation systems in California (11), North Carolina (10), and Oklahoma (16). However, growth of *P. drechsleri* cultures at ≥ 35°C differentiates this species from *P. cryptogea*, and in our work growth or lack or growth at ≥ 35°C also allowed consistent separation of the non-papillate species *P. drechsleri* and *P. megasperma* (Table 1). Gallegly reports the temperature maximum of *P. megasperma* at about 30°C (Gallegly, *unpublished*); however, the temperature maximum does not appear to be well-defined in the literature (5). *P. drechsleri* may also produce hyphal aggregates (Fig. 15).

Fig. 15. Hyphal aggregate of *P. drechsleri*. Bar = 10 µm.

Phytophthora megasperma

P. megasperma was frequently recovered in the Virginia survey. *P. megasperma* produces catenulate hyphal swellings and non-papillate sporangia (Table 1; Figs. 8 and 16). *P. megasperma* is homothallic; however, as noted above, isolates recovered in our survey work failed to produce sexual structures and were misidentified as *P. cryptogea* (3). These isolates produced a SSCP pattern typical of *P. megasperma* (*data not shown*) (9). *P. megasperma* was differentiated from *P. drechsleri* by its lower maximal growth temperature (< 35°C).
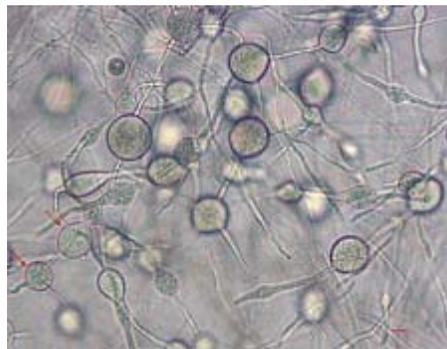

Fig. 16. Catenulate hyphal swellings (arrows) of *P. megasperma*. Bar = 10 µm.

Phytophthora nicotianae

Tufted colony morphology and an arachnoid branching mycelium are characteristic of *P. nicotianae* (Table 1, Fig. 12). Cultures of this species can often be identified with the naked eye simply by looking at colony morphology on isolation media or by a microscopic examination of the mycelium. Sporangia of *P. nicotianae* are non-caducous and easily differentiated from the caducous sporangia of *P. palmivora* (Figs. 11 and 13).

Phytophthora palmivora

P. palmivora is one of four heterothallic species with papillate sporangia that were recovered during this survey (Table 1, Fig. 11). P. *palmivora* produces large chlamydospores (Fig. 17) and is easily distinguished from *P. citrophthora* and *P. nicotianae* by its caducous sporangia (Fig. 11). Comparison of sporangia pedicel length allows easy discrimination of *P. palmivora* and *P. tropicalis*. Pedicels of *P. palmivora* are short (< 5 μm) (Fig. 11), whereas pedicels of *P. tropicalis* are much longer (16 to 120 μm) (Fig. 1).

Fig. 17. Chlamydospores of *P. palmivora*. Bar = 10 μm.

Phytophthora syringae

P. syringae is homothallic, produces semi-papillate, non-caducous sporangia, and paragynous antheridia (Table 1). This species may be confused with *P. citricola*. However, *P. syringae* produces abundant catenulate hyphal swellings (Fig. 18), whereas *P. citricola* produces none.

Fig. 18. Catenulate hyphal swellings (arrows) of *P. syringae*. Bar = 10 μm.

Phytophthora tropicalis

Like *P. capsici* and *P. palmivora*, *P. tropicalis* is heterothallic and produces papillate, caducous sporangia (Table 1, Fig. 1). Comparison of pedicel length between *P. tropicalis* (Fig. 1) and *P. palmivora* (Fig. 11) allow easy separation of these two species, as noted above. Aragaki and Uchida described *P. tropicalis*,

which was previously lumped into *P. capsici* as a somewhat divergent group, as a new species (1). They reported sporangia of *P. tropicalis* as more narrow and mostly tapered at the base and reported little to no growth at ≥ 35°C (1) (Fig. 1). Re-examination of the morphological characteristics and SSCP analysis (*data not shown*) (9) indicated that isolates previously identified in our survey work as *P. capsici* (3) are actually *P. tropicalis*. M. E. Gallegly found that isolates of *P. tropicalis* recovered in the Virginia survey did exhibit mostly tapered sporangial bases and had little to no growth at ≥ 35°C, in concurrence with Aragaki and Uchida (1). Additionally he identified umbellate sympodia (Fig. 2) in aqueous cultures of *P. tropicalis* under light (Gallegly, *unpublished data*). These characteristics are in contrast to analogous characteristics of *P. capsici*: simple sympodial branching habit (not umbellate), sporangial bases mostly rounded, and growth at ≥ 35°C. A large globule may be evident in sporangia of *P. tropicalis* after zoospore release (Fig. 2).

Acknowledgments

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Appendix: Ingredients of P₅ARP, V8 agar, and soil extract

P$_5$ARP. Difco cornmeal agar (17 g/liter); 5 ppm pimaricin (Delvocid Instant, 50% a.i., Gist-Brocades Fermentation Industries, Inc., Charlotte, NC); 250 ppm sodium ampicillin (Bristol Laboratories, Syracuse, NY); 10 ppm rifampicin (Sigma Chemical Co., St. Louis, MO) dissolved in 1 ml DMSO, and 100 ppm PCNB (Terraclor, 75% a.i.).

V8 agar. For 1 liter 20% V8 agar: Add 2 gm CaCO3 to 200 ml V8 juice. Stir for at least 20 min. Centrifuge at 10,000 rpm for 10 min. Transfer supernatant; discard pellet. Mix 200 ml clarified V8 juice with 800 ml distilled H_2O and 15 g Difco Bacto agar (Becton Dickinson, Sparks, MD). Autoclave.

Soil extract. Add 15 g of sandy loam soil to 1 liter distilled H2O. Stir overnight. Allow soil particles to settle out of suspension before transfer of aqueous portion to container. Store at 4°C.