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Differentiating *Phytophthora ramorum* and *P. kernoviae* from Other Species Isolated from Foliage of Rhododendrons

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Abstract

Phytophthora species are among plant pathogens that are the most threatening to agriculture. After the discovery of *P. ramorum*, surveys have identified new species and new reports on rhododendrons. Based upon propagule production, morphology, and colony growth, a dichotomous key was produced that can differentiate *P. ramorum* and *P. kernoviae* from other species known to be pathogenic to aerial plant parts of rhododendrons. These distinctions were made without molecular tools and wide-ranging variables such as propagule sizes and can be made without the need for a large culture collection.

Introduction

The discovery of *Phytophthora ramorum* as an invasive pathogen has prompted an increase in surveys for *Phytophthora* spp. resulting in the identification of several new species. Since 1999, *P. foliorum* (13), *P. gonapodyides* (26), *P. hedraiandra* (28,29), *P. hibernalis* (5), *P. inflata* (31), *P. insolita* (31), *P. kernoviae* (7), *P. ramorum* (37), and *P. tropicalis* (22) have all been newly described on *Rhododendron* spp. This is in addition to the previously known *P. cactorum* (11,29,35), *P. cambivora* (11,35), *P. cinnamomi* (11), *P. citricola* (11,29,35), *P. citrophthora* (29), *P. heveae* (11), *P. nicotianae* (synonym = *P. parasitica*) (11), and *P. syringae* (11). With quarantine regulations in effect for *P. ramorum* and concerns about the importation of *P. kernoviae*, which is known currently only in Great Britain and New Zealand, to North America, it is important to be able to identify clearly these two species and differentiate them from other *Phytophthora* spp. that attack leaves and stems of rhododendrons.

Before the advancement of molecular techniques, keys based upon morphological features were used to differentiate species. However, an often ambiguous overlapping of propagule size and the training needed to identify certain features made confident identification difficult. In addition, a good culture collection is needed for comparison purposes. With the advancement of molecular techniques, separation and identification of species is becoming more reliable. However, sequencing may not always result in clear distinctions when analyzing species that are closely related and other molecular tests may not be specific for an individual species (10). In addition, new *Phytophthora* species are being discovered frequently and there is no assurance that the current "ramorum-specific" primers will not react with these new species (30). This has already been observed with *P. hibernalis* (5) and *P. foliorum* (13). More importantly, not all laboratories have easy access to these molecular tools and it can be time consuming and expensive to send samples to more equipped laboratories. Osterbauer and Trippe (27) compared diagnostic protocols for *P. ramorum* on rhododendron leaves and found 16% of their samples were PCR negative, but culture positive. They also found 19% of their samples were PCR positive, but culture negative. This may be partially accounted for by the fact that PCR will also detect spores that are nonviable or are in some state of

dormancy. In a related study, Sutton et al. (30) concluded that culturing was the single most reliable method for detection of *P. ramorum*. Thus, a method for confident identification based upon stable and simple morphological characteristics needs to be updated.

Several synoptic keys have been published to identify *Phytophthora* spp. (14,20). Recently, Gallegly and Hong (15) compiled 60 known *Phytophthora* spp. together with photographs and DNA fingerprinting. Despite this information, identification through morphology is considered difficult because features are variable and often overlap within species (14). In addition, some recently described species, including *P. ramorum* and *P. kernoviae*, were not included in these older keys. Currently, United States Federal regulations require that all program samples (i.e., collected during surveys of nurseries in the regulated states of California, Washington, and Oregon, or in nurseries of another state as a result of a trace forward) that are symptomatic be sampled and tested for *P. ramorum* (34). Samples will then be considered positive for *P. ramorum* based upon results of a positive PCR or positive culture. Survey and diagnostic samples from nurseries in non-regulated states or not part of a trace forward or trace back can be identified based on morphology and or DNA sequence. It is the attempt of this study to present morphological characteristics that can be used to differentiate *P. ramorum* and *P. kernoviae* from other *Phytophthora* spp. pathogenic to rhododendron leaves and stems. This key will be especially useful to diagnostic and extension laboratories that receive rhododendron leaf samples and are not required to confirm identification through PCR. It could also be useful for differentiating these species from any woody plant.

Species Characteristics

Isolates used. *Phytophthora* species known to be pathogenic to *Rhododendron* were identified through a literature search. Cultures of these *Phytophthora* spp., listed in Table 1, were obtained from various researchers and maintained on 20% clarified V8 agar. Observations were based on examinations of two isolates from each species having, if possible, at least one of the isolates collected from rhododendron. It was justified to use only two isolates since the primary diagnostic features used in this study (e.g., sexual type, sporangia characteristics, etc.) are stable characteristics that are used to define the species and it was sufficient in a recent publication on *Phytophthora* spp. identification (15). Isolates of *P. inflata* and *P. insolita* could not be obtained and characteristics were based solely upon the literature (2,14,17). An unidentified *Phytophthora* taxon labeled Pgchlamydo, isolated from *Rhododendron* (28), was not included in this study since it has not been officially described. Other species isolated only from rhododendron roots (*P. lateralis*, *P. cryptogea*, and *P. megasperma*) (14,21) were not included in this study.

Table 1. List of *Phytophthora* spp. isolates used in this study, their origin, original host material, and source.

***Phytophthora* spp.**	**Isolate**	**Origin**	**Host**	**Source**[x]
P. cactorum	Benson JCW1	NC	Rhododendron	MB (35)
P. cactorum	Hamm 348	WA	Douglas Fir	PH (N/A)
P. cambivora	Benson AJH5	NC	Rhododendron	MB (35)
P. cambivora	Benson HCW3	NC	Unknown	MB (N/A)
P. cinnamomi	Benson 2357	NC	Azalea	MB (N/A)
P. cinnamomi	3267	CA	Walnut	GB (33)
P. citricola	Benson AJH6	NC	Rhododendron	MB (35)
P. citricola	Benson FKP4	NC	Rhododendron	MB (N/A)
P. citrophthora	Reeser 01-02	OR	Rhododendron	JP (35)
P. citrophthora	3E5	VA	Irrigation water	CH, MG (15,23)
P. foliorum	LT192	TN	Rhododendron	KL (13)
P. foliorum	LT1223	CA	Rhododendron	KL (13)
P. gonapodyides	Pgon26	SC	Soil	JH (N/A)
P. gonapodyides	Pgon56	FL	Soil	JH (N/A)
P. hedraiandra	MN832003	MN	Rhododendron	RB (28)
P. hedraiandra	MN1522003	MN	Rhododendron	RB (GenBank DQ139806)
P. heveae	Reeser PC97-251	OR	Rhododendron	JP (33)
P. heveae	HW228	NC	Rhododendron	SJ (N/A)
P. hibernalis	ATCC 32995	CA	*Citrus sinensis*	MC (25)
P. hibernalis	ATCC 64708	New Zealand	*Aquilega vulgaris*	ATCC (25)
P. kernoviae	CSL 2378	England	Rhododendron	KH (GenBank DQ002011)
P. kernoviae	ICMP 14761	New Zealand	*Annona cherimola*	TR (GenBank EU909457)
P. nicotianae	Pn21DJM	FL	Periwinkle	FM (N/A)
P. nicotianae	362	DE	*Solanum tuberosum*	RM (32)
P. ramorum	Pr-52	CA	Rhododendron	DR (13,25)
P. ramorum	PRN-1	the Netherlands	Rhododendron	SW (23)
P. syringae	Kalmia-1	OR	*Kalmia latifolia*	JP (13,25)
P. syringae	Kalmia-2	OR	*Kalmia latifolia*	JP (13,25)
P. tropicalis	31C9	VA	Rhododendron	CH (22)
P. tropicalis	SR10	VA	Soil	SJ (N/A)

[x] Name of originator followed by a reference to the molecular identification in parenthesis (N/A = not available and was identified only on morphology or through RLFP patterns). ATCC = American Type Culture Collection; CH = Chuan Hong; DR = Dave Rizzo; FM = Frank Martin; GB = Greg Browne; JH = Jaesoon Hwang; JP = Jennifer Parke; KH = Kelvin Hughes; KL = Kurt Lamour; MB = Mike Benson; MC = Mike Coffey; MG = Mannon Gallegly; RB = Robert Blanchette; RM = Robert Mulrooney; PH = Phil Hamm; SJ = Steve Jeffers; SW = Sabine Werres; TR = Tod Ramsfield.

Propagule inducement. Cultures of *Phytophthora* spp. were prepared in sterile 10% clarified V8 broth and placed in an incubator at 20°C, either under continuous light (3180 lux) or in the dark. The cultures were observed for the formation of propagules after 3 and 4 days. After 4 days, the cultures were rinsed three times with either sterile 0.1 mM MES buffer, pH 6.2 or sterile 1% soil extract. The cultures were placed back in the incubators either under light or dark at 20°C as they were before rinsing. One day after rinsing, the cultures were again observed for the formation of propagules (Table 2). Some species required longer for production of certain propagules. Caducity of sporangia was determined by a modified procedure described by Al-Hedaithy and Tsao (1). Four days after the sporangia had formed, the plates were sealed with Parafilm (Pechiney Packaging Co., Chicago, IL) and shaken vigorously for 10 sec. The species was determined to have obvious caducity if detached sporangia with consistent pedicel lengths were observed in the solution. The species was rated as noncaducous or not to have obvious caducity if very few sporangia (< 10%) were released and the pedicel lengths were not consistent (1). Photographs were taken of each propagule type that was observed in this study (Figs. 1 to 15).

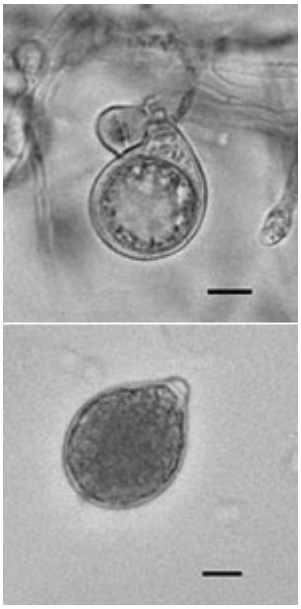

Fig. 1. *Phytophthora cactorum* (**A**) oospore; (**B**) sporangium. Bar = 10 µm.

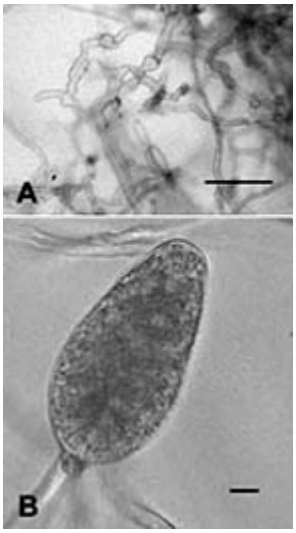

Fig. 2. *Phytophthora cambivora* (**A**) hyphal swellings. Bar = 100 µm; (**B**) sporangium. Bar = 10 µm.

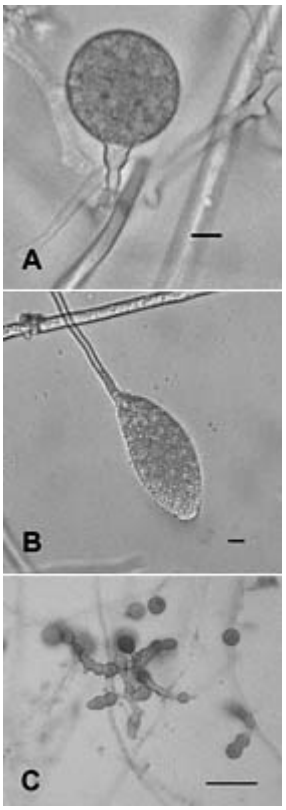

Fig. 3. *Phytophthora cinnamomi* (**A**) chlamydospore. Bar = 10 µm; (**B**) sporangium. Bar = 10 µm; (**C**) hyphal swellings. Bar = 100 µm.

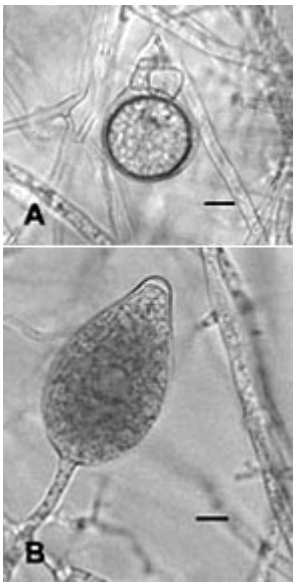

Fig. 4. *Phytophthora citricola* (**A**) oospore; (**B**) sporangium. Bar = 10 µm.

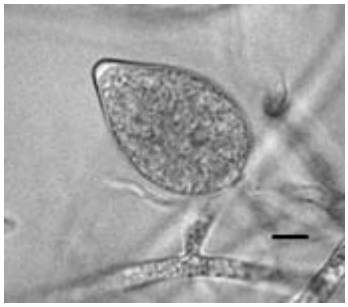

Fig. 5. *Phytophthora citrophthora* sporangium. Bar = 10 µm.

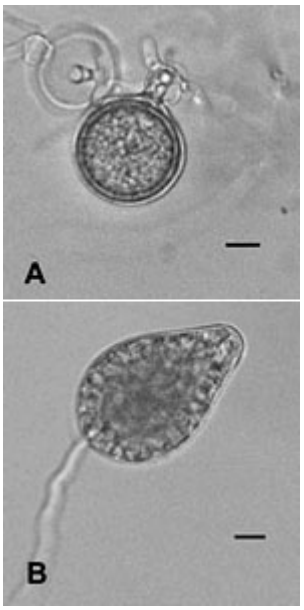

Fig. 6. *Phytophthora foliorum* (**A**) oospore; (**B**) sporangium. Bar = 10 µm.

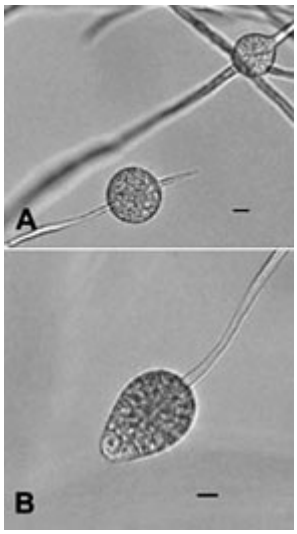

Fig. 7. *Phytophthora gonapodyides* (**A**) chlamydospores; (**B**) sporangium. Bar = 10 µm.

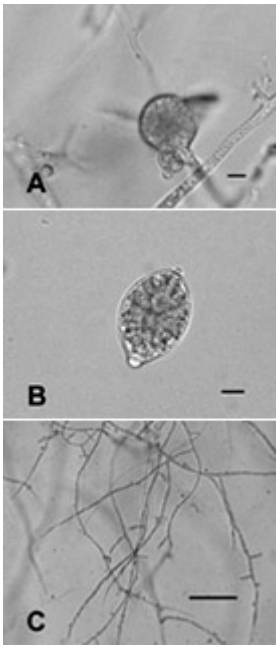

Fig. 8. *Phytophthora hedraiandra* (**A**) oospore; (**B**) sporangium. Bar = 10 µm; (**C**) mycelium after 7 days growth on V8 agar at 4°C. Bar = 100 µm.

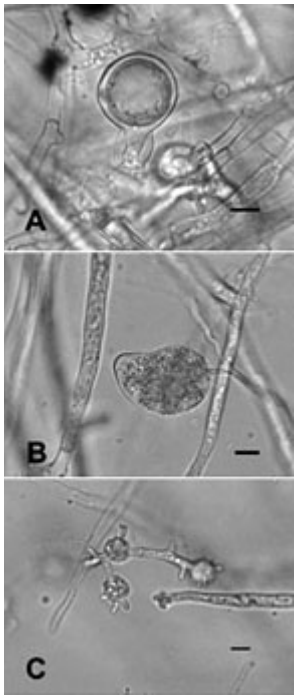

Fig. 9. *Phytophthora heveae* (**A**) oospore; (**B**) sporangium; (**C**) hyphal swelling. Bar = 10 µm.

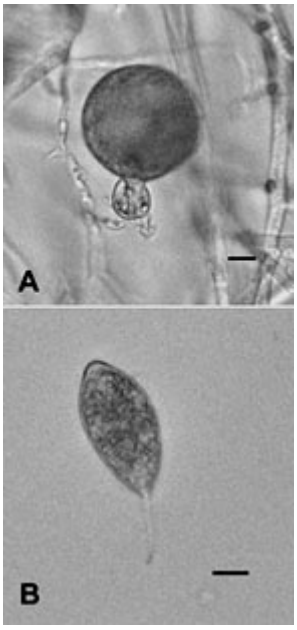

Fig. 10. *Phytophthora hibernalis* (**A**) oospore; (**B**) sporangium. Bar = 10 µm.

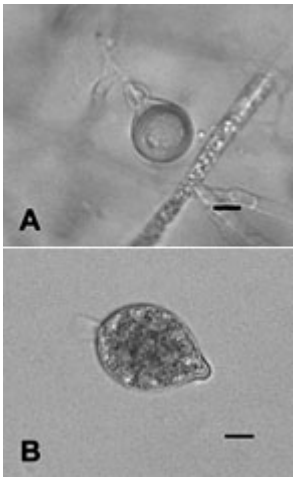

Fig. 11. *Phytophthora kernoviae* (**A**) oospore; (**B**) sporangium. Bar = 10 µm.

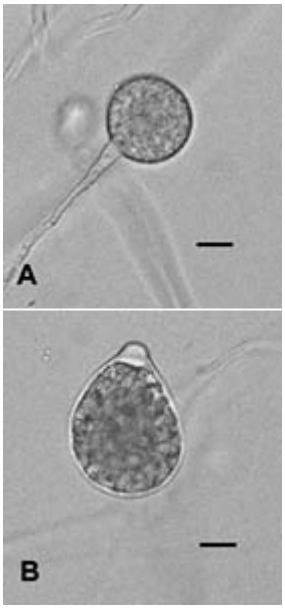

Fig. 12. *Phytophthora nicotianae* (**A**) chlamydospore; (**B**) sporangium. Bar = 10 µm.

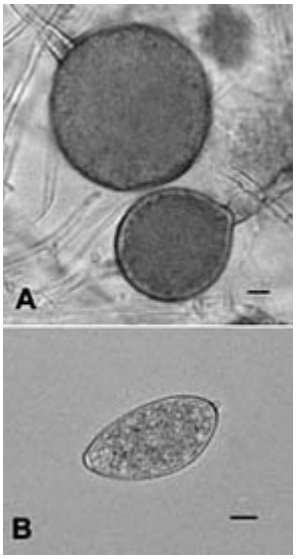

Fig. 13. *Phytophthora ramorum* (**A**) chlamydospores; (**B**) sporangium. Bar = 10 µm.

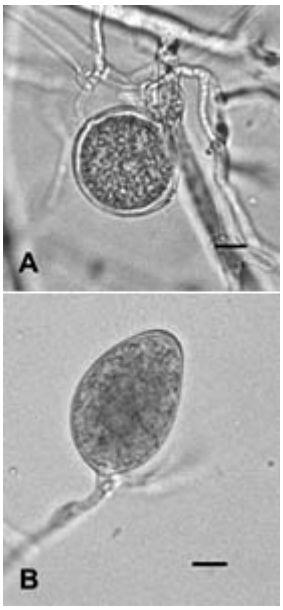

Fig. 14. *Phytophthora syringae* (**A**) oospore; (**B**) sporangium. Bar = 10 µm.

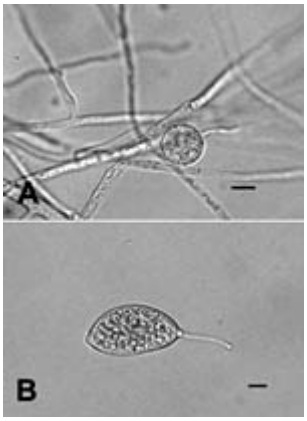

Fig. 15. *Phytophthora tropicalis* (**A**) chlamydospore; (**B**) sporangium. Bar = 10 µm.

Table 2. Propagule type of different *Phytophthora* spp. observed in liquid cultures grown at 20°C.

Species	Condition	Growth in V8 broth[w]		Washing solution[x]	
		3 days	4 days	1 day MES	1 day SE
P. cactorum	Light	Sp[y]	Sp+	Sp+, Oog	Sp+, Oog
	Dark	Oog+	Oog+	Sp, Oog+	Sp, Oog+
P. cambivora	Light	M	M	Sp	Sp+
	Dark	HS	HS	HS	HS, Sp
P. cinnamomi	Light	Ch+, HS+	Ch+, HS+	Ch+, HS+	Ch+, HS+, Sp
	Dark	Ch+, HS+	Ch+, HS+	Ch+, HS+	Ch+, HS+, Sp
P. citricola	Light	M	M	Sp+	Sp+
	Dark	M	Oog	Oog	Sp, Oog
P. citrophthora	Light	Sp+	Sp+	Sp+	Sp+
	Dark	M	Sp	Sp	Sp+
P. foliorum	Light	M	Oog	Oog+	Sp, Oog+
	Dark	M	Oog+	Oog+	Sp, Oog+
P. gonapodyides	Light	M	M	Ch+, Sp	Sp
	Dark	M	M	Ch+, Sp	Sp
P. hedraiandra	Light	Sp+	Sp+	Sp+	Sp+
	Dark	Oog+	Oog+	Oog+, Sp	Oog+, Sp
P. heveae	Light	Oog	Oog+, Sp	Oog+	Oog+, Sp
	Dark	Oog+	Oog+	Oog+	Oog+
P. hibernalis	Light	M	M	Sp	Sp+
	Dark	M	M	Oog	Sp, Oog
P. kernoviae	Light	Sp	Sp+	Sp+	Sp+
	Dark	M	Oog+	Sp, Oog+	Sp+, Oog+
P. nicotianae	Light	Sp	Sp+	Ch+, Sp+	Ch+, Sp+
	Dark	M	Ch, Sp	Ch, Sp	Ch, Sp
P. ramorum	Light	M	Ch[z], Sp+	Sp+	Sp+
	Dark	M	Ch[z], Sp	Sp+	Sp+
P. syringae	Light	M	M	Sp+, Oog+	Sp+, Oog+
	Dark	M	Oog+	Sp, Oog+	Sp+, Oog+
P. tropicalis	Light	Sp+	Sp+	Sp+	Sp+
	Dark	M	M	Sp	Ch, Sp+

[w] Propagule type observed after growing for 3 or 4 days in 10% V8 broth at 20°C under continuous light or in the dark.

[x] Propagule type observed after growing in 10% V8 broth for 4 days, washing three times in either 0.1 mM MES buffer, pH 6.2 or 1% sterile soil extract (SE), and incubating for 1 day at 20°C under continuous light or in the dark.

[y] Propagule type: Ch = chlamydospores; HS = hyphal swellings; M = mycelium only; Oog = oogonia; Sp = sporangia. (+) denotes abundant production of corresponding propagule.

[z] Chlamydospores of *P. ramorum* were first observed 6 days after inoculation in 10% V8 broth.

Colony growth at different temperatures. To confirm previous reports of growth of the *Phytophthora* spp. at different temperatures, V8 agar plates with a plug from an actively growing colony were placed on a thermogradient plate (an aluminum plate with a hot water bath at one end and a cooling bath at the other end) at 2°C intervals from 4° to 10°, 20°, and 26° to 32°C in darkness (25). The desired temperature of the agar medium was confirmed daily by touching

the Type-K probe of an Omega Model HH21 Microprocessor Thermometer (Omega Engineering, Inc.) onto the surface of a V8 agar plate. Cultures were considered to have positive growth if mycelium was observed on the V8 agar plate after 7 days. This was repeated once for each isolate. Although a majority of isolates tested in this study grew within the temperature range reported in the literature (3,7,12,13,14,37), there were some exceptions. Isolates of *P. cambivora*, *P. cinnamomi*, *P. citricola*, and *P. citrophthora* all grew within a narrower temperature range than previously reported (14). The isolate of *P. heveae* used in this study grew at a much lower minimum temperature (4°C) and a lower maximum temperature (26°C) than previously reported (14). Based on these results, growth at different temperatures can be isolate dependent and so was not used as a primary characteristic in this study, except in the case with *P. syringae* separating it from *P. citricola*, *P. foliorum*, and *P. inflata*. *Phytophthora syringae* is reported to have a maximum temperature of growth of 23 to 25°C (14), which was confirmed in this study, while the other three species grow well above 28°C.

Colony morphology on agar media. Colony morphology of the species was compared by growing the isolates on carrot agar, Rye A agar, and 20% clarified V8 agar at 20°C in the dark (14). Photographs of the plates were taken when the colonies reached the edge of the plates (Fig. 16). There is some discussion as to the usefulness of colony patterns for the identification of *Phytophthora* spp. Waterhouse (36) remarks that patterns should be considered as a taxonomic aid, but Erwin and

Ribeiro (14) demonstrate that variability of colony types among isolates of different species make this characteristic not useful for identification beyond supplementary purposes. A majority of papers describing new species [e.g. (7,37)] include colony patterns in the description and so were included in this study for possible reference. Some species had very distinct patterns on all three media types. For example, *P. citricola* showed rosaceous patterns (Fig. 16D) while *P. syringae* was very distinctly stellate (Fig. 16N) and *P. hedraiandra* was petallate (Fig. 16H). *Phytophthora heveae* ranged from stellate on V8 and Rye A agar to rosaceous on carrot agar (Fig. 16J).

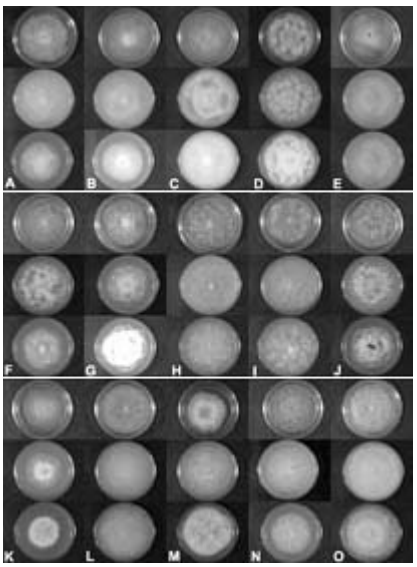

Fig. 16. Colonies of: (**A**) *Phytophthora cactorum*; (**B**) *P. cambivora*; (**C**) *P. cinnamomi*; (**D**) *P. citricola*; (**E**) *P. citrophthora*; (**F**) *P. foliorum*; (**G**) *P. gonapodyides*; (**H**) *P. hedraiandra*; (**I**) *P. heveae*; (**J**) *P. hibernalis*; (**K**) *P. kernoviae*; (**L**) *P. nicotianae*; (**M**) *P. ramorum*; (**N**) *P. syringae*; and (**O**) *P. tropicalis* grown on 20% V8 agar (top row), Rye A agar (middle row), and carrot agar (bottom row).

Discrepancies from previous reports. Although the majority of results in this study agreed with previous reports, several important differences were noted. In the original description (13), *P. foliorum* sporangia were reported as caducous. However, in this study, *P. foliorum* sporangia were not rated as caducuous. Although slightly more than 10% of the sporangia detached into the solution after shaking, the pedicel lengths were highly variable ranging from no pedicel to long detached hyphae (*data not shown*). This is an important criteria

for caducity based upon a study by Al-Hedaithy and Tsao (1). There is also some discrepancy in the literature concerning the caducity of *P. heveae*. Erwin and Ribeiro (14) described caducous sporangia with a pedicel length less than 10 µm, whereas Gallegly and Hong (15) observed noncaducous sporangia. In this study, very few sporangia detached after shaking and obvious caducity was not observed, based upon the criteria mentioned above.

Another notable difference was the production of chlamydospores in the *P. gonapodyides* cultures (Fig. 7A). Previous studies have reported other *P. gonapodyides* isolates to produce chlamydospores (18), but Erwin and Ribeiro (14) describe this species as producing no chlamydospores or hyphal swellings. If *P. gonapodyides* is suspected, but no chlamydospores are formed, then according to the key (Fig. 17) it would fall under the same grouping as *P. citrophthora*. *Phytophthora gonapodyides* can be differentiated from *P. citrophthora* based upon the papillation type of the sporangia, where *P. gonapodyides* is non-papillate and *P. citrophthora* is papillate. Production of chlamydospores has also been used as a distinguishing characteristic between *P. gonapodyides* and the unidentified *Phytophthora* taxon labeled Pgchlamydo (8). However, at this time it is unclear as to whether production or lack of chlamydospores or hyphal swellings is enough to separate these two species. Greslebin et al. (16) analyzed isolates that matched 100% with *P. gonapodyides* sequences, but produced hyphal swellings in culture. At the time of this study, the isolates used were identified as *P. gonapodyides*. But, as the classification of *Phytophthora* spp. evolves, based on new molecular techniques, this identification may change.

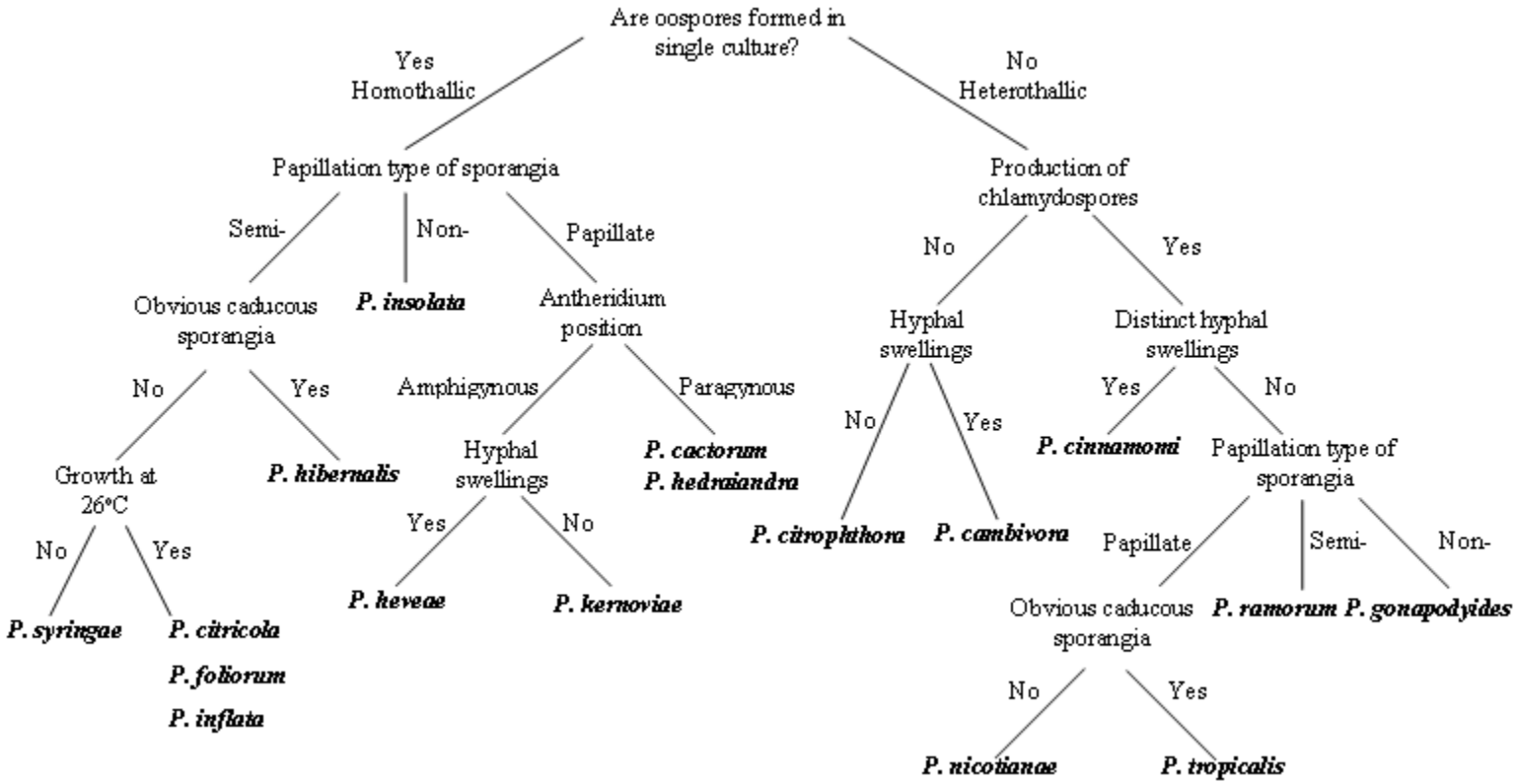

Fig. 17. Dichotomous key using stable characteristics to differentiate *Phytophthora* spp. that are known to be pathogenic to foliar plant parts of *Rhododendron* spp.

Differentiating *P. ramorum* and *P. kernoviae*. Based upon the characteristics outlined below, it is possible to differentiate *P. ramorum* and *P. kernoviae* from other known species that attack the stems and leaves of rhododendrons. A dichotomous key was designed to separate these two species based upon morphological characteristics (Fig. 17).

Oospore production is the first characteristic used to separate *P. ramorum* and *P. kernoviae* from each other. *Phytophthora kernoviae* is homothallic and a single culture produces abundant oospores. On the other hand, *P. ramorum* is heterothallic and is difficult to produce oospores even when the two mating types are combined in a laboratory (6). Oospores of *P. ramorum* have never been observed in nature (19).

If oospores are not formed in single isolate cultures, the lack of chlamydospore production will distinguish *P. citrophthora* and *P. cambivora* from other heterothallic species. Some *P. citrophthora* isolates from cacao in Brazil were reported to produce chlamydospores (14). No chlamydospores were observed in the *P. citrophthora* isolates used in this study. A good characteristic of *P. ramorum* is their very large and distinct chlamydospores. However, there is a range of chlamydospore sizes that could overlap other species and so someone who is unfamiliar with *P. ramorum* and does not have cultures of other species to compare it to, may not be able to confirm the identity. Thus, other characteristics must be used to further distinguish *P. ramorum* from other species. *Phytophthora cinnamomi* can be separated from the group that produces chlamydospores based upon its distinct hyphal swellings (Fig. 3B) and that it does not produce abundant sporangia, if any, in liquid culture (9). Papillae type of the sporangia is then used to differentiate the other species. *Phytophthora ramorum* has semi-papillate sporangia while the other species in this group are either papillate or non-papillate. Sometimes, it is difficult to distinguish papillate from semi-papillate sporangia, so other characteristics may be useful to separate *P. ramorum* from *P. nicotianae* and *P. tropicalis*. All three species produce chlamydospores, but as mentioned above, *P. ramorum* chlamydospores are very large on average compared to the other two species. The average chlamydospore diameter for most isolates of *P. ramorum* is 46 to 60 µm (37), which compare to *P. nicotianae* and *P. tropicalis* chlamydospores that are 30 µm and 27 to 34 µm, respectively (15). *Phytophthora nicotianae* can be differentiated from *P. ramorum* and *P. tropicalis* because it produces non-caducous sporangia. If papillate type and chlamydospore size is in question, *P. tropicalis* can be differentiated from *P. ramorum* based on its colony growth at higher (> 30°C) temperatures, compared to maximum temperature growth at 26 to 30°C for *P. ramorum* (37).

Phytophthora kernoviae can be separated from other homothallic *Phytophthora* spp. based upon three characteristics. Firstly, *P. kernoviae* has papillate sporangia that are shared with *P. heveae*, *P. cactorum*, and *P. hedraiandra*. Secondly, *P. kernoviae* and *P. heveae* have amphigynous antheridia compared to paragynous antheridia characteristic of *P. cactorum* and *P. hedraiandra*. However, in the original description of *P. hedraiandra* (12), antheridia were reported to be occasionally amphigynous. Descriptions in the literature (14) where the position of the antheridium can be occasionally amphigynous or paragynous is the reason that this characteristic is not used to separate other homothallic species. A backup characteristic to separate *P. kernoviae* from *P. cactorum* or *P. hedraiandra* is the pedicel length of the caducous sporangia. *Phytophthora kernoviae* has a medium length pedicel with a range of 5 to 19 µm (7) compared to *P. hedraiandra* and *P. cactorum*, which have a short pedicel length less than 2 and 4 µm, respectively (12,14). This is clearly shown in Figures 1B, 8B, and 11B. In addition, *P. kernoviae* has very little colony growth at 26°C, while *P. cactorum* grows well at 28°C and *P. hedraiandra* can even grow at 30°C. Finally, the lack of hyphal swellings in *P. kernoviae* cultures differentiates it from *P. heveae*, where they are evident (Fig. 9C). Other characteristics also may be helpful in differentiating these two species. Although conflicted in the literature, as mentioned above, *P. kernoviae* has obvious caducous sporangia compared to *P. heveae*, which are ambiguous. Another distinguishing characteristic is that *P. heveae* oospores are markedly

aplerotic (14,15), whereas oospores of *P. kernoviae* are plerotic (7). This characteristic, however, may be difficult to discern.

Differentiating Other Species with Closely Related Characteristics

In the presented dichotomous key, there were two sets of species that could not be differentiated based upon one characteristic. To distinguish these species from one another, the combination of several features were used. These characteristics included growth at different temperature extremes, colony patterns, oospore morphology, and propagule production that were included in the synoptic key by Ho (20), but were noted to be variable within species.

Phytophthora citricola, *P. foliorum*, and *P. inflata* all have similar characteristics outlined in this study and are difficult to separate based upon a single one. To separate *P. inflata*, published results report the formation of intercalary hyphal swellings in aqueous cultures (14). Hyphal swellings have not been observed in *P. citricola* or *P. foliorum* cultures. In addition, colony morphology may provide further confidence in separating *P. inflata* from *P. citricola* and *P. foliorum*. Hall et al. (17) reported a pronounced stellate pattern of *P. inflata* on V8 agar in comparison to the petalloid colonies of *P. citricola* and *P. foliorum* observed in this study (Figs. 16D and 16F). The abundance of sporangia produced in liquid culture may be the best distinguishing characteristic between *P. citricola* and *P. foliorum*. Abundant *P. citricola* sporangia formed in cultures rinsed with soil extract or MES buffer and kept under continuous light, whereas *P. foliorum* produced very few sporangia, regardless of the treatment or condition (Table 2). Donahoo et al. (13) also reported difficulties producing large amounts of *P. foliorum* sporangia. Colony growth at lower temperatures is not a reliable characteristic to separate *P. foliorum* and *P. citricola*. In this study, *P. foliorum* grew slightly at 4° and 6° C, whereas the minimum growth temperature for *P. citricola* was 8°C. However, Erwin and Ribeiro (14) reported the minimum growth temperature for *P. citricola* is 3°C, which demonstrates that this characteristic may be dependent upon which *P. citricola* group the isolate fall under. Gallegly and Hong (15) separated *P. citricola* into three distinct groups based upon single-strand conformation polymorphism (SSCP) analysis but could not define physical characteristics to distinguish them.

Likewise, it is very difficult to separate *P. hedraiandra* from *P. cactorum* (12,24). Some of the unique physical characteristics of *P. hedraiandra* mentioned in the literature (12), such as predominantly sessile antheridia, the absence of tangled hyphae below the antheridia, and the larger oospores are not easy to identify. In this study, *P. hedraiandra* could be distinguished from *P. cactorum* based upon colony morphology growing on agar plates. At 20°C, *P. hedraiandra* produced a definite petalloid colony compared to the more cottony colony of *P. cactorum* (Figs. 16H and 16A). This was more pronounced on the Rye A agar plates. In addition, culturing in an incubator under artificial light inhibited the formation of oogonia in liquid cultures of *P. hedraiandra* 1 day after rinsing with MES buffer or soil extract, while oogonia formed in *P. cactorum* under the same conditions (Table 2).

Finally, *P. insolita* differs from other homothallic species in this study by producing nonpapillate, noncaducous sporangia (14), and sexual structures void of antheridia (15). In addition, this species produces chlamydospores (2), which is a unique characteristic among the other homothallic species in this study.

In conclusion, the data presented here can be useful for differentiating *P. ramorum* and *P. kernoviae* from other *Phytophthora* spp. that are pathogenic to aerial plant parts of rhododendrons. If *P. ramorum* or *P. kernoviae* are suspected, based upon the data presented here, it is important to verify the identification through approved and accepted molecular techniques. However, the key provided in this study will be useful in assisting diagnostic labs and extension agents that may not have the tools or resources available to use molecular techniques in identifying *Phytophthora* spp. for screening purposes.

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