

Phytophthora

A microscopic image of a Phytophthora zoospore, showing its characteristic pear-shaped body, a large circular nucleus, and a long, thin, curved flagellum extending from one end.

Identifying
Species by
Morphology
and DNA
Fingerprints

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CHAPTER 1

The Morphological Key

The morphological key for identifying species of *Phytophthora* begins with patterns of sexuality in the genus. Homothallic species are separated from heterothallic ones, and within the homothallic species there is separation using the type of antheridium; sexual bodies of one species have only oogonia containing oospores. Asexual characters such as type of papillae, caducity, and length of pedicel are used within the sexual groups. Definitive morphological characters are illustrated in Appendix 2. One physiological character, growth or no growth at 35°C, remains a valuable character for separating some of the heterothallic species. Also, the maximum temperature for colony growth is helpful, particularly for identifying low temperature isolates. Waterhouse has evaluated the above characters, and others, for value in species identifications (Waterhouse 1970b).

This dichotomous-like key was first put in writing in 1990 with only 24 species. It was called a quick-fix key because relatively few morphological characters were used. As more species were added to the key it became necessary to use additional characters for separation. Also, single-strand conformation polymorphism (SSCP) analyses revealed DNA differences

among isolates previously regarded as the same species (Kong et al. 2003; Kong et al. 2004a), and this caused closer examinations for additional characters. For example, different SSCP patterns caused the recognition of three groups among isolates received as *P. citricola* and three groups within *P. megasperma*. Other species with multiple SSCP patterns are *P. citrophthora*, *P. drechsleri*, and *P. cryptogea*, but only the groups most closely fitting the original descriptions are presented here. The other groups will be included in a later edition or supplement.

A description with 9 photographs of each species in the key is presented in Chapter 3. The photographs were selected to show the morphology used in identification, with no attempt to show fine details of the structures. The species descriptions are presented alphabetically, even though some diagnosticians prefer presentation in the order in which they appear in the key. *P. italica* is left in the key without a back-up description and photographs, because this species has disappeared from all culture collections. Also, the authorities for *P. italica* (Cacciola et al. 1996) provided a good description.

The morphological key begins on page 2.

HOMOTHALLIC SPECIES

Paragynous antheridia

PAPILLATE SPORANGIA

Caducous

- a. Pedicels short, sporangia ovoid-spherical, with one apex; no hyphal swellings; no amphigyny; antheridia not sessile or short-stalked *P. cactorum*
- b. Sporangial characters like *P. cactorum*; no amphigyny; antheridia sessile or short-stalked and club-shaped *P. hedraiandra*
- c. Pedicels short, sporangia ovoid to broadly ellipsoid, with multiple papillae; caducity often not evident until zoospore release; dome-shaped basal plug in empty sporangium; hyphal swellings present; amphigyny present *P. clandestina*

Noncaducous

- a. No hyphal swellings; no chlamydospores; antheridia club-shaped; occasional amphigyny *P. idaei*
- b. Hyphal swellings present; no chlamydospores; sporangiophores unbranched and long *P. pseudotsugae*
- c. Hyphal swellings present; chlamydospores present; arachnoid mycelium; tooth-shaped projections on antheridia; abundant amphigyny *P. tentaculata*
- d. No hyphal swellings; chlamydospores intercalary and terminal; oogonia of bizarre shapes; antheridia paragynous only; sporangia highly variable in shape; dome-shaped colony on V-8 juice agar; temp. max. <30°C *P. quercina*
- e. No hyphal swellings; chlamydospores in some isolates; oogonia relatively large (33 µm); occasional amphigyny; multiple papillae; irregular sporangia, ovoid to ellipsoid; temp. max. 35°C *P. iranica*
- f. No hyphal swellings; chlamydospores rare; oogonia smaller (22 µm); sporangia uniformly ovoid to obpyriform; no amphigyny; slow growth; temp. max. 35°C (all cultures of this species have been lost; see Erwin and Ribeiro 1996 for good photographs) *P. italica*

SEMIPAPILLATE SPORANGIA (PAPILLAE FLATTENED)

- a. Noncaducous sporangia with more than one apex; sporangial distortion and lateral attachment common; antheridia inflated, >13.0 µm; temp. max. 31°C *P. citricola* I
- b. Noncaducous sporangia, mostly ovoid; antheridia <10 µm; golden brown sex bodies; temp. max. 31°C *P. citricola* II
- c. Noncaducous sporangia, tending toward ellipsoid; oospores with thin walls, many not quite plerotic; many antheridia appear to be perpendicular to the oogonium; temp. max. 31°C *P. citricola* III
- d. Noncaducous sporangia with one apex; sporangial distortion and lateral attachment in some isolates; temp. max. 23°C *P. syringae*
- e. Similar to *P. syringae*, except in occasionally producing amphigynous antheridia and growing at slightly higher temperatures, temp. max. about 27°C *P. pseudosyringae*

- f. Noncaducous sporangia of variable sizes and bizarre shapes;
temp. max. $<27^{\circ}\text{C}$ *P. primulae*
- g. Noncaducous sporangia; oogonia produced at 10°C ; antheridia oblique
and sometimes more than one per oogonium; coiling hyphae in agar *P. porri*

NONPAPILLATE SPORANGIA (ALL NONCADUCOUS)

No amphigyny

- a. Oogonia tapered, usually curved; antheridia affixed to tapered stalks, usually
near bottom of taper; irregular hyphal swellings; no chlamydospores *P. europaea*
- b. Oogonia spherical and terminal but absent in most isolates; chlamydo-
spores abundant and characteristically lateral and sessile on the hyphae *P. lateralis*

Amphigyny present

- a. No chlamydospores, but hyphal swellings and sex bodies always present;
may have small percentage of amphigyny; oogonia about $38\text{ }\mu\text{m}$, with thick
walls; antheridia more tangential than perpendicular; sporangia mostly
ovoid to obpyriform, and large ellipsoid sporangia may occur; temp. max.
about 30°C *P. megasperma* I
- b. No chlamydospores or hyphal swellings; oogonia large ($45\text{ }\mu\text{m}$), sometimes
golden, with mostly aplerotic oospores, which have thinner walls; dome-
shaped plug at base of empty sporangium in some isolates *P. megasperma* II
- c. Hyphal swellings present; oogonia smaller, with plerotic oospores;
antheridia more vertical than horizontal and appear to be perpendicular;
sporangia bluntly ellipsoid *P. megasperma* III
- d. Oogonia about $33\text{ }\mu\text{m}$; both paragyny and 25 to 50% amphigyny occur;
temp. max. about 33°C *P. medicaginis*
- e. Oogonia round, smooth, about $37\text{ }\mu\text{m}$; many antheridia perpendicular or
oblique to oogonial wall, about $12\text{ }\mu\text{m}$ deep; temp. max. about 32°C *P. sojae*
- f. Sex bodies golden brown; oogonia about $40\text{ }\mu\text{m}$; sporangia small, about
 $32 \times 24\text{ }\mu\text{m}$ *P. trifolii*
- g. Oogonia $>40\text{ }\mu\text{m}$; small percentage of amphigyny; large hyphal swellings
with radiating hyphae; sporangia mostly ovoid, $53 \times 40\text{ }\mu\text{m}$; temp. max.
about 32°C *P. humicola*
- h. Oogonia about $35\text{ }\mu\text{m}$; small percentage of amphigyny; hyphal swellings
with radiating hyphae; sporangia large, ellipsoid, $77 \times 40\text{ }\mu\text{m}$; temp. max.
about 32°C *P. pistaciae*

Amphigynous antheridia

PAPILLATE SPORANGIA

- a. Oogonial stalk tapered, oogonium smooth *P. heveae*
- b. Oogonial stalk tapered, oogonium ornamented *P. katsurae*
- c. Oogonial stalk not tapered *P. boehmeriae*

SEMIPAPILLATE SPORANGIA (PAPILLAE FLATTENED)

- a. Caducous sporangia with long pedicels; temp. max. 25°C *P. hibernalis*
- b. Caducous sporangia with medium pedicels; sporangia almost spherical to ovoid, about 30 × 25 µm; small sex bodies, about 25 µm; temp. max. 25°C *P. ilicis*
- c. Caducous sporangia with medium pedicels; sporangia about 40 × 25 µm, sometimes sickle-shaped; oogonia about 36 µm; temp. max. <25°C *P. nemorosa*
- d. Caducous sporangia with short pedicels; temp. max. 25°C *P. phaseoli*
- e. Noncaducous sporangia, about 50 × 34 µm; temp. max. <25°C *P. psychrophila*

NONPAPILLATE SPORANGIA

- a. Oogonia smooth-walled; sporangia ellipsoid, often constricted in the middle *P. erythroseptica*
- b. Oogonia ornamented; sporangia not constricted but of variable shapes *P. alni*
- c. Sex bodies golden brown; oospores aplerotic; sporangia mostly ellipsoid; not pathogenic on *Rubus* spp.; temp. max. slightly >25°C *P. fragariae*
- d. Sex bodies golden brown; oospores mostly plerotic; sporangia mostly ovoid; not pathogenic on *Fragaria* spp.; temp. max. <30°C *P. rubi*

Antheridia not formed

- *P. insolita*

HETEROTHALLIC SPECIES

PAPILLATE SPORANGIA

Colony growth at 35°C or higher

- a. Noncaducous sporangia, usually ovoid to spherical, usually with one papilla; chlamydospores usually present; arachnoid mycelium in most isolates *P. nicotianae*
- b. Caducous sporangia, many with two or more papillae, distorted shapes, long pedicels (up to 100 µm long); usually no chlamydospores *P. capsici*

No colony growth at 35°C, or very slight growth

- a. Caducous sporangia with short pedicels; sporangia of variable shape, ovoid to ellipsoid, av. l/w ratio <1.6:1; chlamydospores abundant *P. palmivora*
- b. Caducous sporangia with medium pedicels 5 to 10 µm long; sporangia mostly ovoid or obpyriform but some ellipsoid, av. l/w ratio <1.6:1; chlamydospores in most isolates; tapered oogonial stalk *P. megakarya*
- c. Caducous sporangia with medium pedicels 10 to 20 µm long (av. near 20 µm); sporangia ellipsoid or elongated but some obpyriform and ovoid; some chlamydospores *P. meadii*
- d. Caducous sporangia with medium pedicels; sporangia ovoid, av. l/w ratio >1.6:1; small sporangia formed in botryose clumps on agar; chlamydospores sparse *P. botryosa*
- e. Caducous sporangia with long pedicels (usually >50 µm) and characteristic tapered bases; umbellate sympodia are common *P. tropicalis*

- f. Noncaducous sporangia, ovoid to almost spherical; chlamydospores present; sex bodies golden brown on hemp-seed agar *P. mexicana*
- g. Noncaducous sporangia, ovoid or obpyriform to spherical on agar and having bizarre distorted shapes in water, with more than one apex; chlamydospores in some isolates; finely radiate colony on agar; sex bodies rare *P. citrophthora*

SEMIPAPILLATE SPORANGIA (PAPILLAE FLATTENED)

No colony growth at 35°C

- a. Caducous sporangia with medium pedicels (about 12 µm); chlamydospores usually produced; pathogenic on leaves of taro; temp. max. <35°C *P. colocasiae*
- b. Caducous sporangia with short pedicels; no chlamydospores; pathogenic on *Solanum* and *Lycopersicon* species; temp. max. 26°C *P. infestans*
- c. Caducous sporangia with short pedicels; no chlamydospores; host specific on four o'clocks (*Mirabilis jalapa* L.) *P. mirabilis*
- d. Caducous sporangia with short pedicels; chlamydospores abundant (intercalary and terminal); pathogenic on many woody hosts *P. ramorum*

NONPAPILLATE SPORANGIA (ALL NONCADUCOUS)

Colony growth at 35°C or above

- a. Many sporangia with tapered bases; hyphal swellings catenulate or angular, giving a net-like appearance, oospores plerotic, about 26 µm in diameter *P. drechsleri*
- b. Sporangia mostly with rounded bases; small hyphal swellings; oospores mostly applerotic, 36 µm in diameter *P. inundata*
- c. Sporangia mostly with rounded bases; no hyphal swellings *P. melonis*

No colony growth at 35°C

- a. Like *P. drechsleri* above *P. cryptogea*
- b. No hyphal swellings; no chlamydospores; many bluntly ellipsoid sporangia, some with constrictions, formed in water and agar; plerotic oospores; oogonia not tapered *P. cajani*
- c. Abundant botryose hyphal swellings in agar culture; coralloid hyphae; oogonia tapered within the antheridium; sporangia formed only in water, av. l/w ratio >1.6:1 *P. cinnamomi* var. *cinnamomi*
- d. Hyphal swellings not botryose; sporangia bluntly ellipsoid; oogonia not tapered within the antheridium; temp. max. 30°C *P. cinnamomi* var. *parvispora*
- e. Hyphal swellings not botryose; hyphae not coralloid; sporangia formed only in water, av. l/w ratio <1.6:1; oogonia ornamented with bullate protuberances *P. cambivora*

APPENDIXES TO CHAPTER 1

1. General Procedures for Morphological Identification

When a new isolate is received it is subcultured on lima bean agar and checked for contamination. If clean the culture is examined for the sexual stage. If sexual bodies are not present it is paired with A¹ and A² testers (mostly *P. meadii* but *P. nicotianae*, *P. capsici*, *P. drechsleri*, *P. cinnamomi*, and *P. cryptogea* have been used), and the isolate is grown on other media, hemp seed agar, carrot juice agar, and clarified 10% V-8 juice agar.

Should the isolate contain contaminants, steps taken to eliminate them include growing it on media containing antibiotics and using the pancake technique (Sleeth 1945). The antibiotic mixture used most is 3P+R (pimaricin, 100 ppm; penicillin G, 50 ppm; polymyxin B, 50 ppm; and rifampicin, 10 ppm). Ampicillin at 250 ppm, vancomycin at 200 ppm, hymexazol at 20 ppm and pentachloronitrobenzene (PCNB) at 100 ppm are used occasionally. Sometimes the pancake technique is performed using agar containing antibiotics. Stepwise procedures are as follows:

1. New isolates were checked for growth on lima bean agar at 20, 25, 30, and 35°C.

2. Disks 6 mm in diameter were cut from the margins of 3-day-old cultures on lima bean agar, placed in a Petri plate (four disks per 100-mm plate or two disks per 60-mm plate) and covered with sterile deionized water or sterile 10% soil extract. Some slow-growing isolates were more than 3 days old when disks were taken.

3. For sporangium production, the water-covered disks were placed under cool-white fluorescent lamps at about 100 foot candles and 20 to 22°C.

4. Most species produce sporangia overnight (within 24 hours), whereas others may require 3 days. Sometimes water cultures were sprinkled with non-sterile soil to induce sporangium formation.

5. To determine caducity a disk bearing sporangia was inverted on a microscope slide in a drop of water and vigorously agitated with a teasing needle. Measurements were made with a calibrated microscope to determine pedicel length if an isolate was caducous.

6. The agar cultures of single and paired isolates were grown in the dark at 20 to 22°C for the production of sex bodies. A few species required special techniques as described in the species descriptions.

7. Morphological characters were examined with a compound light microscope and measured with a calibrated ocular micrometer. A standard number of measurements were not made. If an isolate was received with a species name and random measurements fit those in the species description, then the authority's measurements were used.

8. The various morphological stages were photographed using 43 and 97× objectives. The microscope used was a Bausch and Lomb zoom with photographic capabilities. The film used was 35-mm Kodachrome 64 or T-Max 100 black and white. Because this project was started in the 1980s, before digital technology was available, the older film technique was continued for uniformity.

9. The 35-mm slides were scanned with a Polaroid Sprint Scan 35 using Photoshop and stored on the hard drive. Zip disks were used for backup.

10. A 10-μm scale bar was incorporated into each scanned image in Photoshop.

11. Once the morphology had been determined, a new isolate was placed to species using the new dichotomous key above.

2. Illustration of Definitive Morphological Characters

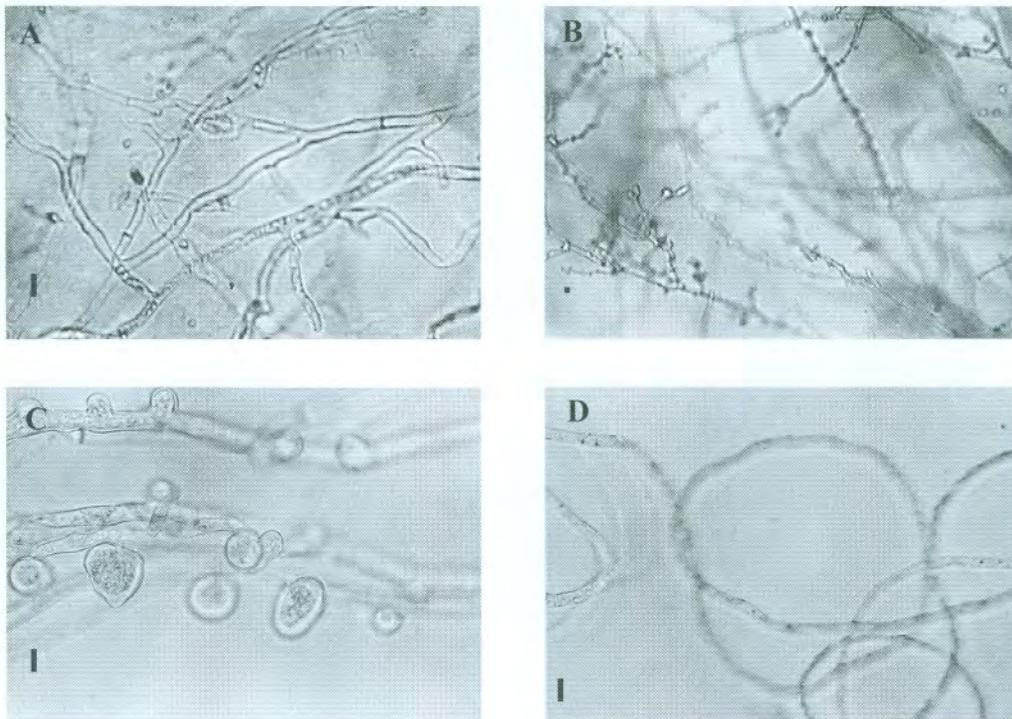


Figure 1.1. Mycelium morphology. **A**, Uniform mycelium of *Phytophthora ilicis*. **B** and **C**, Coralloid and knobby mycelium of *P. cinnamomi* var. *parvispora*. **D**, Coiled mycelium of *P. porri*.

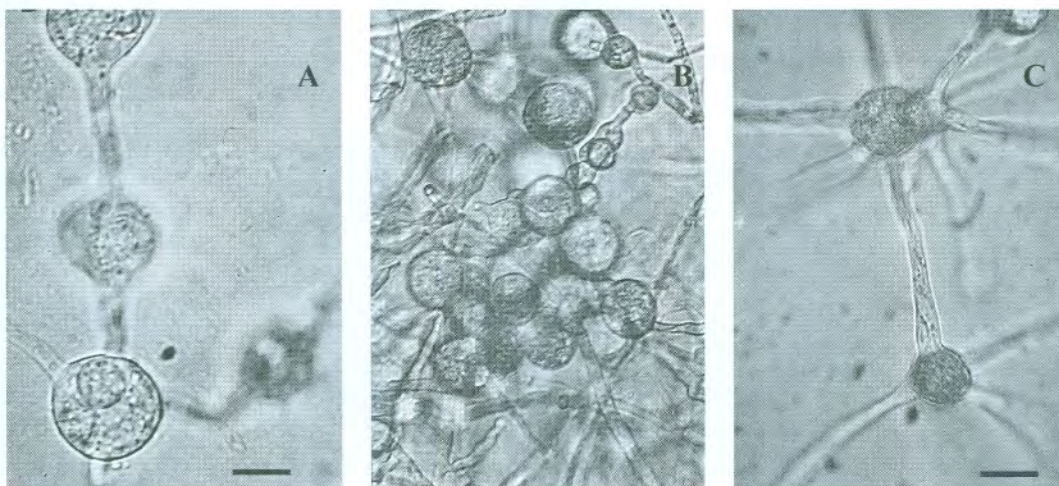


Figure 1.2. Hyphal swellings. **A** and **B**, Catenulate hyphal swellings and clumps of hyphal swellings of *Phytophthora cryptogea*. **C**, Hyphal swellings of *P. megasperma* with radiating hyphae, sometimes called spikes.

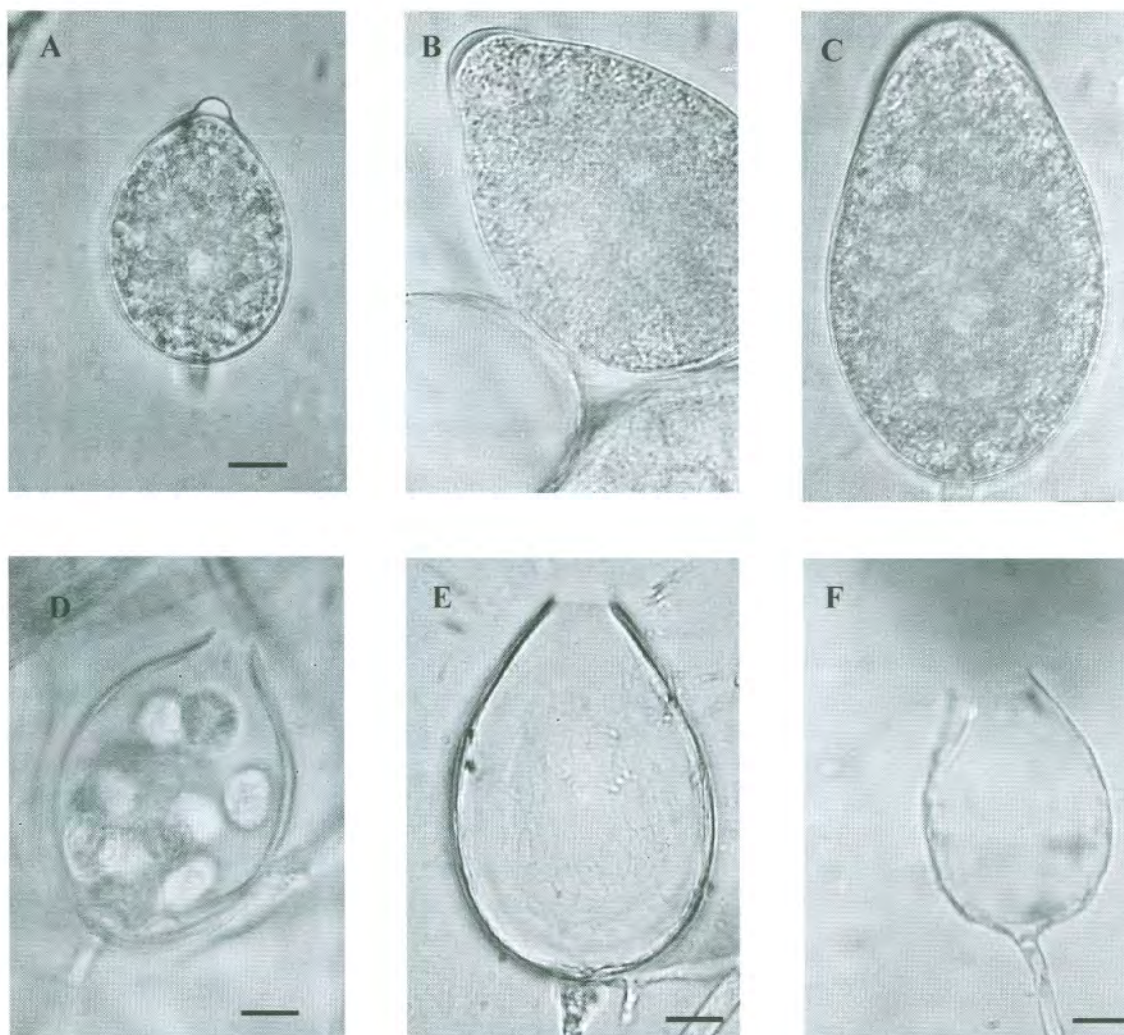


Figure 1.3. Sporangium morphology: papillae and width of exit pore. **A**, Papillate sporangium of *Phytophthora cactorum*. **B**, Semipapillate sporangium of *P. citricola* I. **C**, Nonpapillate sporangium of *P. pistaciae*. **D**, Narrow exit pore of *P. cactorum*. **E**, Medium exit pore of *P. citricola* I. **F**, Wide exit pore of *P. lateralis*.

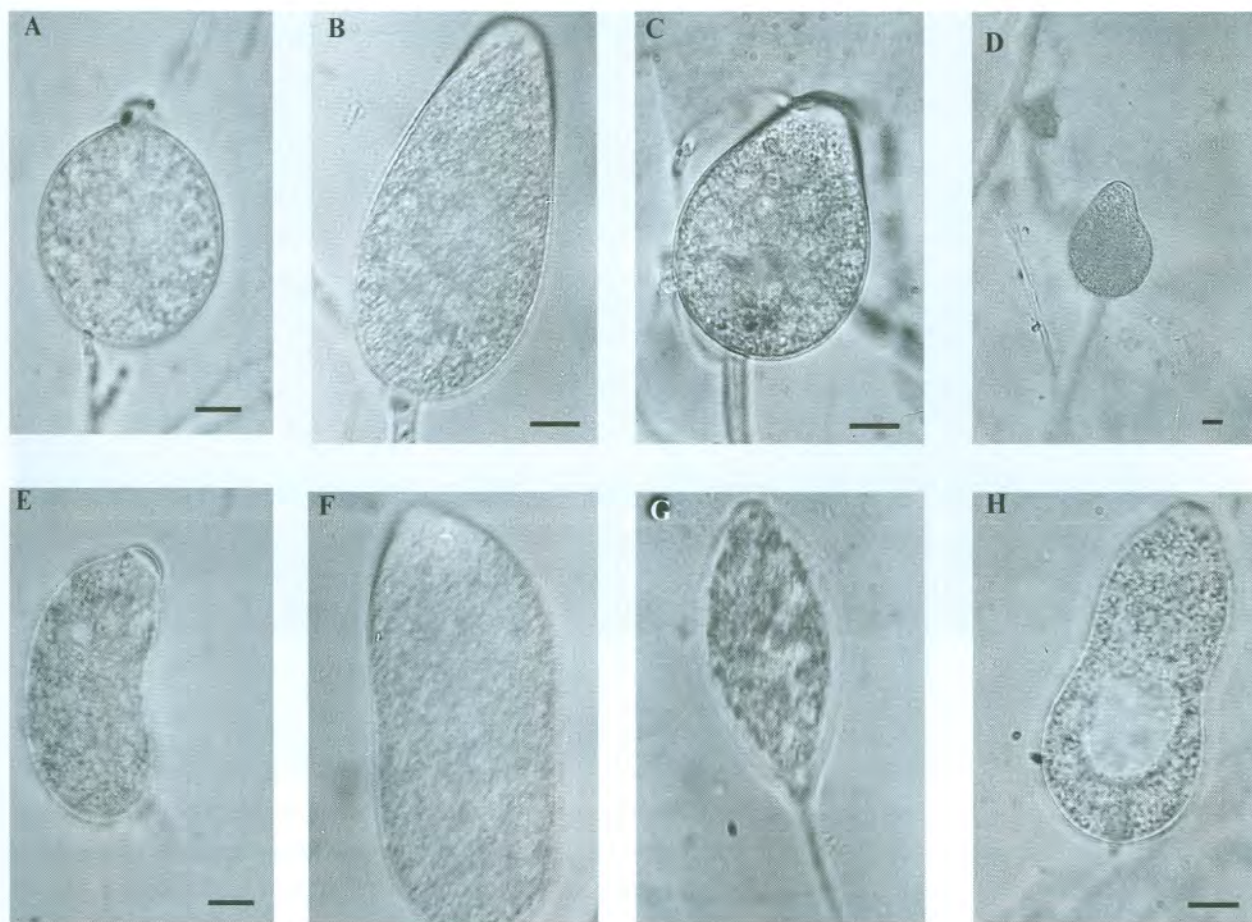


Figure 1.4. Sporangium morphology: shape. **A**, Spheroid sporangium of *Phytophthora nicotianae*. **B** and **C**, Ellipsoid and ovoid sporangia of *P. megasperma* I. **D**, Obpyriform sporangium of *P. cajani*. **E**, Sick-shaped sporangium of *P. nemorosa*. **F**, Bluntly ellipsoid sporangium of *P. fragariae*. **G**, Sporangium with tapered base, *P. hibernalis*. **H**, Sporangium with constricted middle, *P. erythroseptica*.

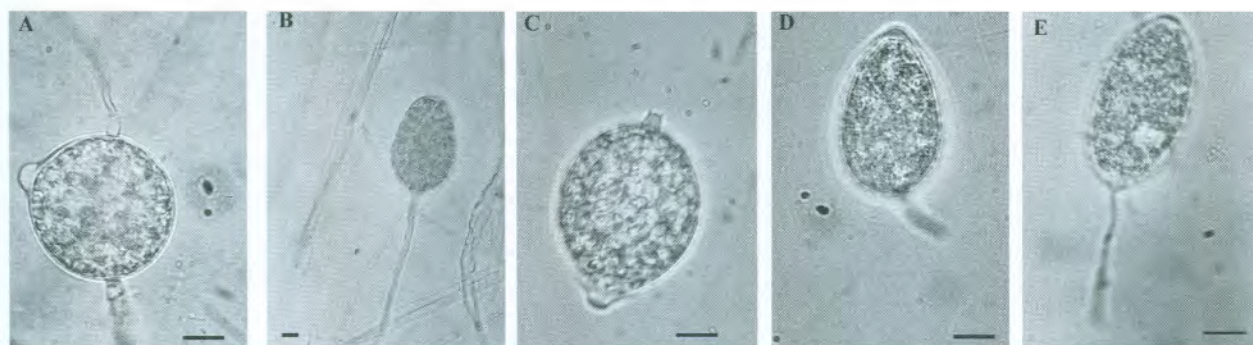


Figure 1.5. Sporangium morphology: positioning, caducity, and pedicel length. **A**, Intercalary sporangium of *Phytophthora pseudotsugae*. **B**, Terminal, noncaducous sporangium of *P. melonis*. **C**, Caducous sporangium with short pedicel, *P. cactorum*. **D**, Caducous sporangium with medium pedicel, *P. megakarya*. **E**, Caducous sporangium with long pedicel, *P. hibernalis*. A 10- μ m bar is included in **C**, **D**, and **E** to aid in determining the range of pedicel lengths: short (<5 μ m), medium (5–20 μ m), and long (>20 μ m).

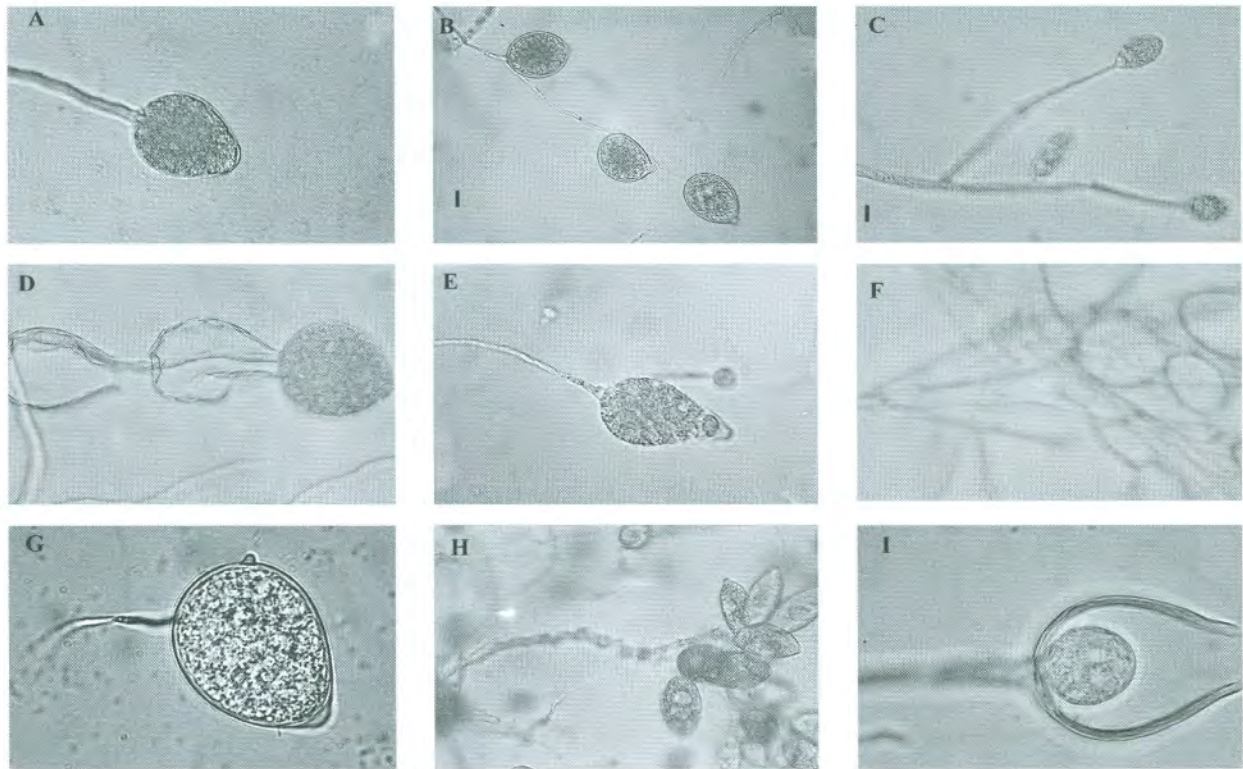


Figure 1.6. Sporangiophore morphology. **A**, Unbranched sporangiophore of *Phytophthora europaea*. **B**, Sporangiophores in a simple sympodium of *P. cactorum*. **C**, Sporangiophores in a compound sympodium of *P. mirabilis*. **D**, Internal proliferation of sporangiophore of *P. inundata*. **E**, External proliferation of sporangiophore of *P. clandestina*. **F**, Sporangiophores in an umbellate sympodium of *P. tropicalis*. **G**, Offset attachment of sporangiophore of *P. capsici*. **H**, Sporangiophore producing botryose clumps of sporangia of *P. botryosa*. **I**, Nesting from sporangiophore of *P. insolita*.



Figure 1.7. Chlamydospore morphology. **A**, Terminal chlamydospore of *Phytophthora nicotianae*. **B**, Intercalary chlamydospores of *P. tropicalis*. **C**, Lateral chlamydospore of *P. lateralis*. **D**, Clustered chlamydospores of *P. cinnamomi*.

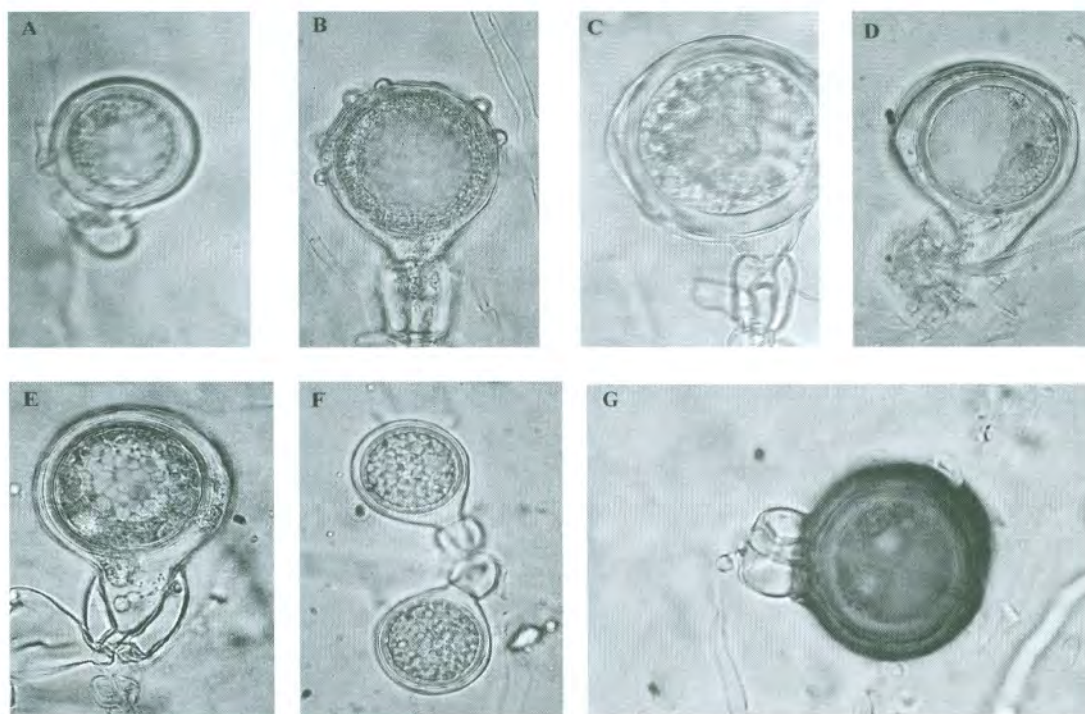


Figure 1.8. Characteristics of oogonia. **A**, Smooth globose oogonium of *Phytophthora tentaculata*. **B**, Ornamented oogonium of *P. cambivora*. **C**, Oogonium with wavy wall, *P. alni*. **D**, Hooked oogonium of *P. europaea*. **E**, Oogonium with a tapered base within an antheridium of *P. cinnamomi*. **F**, Oogonia with a tapered base above the antheridium of *P. heveae*. **G**, Pigmented oogonium of *P. tropicalis*.

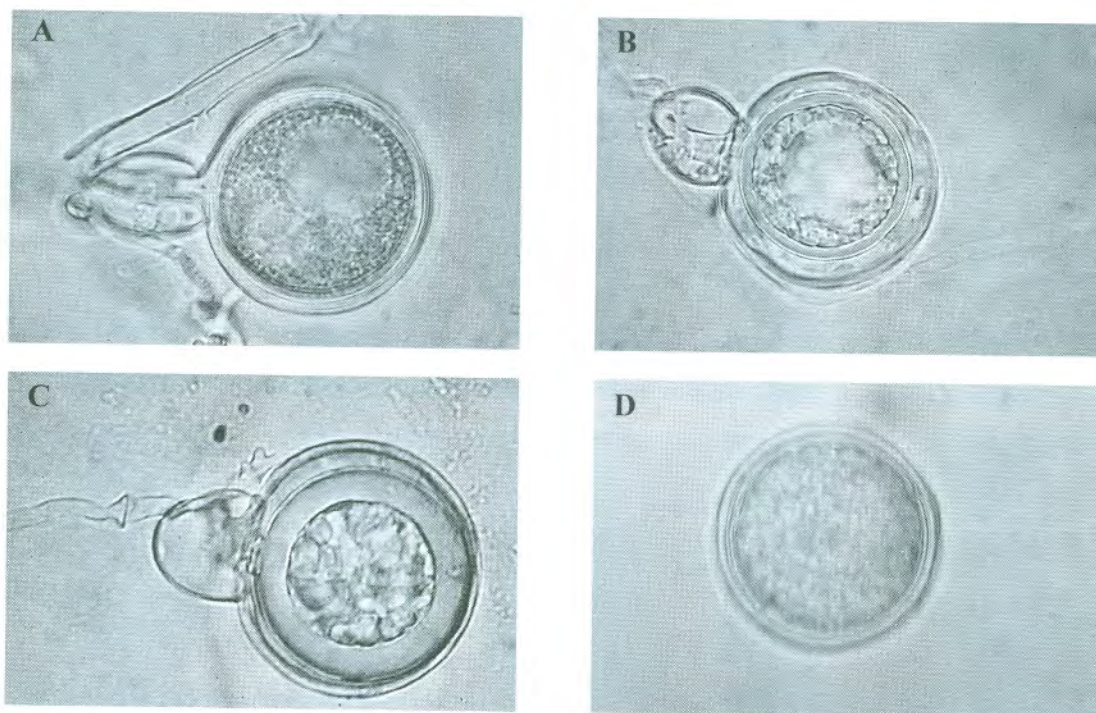


Figure 1.9. Oospore morphology. **A**, Plerotic oospore of *Phytophthora nemorosa*. **B**, Aplerotic oospore of *P. pistaciae*. **C**, Thick-walled oospore of *P. cryptogea*. **D**, Thin-walled oospore of *P. cajani*.

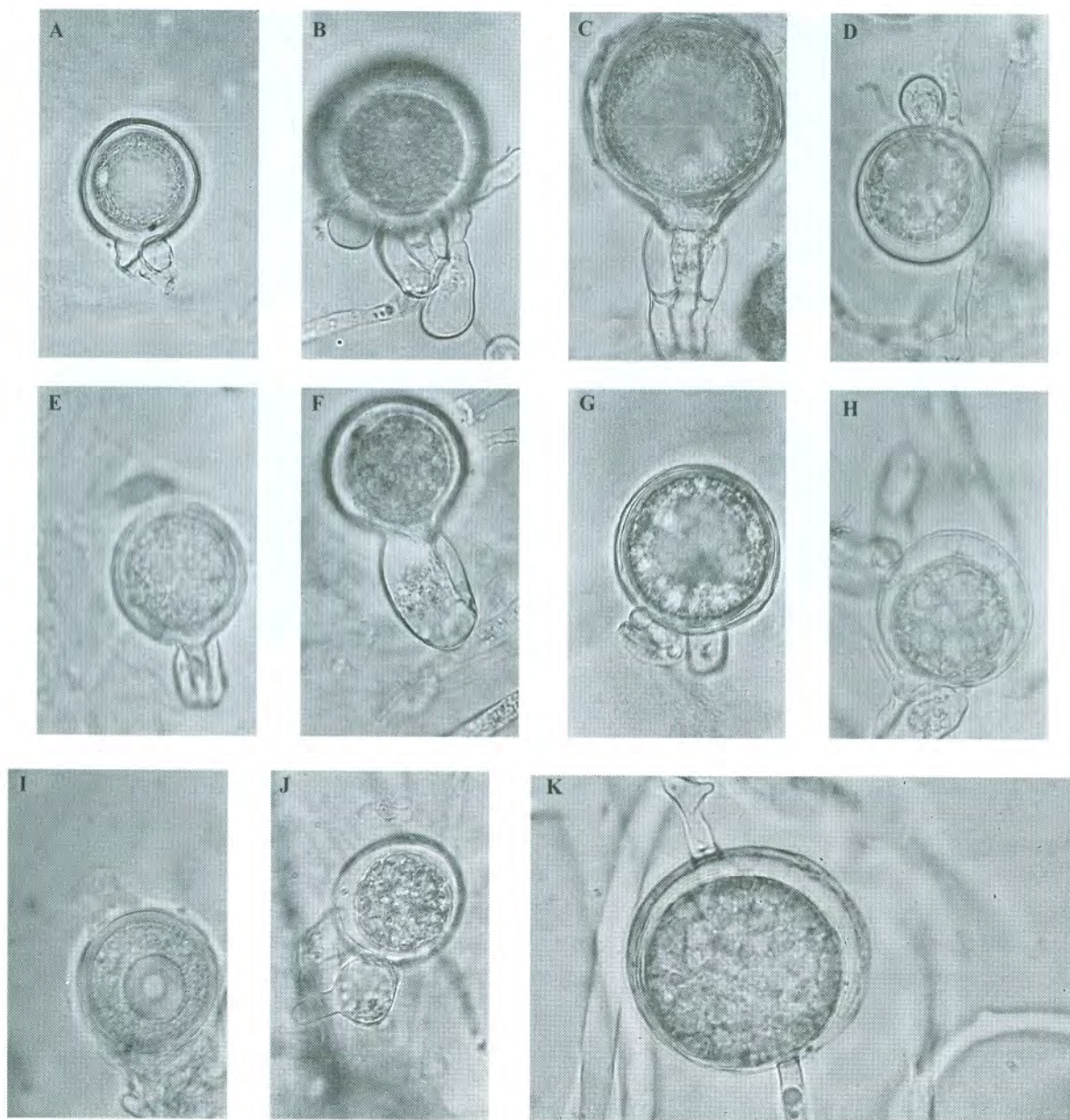


Figure 1.10. Antheridial morphology and configurations. **A**, Paragynous antheridium of *Phytophthora citricola* II. **B**, Amphigynous one-cell antheridium of *P. inundata*. **C**, Amphigynous two-cell antheridium of *P. cambivora*. **D**, Spherical antheridium of *P. cactorum*. **E**, Rectangular antheridium of *P. psychrophila*. **F**, Elongated antheridium of *P. infestans*. **G**, Perpendicular antheridium of *P. megasperma* III. **H**, Oblique antheridia of *P. porri*. **I**, Antheridium tangled in hyphae of *P. syringae*. **J**, Club-shaped antheridium of *P. hedraiandra*. **K**, No antheridium, *P. insolita*.

3. Growth Media and Methods

Lima bean agar seems to be the overall best medium for growth of all the species, particularly the slow-growing low-temperature isolates. The medium as used in our studies is made as follows from 284 g (a 10-ounce box) of frozen baby lima beans, 40 g of agar, and 2000 ml of distilled water.

1. The lima beans are placed in a liter of distilled water in a 2-liter flask, and the agar in another liter, and then autoclaved for 20 minutes at 121°C.
2. After slow exhaust the liquid from the lima beans is poured into the melted agar through three layers of cheesecloth and brought to a volume of 2 liters.
3. The thoroughly mixed melted agar and lima bean extract is dispensed into 8-ounce medicine bottles, 150 ml per bottle.
4. The agar in these bottles is reautoclaved for 15 minutes at 121°C and stored in the dark in a refrigerator.
5. Each bottle of agar, when melted by a microwave oven, will pour about eight 100-mm Petri plates or about twenty 60-mm plates.

Hemp seed agar is usually the best medium for production of the sexual stages, providing that isolates of a species will grow on this medium. Several of the fastidious species, such as *P. infestans*, will not grow on hemp seed agar. Our hemp seed was obtained in 1965 from a supplier in New Jersey (company name lost) and preserved in a large desiccator. The agar is made as follows:

1. Hemp seed (50 g) is soaked in 500 ml of distilled water overnight.
2. The soaked hemp seed is autoclaved at 121°C for 20 minutes, along with 20 g of agar suspended in another 500 ml of distilled water.
3. The heat-extracted liquid from the hemp seeds is filtered through cheesecloth into the melted agar and brought to a volume of 1 liter.
4. Dispensing and reautoclaving is as described above for lima bean agar.

Carrot juice agar is made from canned carrot juice, 100 ml per liter. The juice is mixed with 3 g of calcium carbonate and then decanted into a liter of distilled water containing 20 g of agar. Further handling is as described above. This medium is useful for producing sexual bodies of some species.

V8 juice agar is used only occasionally, because of difficulty with microscopic observations. Even on cleared 10% V8 juice agar the mycelium of many species is so thick that sex bodies have to be teased out of the colony. Also, it is a common practice to follow progress of organ production in a culture by looking through the bottom of Petri plates. This cannot be done with the commonly used V8 juice agars. This agar, along with cornmeal and potato dextrose agars, are seldom used but can be prepared as described in Erwin and Ribeiro (1996).

Rye seed lima bean agar is used for maintenance of stock cultures. The use of this mixed ingredient agar began when it was found to be excellent for *P. infestans*. The older isolates of this species grew better on lima bean agar whereas the newer isolates seemed to grow better on rye seed agar. When the mixture is used, all isolates of *P. infestans* grew well, as did isolates of the slow-growing cool-temperature species. This medium is prepared by grinding 50 g of rye seed in water in a Waring blender for 5 minutes, then dumping the contents into the flask containing the lima beans, and then following the procedures described above for lima bean agar. This medium is dispensed into 125 × 16 mm screw-cap tubes when used for stock cultures.

Stock cultures are maintained in 20°C incubators, one set covered by sterilized heavyweight mineral oil and transferred every 2 to 3 years. Another set without oil is transferred every 6 months. Our key isolates are being deposited with the American Type Culture Collection.

The polycarbonate technique (Ko 1978) is used to produce selfed sexual stages of a species. Our adaptation of this technique is the use of hemp-seed agar in 60-mm plates, when the species will grow on it. After the isolate being studied grows for 1 or 2 days on the hemp seed agar, a 45-mm polycarbonate membrane (0.4 µm pore size) is placed on top. Then an 18-mm disk of hemp seed agar is placed on the center of the membrane. The 18-mm disk is inoculated with either an A¹ or an A² isolate. We use *P. meadii* mostly. The cultures are examined after about 10 days. Selfed sex bodies are usually produced under the membrane and directly under where the 18-mm disc was placed.

CHAPTER 2

The DNA Fingerprint Key

A molecular fingerprint key to *Phytophthora* species has long been sought by numerous mycologists and plant pathologists to increase the speed and accuracy of isolate identification and reduce the requirements of training and work experience for such tasks. An array of molecular technologies including polymerase chain reaction (PCR) has proven to be advantageous over morphology-based identification in terms of speed and expertise requirements. However, none had shown an adequate resolution until we developed a single-strand conformational polymorphism (SSCP) analysis of PCR-amplified ribosomal DNA internal transcribed spacer 1 (Kong et al. 2003). We have since evaluated the PCR-SSCP with isolates of additional species and found that it can differentiate a number of other taxa, in addition to the 39 species demonstrated previously (Kong et al. 2003; Kong et al. 2004a). This chapter presents a PCR-SSCP key we have developed to date and provides stepwise procedures employed in developing the DNA fingerprints of individual species. Also, a partial list of other molecular fingerprinting techniques that may be complementary to the PCR-SSCP is presented.

A total of 59 species are included in this key. A typical PCR-SSCP pattern consists of upper and lower bands (Kong et al. 2003). Occasionally, the upper or lower bands may split into two that are close to each other. In these cases, the two bands are treated as a band set. Individual species are aligned in the key by the location of their upper bands then lower bands (Figure 2.1). DNA fingerprints of individual species are characterized by the locations of upper and lower bands and the distance between them. *P. cajani* and *P. melonis* are retained as separate taxa although they have been considered to be conspecific with *P. drechsleri* (Kannaiyan et al. 1980; Ho 1986). These two species have distinct PCR-SSCP patterns and morphological characters different from those of *P. drechsleri*. Each PCR-SSCP pattern seems to represent one species within the genus *Phytophthora*, with a few excep-

tions (e.g., *P. cactorum* and *P. pseudotsugae*). As a result, every PCR-SSCP subgroup within each species complex of *P. citricola* and *P. megasperma* is treated as a separate taxon in our keys. Some PCR-SSCP subgroups are being named by originating colleagues of respective key isolates, and others are being named by ourselves. Almost all the species included in the keys have distinct PCR-SSCP patterns. The only exceptions are (i) *P. cactorum* and *P. pseudotsugae* (Kong et al. 2003); (ii) *P. cryptogea*, *P. erythroseptica*, and *P. megasperma* II; (iii) *P. fragariae* and *P. rubi*; (iv) *P. infestans* and *P. mirabilis*; (v) *P. humicola* and *P. inundata* (Ho et al. 2006); and (vi) *P. porri* and *P. primulae*. Each of these species pairs and triplets produces an identical or very similar pattern.

To assist in measuring and describing the migration of individual conformations associated with a given species, a single-strand DNA (ssDNA) ladder (Kong et al. 2003) is included in the key. This ladder consists of 14 obvious bands or band sets. Each band or band set is considered as a marker. These markers are labeled numerically, assigning 1 to the top and 14 to the bottom band or band set. The distance between two markers is divided into 10 equal sections to facilitate describing the location of SSCP bands of interest. For example, if a band of interest migrates to the eighth section between markers 5 and 6, the location of the band is estimated as 5.8. Characterization of PCR-SSCP patterns of individual species is provided in Table 2.1 to facilitate use of this key for species identification. Electrophoresis side by side with reference isolates of similar PCR-SSCP patterns often adds ease and accuracy to species identification, especially when difficult isolates are encountered and new species are suspected.

The PCR-SSCP is one of the characters with the highest resolution found to date for species identification within the genus *Phytophthora*. The mechanism behind this exceptional resolution is that DNA fragments of the same or similar size but different se-

Figure 2.1. Alignment of single-strand conformational polymorphism (SSCP) patterns of fifty-nine species of *Phytophthora* by locations of their upper and lower bands. Species names are listed at the top of their respective lanes. ssL = single-stranded DNA ladder.

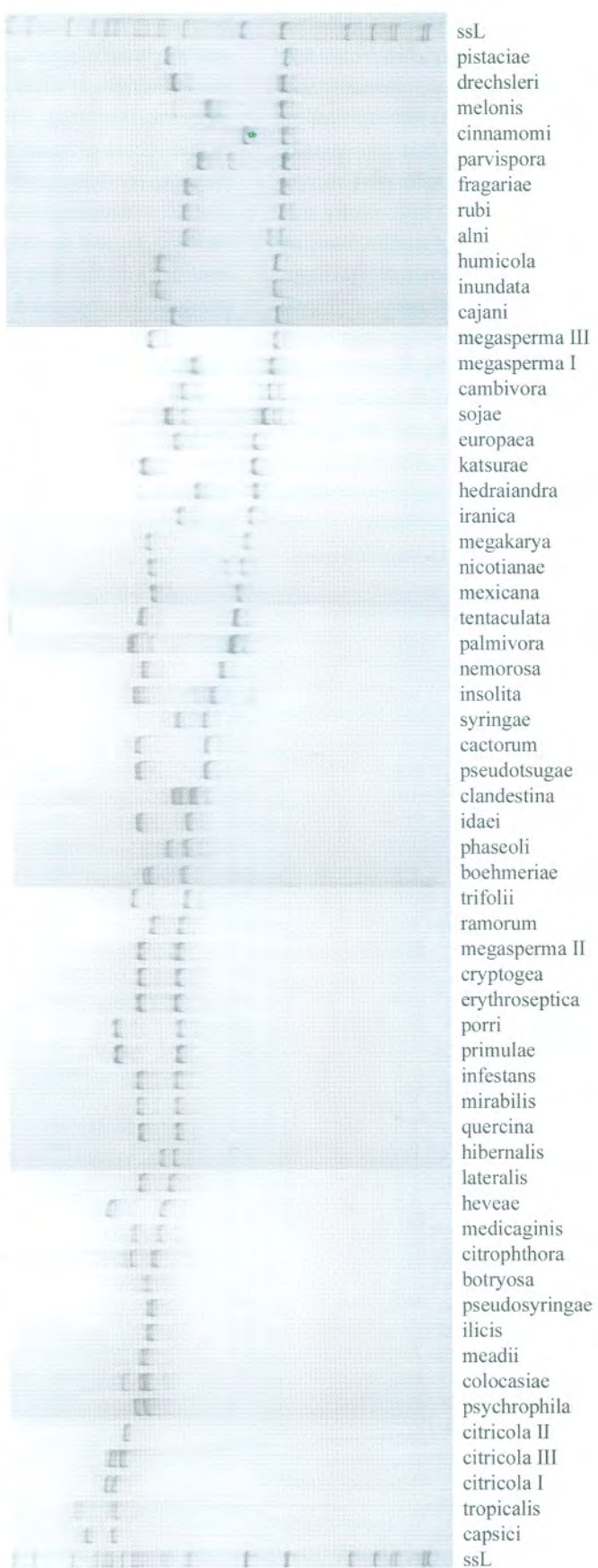


Table 2.1. Characterization of single-strand conformation polymorphism (SSCP) of ribosomal DNA internal transcribed spacer 1 amplified with primers ITS6 and ITS7 (arranged by band location)

Species	SSCP identifier ^a	Band location ^b		Morpho-group ^c	Phylogenetic clade ^d
		Upper	Lower		
<i>P. pistaciae</i>	Pis	4.8	7.6	V	...
<i>P. drechsleri</i>	Dre	4.8	7.2	VI	8a
<i>P. melonis</i>	Mel	4.9	6.7	VI	7b
<i>P. cinnamomi</i> var. <i>cinnamomi</i>	Cin	5.0	6.0	VI	7a (7c)
<i>P. cinnamomi</i> var. <i>parvispora</i>	Par	5.0	6.8	VI	...
<i>P. fragariae</i>	Fra	5.0	7.2	V	7a
<i>P. rubi</i>	Rub	5.0	7.2	V	7a
<i>P. alni</i>	Aln	5.1	7.2	V	(7a)
<i>P. humicola</i>	Hum	5.1	8.2	V	6
<i>P. inundata</i>	Inu	5.1	8.2	VI	...
<i>P. cajani</i>	Caj	5.2	7.4	VI	7b
<i>P. megasperma</i> III	Meg III	5.2	8.4	V	...
<i>P. megasperma</i> I	Meg I	5.3	6.8	V	6
<i>P. cambivora</i>	Cam	5.3	7.1	VI	7a
<i>P. sojae</i>	Soj	5.3	7.8	V	7b
<i>P. europaea</i>	Eur	5.5	7.3	V	7a
<i>P. katsurae</i>	Kat	5.7	8.7	II	5
<i>P. hedraiaandra</i>	Hed	5.8	6.7	I	...
<i>P. iranica</i>	Ira	5.8	7.3	I	1b
<i>P. megakarya</i>	Mek	6.0	8.4	II	4
<i>P. nicotianae</i>	Nic	6.1	8.6	II	1b (1d)
<i>P. mexicana</i>	Mex	6.2	8.4	II	...
<i>P. tentaculata</i>	Ten	6.2	8.7	I	1b
<i>P. palmivora</i>	Pal	6.2	9.2	II	4
<i>P. nemorosa</i>	Nem	6.4	8.4	IV	...
<i>P. insolita</i>	Ins	6.4	8.8	V	10 (8f)
<i>P. syringae</i>	Syr	6.6	7.4	III	8b
<i>P. cactorum</i>	Cac	6.7	8.9	I	1a
<i>P. pseudotsugae</i>	Pst	6.7	8.9	I	1a
<i>P. clandestina</i>	Cla	6.8	7.7	I	1b
<i>P. idaei</i>	Ida	6.8	8.9	I	1a

(continued on next page)

quences assume different conformations, resulting in distinct migration patterns on polyacrylamide gels (Orita et al. 1989). Formation of DNA conformations or secondary structures, however, is subject to DNA denaturation and electrophoresis conditions (Sambrook and Russell 2001). Thus it is important to strictly follow the PCR-SSCP protocol to realize the full power of this key. Stepwise procedures employed in developing the standard DNA fingerprints are provided in the appendixes to assist users from DNA amplification to gel staining and documentation.

While being evaluated with additional species and isolates, the PCR-SSCP itself has been a useful tool in developing the morphological key. We used this technique to double check the identity of all incoming isolates, which has expedited the selection of key isolates for some species. We also used it as a guiding

tool to examine individual subgroups within species complexes (different SSCP patterns) in search of morphological differences among them. Application of this fingerprinting technique allowed us to identify many other species of *Phytophthora* in addition to *P. ramorum* during the statewide survey of ornamental nurseries, surrounding forests and waterways. This technique has proven to be an asset in many other research projects investigating population diversity and ecology of *Phytophthora* species and routine diagnostic service.

Like all other characters (morphological, physiological and molecular), the PCR-SSCP has its own limitations and is far from perfection. It can be as sensitive as DNA sequencing; however, its sensitivity is limited to the target DNA fragment or choice of PCR primers. Strategies to differentiate those indistinguish-

Table 2.1. (continued)

Species	SSCP identifier ^a	Band location ^b		Morpho-group ^c	Phylogenetic clade ^d
		Upper	Lower		
<i>P. phaseoli</i>	Pha	6.9	7.8	IV	1c
<i>P. boehmeriae</i>	Boe	6.9	8.6	II	(8f)
<i>P. trifolii</i>	Tri	6.9	8.9	V	8a
<i>P. ramorum</i>	Ram	7.0	8.2	IV	(8d)
<i>P. megasperma</i> II	Meg II	7.2	8.5	V	...
<i>P. cryptogea</i>	Cry	7.2	8.5	VI	8a
<i>P. erythroseptica</i>	Ery	7.2	8.5	VI	8a
<i>P. porri</i>	Por	7.2	9.5	III	8b
<i>P. primulae</i>	Pri	7.2	9.5	III	8b
<i>P. infestans</i>	Inf	7.2	8.6	IV	1c
<i>P. mirabilis</i>	Mir	7.2	8.6	IV	1c
<i>P. quercina</i>	Que	7.3	8.5	I	3
<i>P. hibernalis</i>	Hib	7.4	7.9	IV	(8c)
<i>P. lateralis</i>	Lat	7.6	8.6	V	8a (8d)
<i>P. heveae</i>	Hev	7.7	10.2	II	5
<i>P. medicaginis</i>	Med	7.9	8.8	V	8a
<i>P. citrophthora</i>	Cip	7.9	9.0	II	2 (2a)
<i>P. botryosa</i>	Bot	8.0	8.6	II	2 (4)
<i>P. pseudosyringae</i>	Pss	8.1	8.4	III	...
<i>P. ilicis</i>	Ili	8.2	8.4	IV	3
<i>P. meadii</i>	Mea	8.2	8.6	II	(2a)
<i>P. colocasiae</i>	Col	8.2	8.9	IV	2 (2a)
<i>P. psychrophila</i>	Psy	8.4	8.6	IV	3
<i>P. citricola</i> II	Cil II	8.8	9.3	III	2 (2a)
<i>P. citricola</i> III	Cil III	8.9	10.3	III	2
<i>P. citricola</i> I	Cil I	9.3	10.3	III	2 (2a)
<i>P. tropicalis</i>	Tro	9.4	11.5	II	(2a)
<i>P. capsici</i>	Cap	9.6	11.1	II	2

^a The SSCP identity of individual species is named as described previously (Kong et al. 2003).

^b Measurements always are taken at a prominent band in case of a band set.

^c Morphogroups as designated previously (Waterhouse 1963).

^d Phylogenetic clades as designated by Cooke and associates (Cooke et al. 2000); modifications by Kroon and associates (Kroon et al. 2004) if present are listed in parentheses.

able species pairs with the current DNA fingerprints include (i) use of morphological characters, (ii) host specificity and chemical tolerance or requirement for growth, (iii) other molecular fingerprinting techniques, including restriction fragment length polymorphism (RFLP) and amplified fragment length polymorphism (AFLP) analyses of internal transcribed spacers (ITS) and/or mitochondrial DNA (Förster et al. 1989; Ristaino et al. 1998; Ivors et al. 2004; Martin and Tooley 2004), (iv) DNA sequencing (Förster et al. 1990; Cooke and Duncan 1997; Cooke et al. 2000; Martin and Tooley 2003; Kroon et al. 2004), and (v) identification of additional DNA fragments for SSCP analysis. For example, one can easily differentiate *P. cactorum* and *P. pseudotsugae* with the morphological key in Chapter 1. Similarly, one can distinguish *P. infestans* from *P. mirabilis* by their host specificity and responses to nitrate and hymexazol in growth media

(Galindo and Hohl 1985). A partial list of other molecular fingerprinting techniques that are complementary to the PCR-SSCP is provided in the appendixes. DNA sequencing has been increasingly used in identification and description of new species such as *P. ramorum* (Werres et al. 2001), *P. ipomoeae* (Flier et al. 2002), *P. brassicae* (Man in 't Veld et al. 2002), and *P. rubi* (Man in 't Veld 2007).

Users of this key must realize that all DNA fingerprinting techniques including the PCR-SSCP are only as good as the diversity and quality of isolates used during technical development. While using the power of PCR-SSCP in research and survey projects, we always select a small number of isolates representing different patterns, examine them morphologically to confirm their identities and minimize the risk of misidentification by fingerprint alone. It is advisable that users of this key exercise the same caution.

APPENDIXES TO CHAPTER 2

1. The PCR-SSCP Protocol

Primers

Forward (ITS6):

5'-GAA GGT GAA GTC GTA ACA AGG-3'

Reverse (ITS7):

5'-AGC GTT CTT CAT CGA TGT GC-3'

DNA amplification

Recipe of PCR mix for **one** reaction in 25 μ l volume:

Sterile nanopure H ₂ O	16.4 (μ l)
10 \times buffer	2.5
2 mM dNTPs	2.0
10 μ M ITS6F primer	1.0
10 μ M ITS7R primer	1.0
<i>Taq</i> polymerase at 5 U/ μ l (TaKaRa, Shuzo, Japan)	0.1
DNA template	2.0

Thermocycler:

Eppendorf Mastercycler Gradient

PCR program (Lid temp: 100°C):

2 minutes at 96°C

30 cycles of:

30 seconds at 94°C

45 seconds at 55°C

1 minute at 72°C

10 minutes at 72°C

Acrylamide (8%) gel electrophoresis

Recipe of acrylamide gel mix for two minigels:

5.9 ml of nanopure H₂O

2.0 ml of 5 \times TBE buffer (make sure there is no precipitate)

2.0 ml of 40% acrylamide:bis 29:1

100 μ l of 10% ammonium per sulfate (make fresh)

10 μ l of TEMED

Electrophoresis unit:

BioRad Mini PROTEAN 3 vertical system

Steps to prepare and run a gel:

1. Thoroughly clean glass plates with 70% ethanol. Make sure they are squeaky clean, with no grease spots.

2. Assemble the plates on a casting stand; have combs ready.
3. Quickly pipette acrylamide mix between glass plates, all the way to the top, immediately insert comb, check for bubbles and adjust comb as necessary to remove them.
4. Allow polymerization for a minimum of 60 minutes at room temperature; the plates can go into a refrigerator overnight.
5. During this time set up PCR products to be analyzed: generally mix 1 to 3 μ l of product with 9 to 7 μ l of a denaturing buffer (for 10 ml, mix 9.5 ml of superpure deionized formamide, 0.4 ml of 0.5M mM EDTA, and 0.005 g of bromophenol blue, aliquot 1 ml per tube; store at -20°C).
6. Also prepare DNA ladder by adding 2 μ l of diluted PhX174/*Hae* III (1:5) and 2 μ l of PCR product of *P. cinnamomi* amplified using primers ITS6 and ITS7 to 36 μ l of SSCP loading dye.
7. Set up gels in a vertical unit with chilled (4°C) 1 \times TBE buffer, to fill just under the base of the well.
8. Heat PCR samples at 95°C for 10 minutes (a thermal cycler can be used), immediately place them on ice to cool about 1 minute, and then load 1 to 3 μ l per lane (use a 2- μ l ladder on each outside lane).
9. Run 220V for 2 hours at room temperature (about 23°C).

Silver staining and gel documentation

1. Rinse the gel briefly in nanopure H₂O in a plastic Tupperware container to remove the gel from the glass plate.
2. Pour off the water carefully, add enough 10% ethanol to submerge the gel, and incubate with gentle agitation for 10 min.
3. During this time prepare silver stain by diluting 800 μ l of 100 \times stock (20% silver nitrate in nanopure water) in 80 ml of nanopure water (enough for two minigels).
4. Pour off the ethanol and add 1% nitric acid, shake gently, and incubate 3 min.

5. Pour off HNO_3 and rinse twice with nanopure water.
6. Add silver stain and incubate for 20 minutes.
7. During this time prepare developer by mixing 80 ml of 3% Na_2CO_3 with 80 μl of 37% formaldehyde; have 60-ml stop solution ready (10% glacial acetic acid).
8. When staining is complete, rinse the gel three times with nanopure water, then add a small volume (20 to 30 ml) of developer. When color change occurs, pour off and add remaining developer solution.
9. Gently agitate and watch for bands to develop. Once desired intensity has been reached **immediately** add 10% acetic acid to the container, mix thoroughly to fix, then pour off and rinse twice with nanopure water.
10. Photograph the gel with a digital camera or gel documentation system.

2. A Partial List of Other Molecular Fingerprinting Techniques

Other major molecular fingerprinting techniques employed in description of new species are either protein- or DNA-based. The protein techniques include allozyme and isozyme analyses. Listed under each of these techniques are the enzymes, followed by the name of a new species of *Phytophthora* identified and a respective reference. DNA techniques target the ribosomal internal transcribed spacers unless otherwise specified. Under each technique is a list of commonly employed restriction enzymes, followed by the name of a new species of *Phytophthora* identified and a reference.

Allozyme

- Glucose-6-phosphate isomerase (*Gpi*, EC 5.3.1.9)
P. ipomoeae (Flier et al. 2002)
P. rubi (Man in 't Veld 2007)
- Peptidase (*Pep*, EC 3.4.3.1)
P. ipomoeae (Flier et al. 2002)

Isozyme

- Aconitase (ACO, EC 4.2.1.3)
P. rubi (Man in 't Veld 2007)
- Isocitrate dehydrogenase (IDH, EC 1.1.1.42)
P. brassicae (Man in 't Veld et al. 2002)
P. multivesiculata (Ilieva et al. 1998)
P. rubi (Man in 't Veld 2007)
- Lactate dehydrogenase (LDH, EC 1.1.1.27)
P. brassicae (Man in 't Veld et al. 2002)
- Malate dehydrogenase (MDH, EC 1.1.1.37)
P. brassicae (Man in 't Veld et al. 2002)
P. multivesiculata (Ilieva et al. 1998)
P. ramorum (Werres et al. 2001)
P. rubi (Man in 't Veld 2007)

- Malic enzyme (MDHP, EC 1.1.1.40)
P. multivesiculata (Ilieva et al. 1998)
P. ramorum (Werres et al. 2001)
- Phosphogluconate dehydrogenase (PGD, EC 1.1.1.44)
P. rubi (Man in 't Veld 2007)

Restriction fragment length polymorphism (RFLP)

- Alu* I
P. pistaciae (Mirabolfathy et al. 2001)
P. quercina (Cooke et al. 1999)
- Msp* I
P. pistaciae (Mirabolfathy et al. 2001)
P. quercina (Cooke et al. 1999)
- Taq* I
P. pistaciae (Mirabolfathy et al. 2001)
P. quercina (Cooke et al. 1999)

Amplified fragment length polymorphism (AFLP)

- Eco*R I
P. ramorum (Werres et al. 2001)
- Msp* I
P. ramorum (Werres et al. 2001)

Mitochondrial DNA (mtDNA) haplotype

- P1 (digested with *Cfo* I)
P. ipomoeae (Flier et al. 2002)
- P2 (digested with *Msp* I)
P. ipomoeae (Flier et al. 2002)
- P3 (digested with *Eco*R I)
P. ipomoeae (Flier et al. 2002)
- P4 (digested with *Eco*R I)
P. ipomoeae (Flier et al. 2002)

CHAPTER 3

Morphological Characters and DNA Fingerprints of Individual Species

This chapter presents the morphological details and DNA fingerprints necessary to create the keys presented in Chapters 1 and 2. In this sense Chapter 3 is the heart of this publication. The morphological description is used to place a species in the Chapter 1 key and the DNA fingerprint is used to place a species in the Chapter 2 key. The reverse of this is also true; the descriptions and fingerprints, along with the photographs, back up the identifications made first by using the keys in Chapters 1 and 2.

We have searched diligently to find cultures of bona fide species, and have cultured those received to produce the various morphological stages. Thus, all photographs are of live stages without stains. The DNA for the SSCP fingerprint was extracted from live cultures, also. The morphological descriptions and photographs were always compared with those in the original description of a species.

All of the photographs, selected to show the morphology necessary for identification, were taken with the same camera using the same microscope. Most were taken with the 97 \times oil immersion objective or with the 43 \times dry objective. Thus, the sizes of the various organs can be compared among the species throughout this chapter. A rough determination of dimensions in μm can be made by multiplying the size in mm of the 97 \times photographs by a factor of 1.25. A 10- μm indicator bar is located on each photograph for assistance in size determinations.

A typical PCR-SSCP pattern is illustrated in Figure 3.1. Lanes A and D are single-strand DNA (ssDNA) ladders, consisting of fourteen markers (Kong et al. 2003; Kong et al. 2004b). Markers 5 and 6 are migration indicators of the conformations from the PCR product of *P. cinnamomi* incorporated in the ladder. These two, along with markers 1 to 4, 11 to 14, always are intense and sharp, whereas one or two other markers sometimes may be less intense, depending on

the electrophoresis conditions. Each of markers 1, 2, and 4 often appears in a band set of two. Markers 8 to 10 do the same but at less intensity. All markers are clearly indicated on the right ladder to aid in determining the migration pattern of DNA conformations of each species.

Two middle lanes (B and C) generally show the migration pattern of two key isolates as listed in the appendix at the end of this chapter. Exceptions were made for *P. cinnamomi* var. *parvispora*, *P. idaei*, *P. ilicis*, *P. iranica*, and *P. megakarya*. We found only one authentic isolate for each of these species and in these cases the two middle lanes show the migration pattern of the same isolate. The PCR-SSCP pattern of each species consists of upper and lower bands. Some may show in a band set of two like the upper bands in Fig-

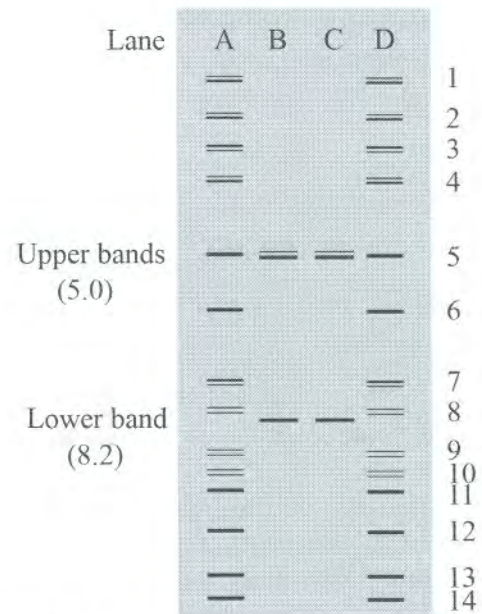


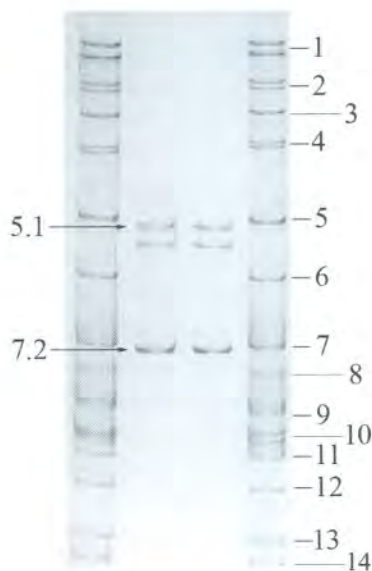
Figure 3.1. Illustration of a typical PCR-SSCP fingerprint of *Phytophthora* species.

ure 3.1. One of these two bands often is more intense than the other. Others also may appear in a single intense band as illustrated in Figure 3.1 (lower band), depending on the species and at a lesser degree the electrophoresis conditions. Locations of both upper and lower bands are measured against the ssDNA ladder. In case of band sets, the location is measured at the middle between the two bands. The locations of both bands characteristic of each species are indicated on the left of the gel picture for reference.

The descriptions and photographs of morphological characters, and the DNA fingerprint, should be helpful in identifying species using the dichotomous (Chapter 1) and DNA fingerprint (Chapter 2) keys. Though the Chapter 1 key uses a minimum of morphological traits, only those necessary for separation, the descriptions and photographs provide more detail. For still further detail one must go to Erwin and Ribeiro (1996), or to the original descriptions by the authorities if the species was named after 1996.

Phytophthora alni

Brasier and S. A. Kirk (2004)



P. alni SSCP fingerprint

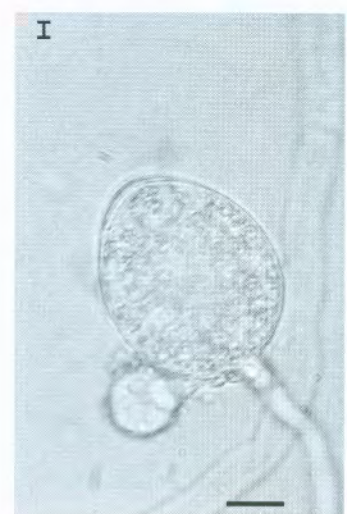
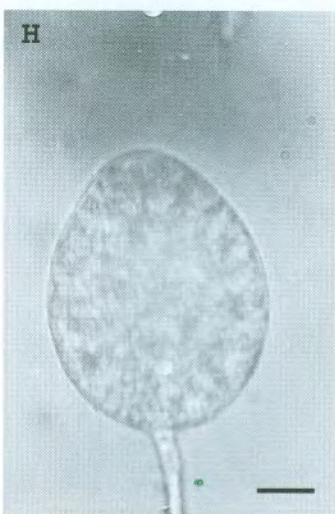
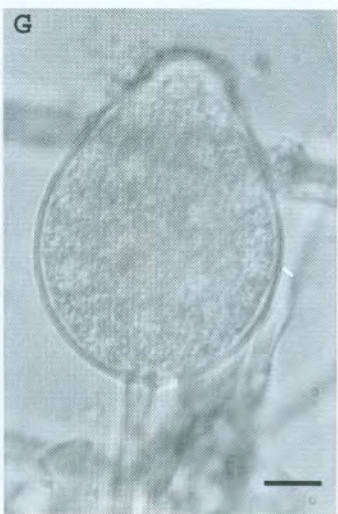
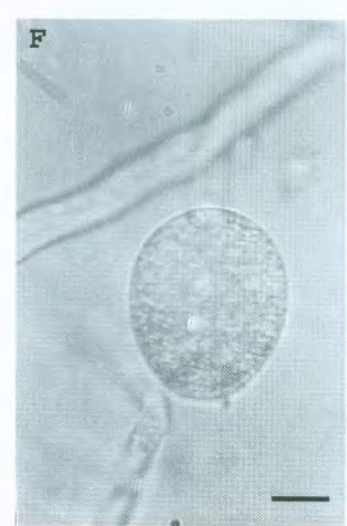
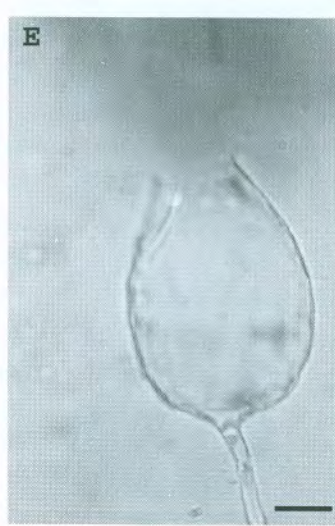
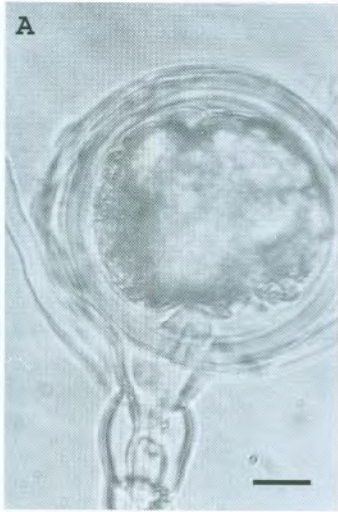
Brasier and Kirk described three subspecies when *Phytophthora alni* was named: *alni*, *uniformis*, and *multiformis* (Brasier et al. 2004). The *alni* subspecies was most prevalent, comprising 89% of 200 isolates examined. The *uniformis* variant was more uniform, as the name implies, whereas the *multiformis* variant was more variable. Molecular and chromosomal differences occurred as well. The *alni* variant was presumed to be an allopolyploid. The authors proposed that this species was a hybrid between *P. cambivora* and *P. fragariae*. *P. alni* is a destructive pathogen to alder (*Alnus* spp.) and is widespread in Europe. It is rather specific to alder. Only the *alni* subspecies is described here.

DESCRIPTION

This new species is a **homothallic** species, and this one has predominantly **amphigynous** antheridia. The oogonia are ornamented with wavy walls and some bullate protuberances like those of *P. cambivora*. They vary in diameter from about 41 to 50 μm . Oospores are **plerotic** and thick-walled (3 to 5 μm) and have diameters of about 37 to 48 μm . The oogonia have tapered stalks, and some are hook shaped. Antheridia measure about $20 \times 16 \mu\text{m}$ except for the long bicellular ones, which can be up to 35 μm long. Sporangia are **noncaducous** and **non-papillate**. They are borne singly on long sporangiophores with shapes variable from ovoid to obpyriform to ellipsoid. They average about $50 \times 40 \mu\text{m}$. The exit pore is broad. Nesting and internal proliferation occur. Chlamydospores and hyphal swellings have not been reported. The maximum temperature for colony growth is about 29°C.

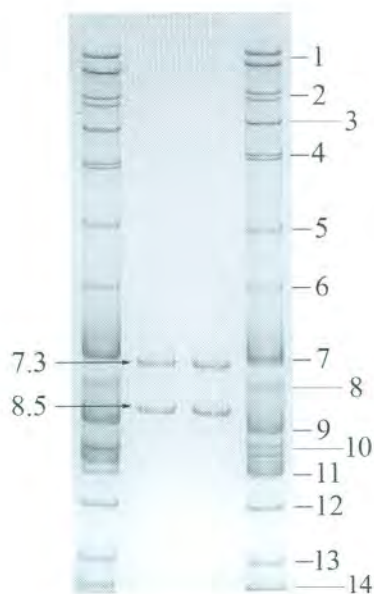
FIGURES (facing page)

- A–C Sex bodies with amphigynous antheridia, ornamented oogonia, and one septate antheridium.
- D Remains from nesting and internal proliferation.
- E An empty sporangium with a wide exit pore.
- F–I Sporangia varying in size.



Phytophthora quercina

T. Jung (1999)



P. quercina SSCP fingerprint

Phytophthora quercina has been associated with oak decline in central and southern Europe, according to Jung et al. (1999), who named the pathogen. It was isolated from necrotic fine roots of *Quercus robur* L., *Q. petraea* L., *Q. cerris* L., *Q. pubescens* Willd., and *Q. ilex* L. in sites throughout Germany, Hungary, Italy, and France (Jung et al. 1999). In pathogenicity tests, *P. quercina* was more pathogenic on *Q. robur* than any other species of *Phytophthora* isolated from declining oaks. *P. quercina* was recently reported in the Missouri Ozark forest in the United States (Schwingle et al. 2007).

DESCRIPTION

This **homothallic** species readily forms sexual bodies in single culture on lima bean and V8 juice agars. A characteristic of *P. quercina* is the bizarre shapes of oogonia, from globose to ovoid to ellipsoid and finally to markedly elongate. The oospores within oogonia with bizarre shapes are **aplerotic**. Size of oogonia average about 32 μm in diameter, but elongated ones may reach 52 μm in length. Oospores average about 27 μm in diameter and have walls about 2.5 μm thick. Antheridia are always **paragynous** and measure about 11 μm tangential and 14 μm perpendicular to the oogonial walls; they are located adjacent to the oogonial stalks. Sporangia are produced abundantly on agar disks in water and in small numbers on agar. They are **papillate**, **noncaducous**, and variable in shape from ovoid to ellipsoid to bluntly ellipsoid. When the papilla is on the side, sporangia may appear sickle-shaped. Sporangia are formed on simple and lax sympodia. The ellipsoid sporangia are usually large, about 65 \times 37 μm , whereas the ovoid ones measure about 45 \times 34 μm . Chlamydospores are both terminal and intercalary, averaging about 27 μm in diameter. Hyphal swellings have not been reported. *P. quercina* is distinguished by bizarre oogonial shapes with only paragynous antheridia, by highly variable shapes of sporangia, and by dome-shaped fluffy colonies on V8 juice agar. The maximum temperature for growth is <30°C.

FIGURES (facing page)

- A–C Sexual bodies with aplerotic oospores and paragynous antheridia.
- D Group of chlamydospores.
- E Intercalary and terminal chlamydospore.
- F Ellipsoid sporangium.
- G, H Ovoid sporangia.
- I Bluntly ellipsoid sporangium.

