Phytophthora quercina sp. nov., causing root rot of European oaks

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In a 3 year study of oak decline in Central and Southern Europe, a papillate homothallic *Phytophthora* species was isolated consistently, with other *Phytophthora* spp., from necrotic fine roots by direct plating on to selective agar medium and from rhizosphere soil samples by baiting with leaves of *Quercus robur*. The morphology, physiology, RAPD banding patterns and pathogenicity against apple fruits of this *Phytophthora* sp. are described and compared with those of other papillate *Phytophthora* species from Waterhouse's Group I, namely *P. cactorum*, *P. clandestina*, *P. idaei*, *P. iranica*, *P. pseudotsugae* and *P. tentaculata*, and semi-papillate Group III *P. citricola*. The papillate *Phytophthora* isolates from oak differed from all other Group I species by their uniform, dome-shaped and cottonwool-like colony growth pattern on V8 juice agar and malt extract agar, the frequent occurrence of sympodially branched primary hyphae, a high proportion of elongated, ellipsoid or ovoid oogonia, the absence of amphigynous antheridia and RAPD banding patterns. Additionally, there was no other species in Group I with as much variation in size and shape of the sporangia or large proportion of sporangia with a curved apex, hyphal projections, lateral displacement of the papilla and lateral attachment to the sporangiophore. In pathogenicity tests with infested soil, the isolates proved to be more pathogenic to *Q. robur* than any other *Phytophthora* sp. recovered from declining oaks in Central Europe. Based on their unique combination of cultural, sporangial and gametangial morphology, pathogenicity and close association with *Quercus* but not other trees, the papillate *Phytophthora* isolates from oak are described as *Phytophthora quercina* sp. nov.

Oak decline is a serious and frequently recurring threat to European forestry. Above-ground symptoms include dieback of branches and parts of the crown, formation of epicormic shoots, high transparency of the crown, yellowing and wilting of leaves and tarry exudates from the bark (Siwecki & Liese, 1991; Luisi, Lerario & Vannini, 1993), all symptoms indicative of water stress and poor nutrition. Roots of declining and healthy trees in 33 stands of *Quercus robur* L., *Q. petraea* (Mattuscka) Liebl., *Q. cerris* L., *Q. pubescens* Willd. and *Q. ilex* L. in Germany, Switzerland, Hungary, Slovenia, Italy and France were examined for the presence of *Phytophthora* species. For the first time in Central Europe, several *Phytophthora* species including *P. citricola*, *P. cactorum*, *P. cambivora*, *P. gonapodyides* and *P. undulata* and an unidentified species, *Phytophthora* sp. 2, were isolated from root and soil samples of most stands investigated. Another unknown but papillate *Phytophthora* species, possessing a unique combination of sporangial, gametangial and vegetative characters was also isolated frequently from necrotic fine roots and rhizosphere soil containing necrotic fine roots of all five oak species from sites throughout Germany, Hungary, Italy (Blaschke & Jung, 1996; Jung 1996; Jung, Blaschke & Neumann, 1996) and France (Jung, unpublished) but not Slovenia and Switzerland. This species was assigned to Group I of *Phytophthora* (Stamps *et al*., 1990) by Jung *et al*. (1996).

This paper describes this last species as *Phytophthora quercina* sp. nov. and compares its morphology, physiology and pathogenicity to apple fruits with that of other Group I *Phytophthora* spp. (Stamps *et al*., 1990; Kröber & Marwitz, 1993; Kennedy & Duncan, 1995; Cacciola, Magnano Di San Lio & Belisario, 1996). The pathogenicity to oak seedlings of this and all other *Phytophthora* species recovered from declining oak stands in Central Europe was tested by a soil infestation test. Molecular evidence is used increasingly to sustain the specific status of new species in *Phytophthora* (Hamm & Hansen, 1983; Kennedy & Duncan, 1995; Cacciola *et al*., 1996). Randomly amplified polymorphic DNAs (RAPDs) have proven to be valuable in *Phytophthora* in their ability to distinguish among isolates of closely related species and subspecies (Cooke *et al*., 1996). A full analysis of RAPDs and ITS1 and ITS2 sequences of *P. quercina* and other Group I Phytophthoras is published concurrently (Cooke *et al*., 1999).

MATERIALS AND METHODS

Phytophthora *isolates examined*

Details of all isolates examined or tested are given in Table 1. The *P. quercina* isolates were obtained from five European oak species growing at eight sites in Germany, Italy and Hungary. Ten of them were compared morphologically and culturally

Table 1. Species and isolates of *Phytophthora* spp. studied

	Group*	IFB-no.†	Other references†	Origin	Host	Source†
P. quercina	I	QUE I	CBS 783.95	Germany	*Quercus robur*	IFB
T. Jung	I	QUE 2		Germany	*Q. robur*	IFB
	I	QUE 3	CBS 784.95	Germany	*Q. robur*	IFB
	I	QUE 4	CBS 782.95	Germany	*Q. robur*	IFB
	I	QUE 5	CBS 788.95	Germany	*Q. robur*	IFB
	I	QUE 6	CBS 789.95	Germany	*Q. cerris*	IFB
	I	QUE 7	CBS 781.95	Hungary	*Q. petraea*	IFB
	I	QUE 8	CBS 785.95	Italy	*Q. ilex*	IFB
	I	QUE 10	CBS 787.95	Italy	*Q. pubescens*	IFB
	I	QUE 13		Italy	*Q. pubescens*	IFB
P. cactorum	I	CAC 1		Germany	*Q. robur*	IFB
(Lebert & Cohen)	I	CAC 2		Germany	*Q. petraea*	IFB
J. Schröt.	I	CAC 3472	TU-3472	Germany	*Fragaria vesca*	V. Zinkernagel, TU
	I	CAC 4810	TU-4810	Germany	*Malus domestica*	V. Zinkernagel, TU
	I	CAC 23	SCRI-CAC 23	England	*M. domestica*	SCRI
	I	CAC 30	SCRI-CAC 30	England	*Ribes uva-crispa*	SCRI
	I	CAC 33	SCRI-CAC 33	England	*M. domestica*	SCRI
P. clandestina P.A. Taylor, Pascoe & F.C. Greenh.	I	CLA 2	IMI 287317 (SCRI-CLA 2)	Australia	*Trifolium subterraneum*	P. A. Taylor‡
P. idaei	I	IDA 1	SCRI-IDA 1	Scotland	*Rubus idaeus*	SCRI
D. M. Kenn.		IDA 3	SCRI-IDA 3	Scotland	*Rubus idaeus*	SCRI
		IDΛ 4	SCRI-IDA 4	England	*R. idaeus*	SCRI
P. iranica Ershad	I	IRA 1	IMI 158964	Iran	*Solanum melongena*	D. Ershad[3]
P. pseudotsugae Hamm & E. M. Hansen	I	PSE I	SCRI-PSE 1	USA	*Pseudotsugae menziesii*	P. Hamm‡
P. tentaculata Kröber & R. Marwitz	I	TEN I	CBS-552.96	Germany	*Chrysanthemum leucanthemum*	H. Kröber¶
P. citricola	III	CIT 9		Germany	*Q. robur*	IFB
Sawada	III	CIT 11		Germany	*Q. robur*	IFB
	III	CIT 30		Italy	*Q. robur*	IFB
	III	CIT 35		Slovenia	*Q. petraea*	IFB
	III	CIT 40		Germany	*Q. petraea*	IFB
P. ilicis Buddenh.	IV	ILI 1		Great Britain	*Ilex aquifolium*	IMI
Phytophthora sp. 2	IV	*P.* sp2/1	CBS 803.95	Germany	*Q. robur*	IFB
P. cambivora	VI	CAM 3		Germany	*Q. robur*	IFB
P. cambivora	VI	CAM 3		Germany	*Q. petraea*	IFB
(Petri) Buisman	VI	Cam 5		Germany	*Q. petraea*	IFB
P. gonapodyides (H. E. Petersen) Buisman	VI	GON 13		Germany	*Q. robur*	IFB
P. undulata (H. E. Petersen) M. W. Dick	VI	UND 5		Germany	*Q. petraea*	IFB

* Groups according to Stamps *et al.* (1990).

† IFB, = Institute of Forest Botany, Phytopathology, University of Munich, Freising; TU, Technical University of Munich, Institute of Phytopathology, Freising; SCRI, Scottish Crop Research Institute, Dundee; IMI, International Mycological Institute, Egham, Surrey; CBS, Centraalbureau voor Schimmelcultures, Baarn.

‡ Original source, cultures supplied by IMI.

¶ Original source, culture supplied by CBS.

with isolates of other Group I species and with isolates of *P. citricola* from Group III (see Tables 1–4). Isolates of *P. quercina* and *P. citricola*, together with isolates of *P. cambivora*, *P. gonapodyides* and *P. undulata* from oak, were also tested for their pathogenicity to oak (Table 5).

Isolation methods

Isolations of *Phytophthora* spp. from necrotic fine roots of oak were made at 20 °C by direct plating on to a selective agar medium (Tsao, 1983) PARPNH (V8A, see below, amended with 10 μg ml^{-1} pimaricin, 200 μg ml^{-1} ampicillin, 10 μg ml^{-1} rifampicin, 25 μg ml^{-1} PCNB, 50 μg ml^{-1} nystatin, and 50 μg ml^{-1} hymexazol) as described by Jung *et al.* (1996) or by using apple traps (Brasier & Strouts, 1976). In addition, soil samples containing necrotic fine roots were flooded to a depth of 3 cm with demineralized water and baited with 2–5 d-old leaves of *Q. robur* seedlings floated on the surface of the water. Apple (cv. Cox's Orange Pippin) or pear fruits (various cvs) were also used as baits. In both cases, isolations were made from the leaflets and fruits on to PARPNH as described above (Jung *et al.*, 1996).

Morphology and physiology

Colony morphology and growth rate. Isolates were grown at 20° on V8-juice agar (V8A – 16 g agar, 3 g $CaCO_3$, 100 ml V8 juice (Campbell's) and 900 ml distilled water), Sigma malt-extract agar (MEA), Sigma cornmeal agar (CMA) and Sigma potato-dextrose agar (PDA). Petri dishes (9 cm diam.) con-

taining 16 ml of the test media were inoculated with 5–7 mm diam. discs cut from the edge of a 5–10-d-old culture. The discs were placed upside down in the centre of each plate, and the plates were incubated in the dark. Colony morphology on V8A, MEA and CMA was noted after 7 d, and on PDA after 14 d. Growth rate measurements were made after the onset of growth along two lines intersecting at right angles at the centre of the inoculum (Hall, 1993). Growth rate (mm d^{-1}) was recorded on all media after 5 d and, in the case of slow growing species on PDA, additionally after 10 d. For temperature–growth relationships, V8A plates were incubated for 24 h at 20° to stimulate onset of growth, and then transferred to 5, 10, 15, 17·5, 20, 25, 27·5, 30 and 35°. Growth rate was recorded 5 d after the onset of linear growth (Kröber, 1985), and an additional measurement was made after 10 d in the case of slow growing isolates at 5 and 10°. Tests were repeated twice using three replicate plates per isolate.

Sporangia. One disc (15 mm diam.), cut from the growing edge of a 5–7-d-old culture grown on V8A at 20° in the dark, was placed in a 5 cm Petri dish and flooded, just over its surface, with non-sterile soil extract water (200 g soil from a *Q. robur* stand suspended in 1 l de-ionized water for 24 h at room temperature and then filtered). After incubation at 20° in the dark for 24–72 h, dimensions and characteristic features of 50 fully mature sporangia and diameters of 25 sporangiophores and encysted zoospores, chosen at random, were determined at × 400 magnification for each isolate.

Oogonia, oospores and antheridia. Measurements were made at × 400 magnification at the surface of 15 mm discs cut from the centre of 14–21-d-old MEA cultures grown in the dark. *P. clandestina* did not produce gametangia on MEA and 21-d-old V8A cultures were used. The diameters of main hyphae were recorded at the growing edge of 5-d-old MEA cultures. For each isolate 50 oogonia, oospores and antheridia and 25 main hyphae chosen at random, were measured.

Sensitivity to malachite green. Malachite green was added to CMA at 125 µg ml^{-1}. Growth rate was recorded 5 d after onset of growth and expressed as a percentage of the unamended CMA controls. This and the other growth tests that follow were carried out at 20° in the dark according to Kennedy & Duncan (1995). Tests were repeated twice using three replicate plates per isolate.

Sensitivity to hymexazol. Stock solutions with different concentrations of hymexazol (HMI) (Sankyo Chemical Company, Tokyo, Japan) were added to V8A to give final concentrations ranging from 10 to 500 µg ml^{-1}. The control received only sterile water. Growth rate was recorded 5 d after onset of growth and expressed as percentage of the unamended V8A controls.

Ability to use nitrate as sole nitrogen source. Asparagine (P3) and nitrate (P4) agar media (Hohl, 1975) were inoculated with 5 mm diam. discs of V8A taken from the edge of growing cultures. Growth rate was recorded 10 and 15 d after inoculation.

Pigment production. Isolates were grown on casein hydrolysate tyrosine (CHT) agar (Shepherd, 1976) and pigment production recorded after 28 d.

Pathogenicity

Pedunculate oak. Pathogenicity of *P. quercina* and other *Phytophthora* species isolated from oak to roots of *Q. robur* was determined using a soil infestation test according to Matheron & Mircetich (1985) and Jung *et al*. (1996). A 4–6-wk-old vermiculite/oat grain/V8 juice inoculum was mixed with an autoclaved soil mixture (1:1:1, v/v/v of peat, vermiculite and sand) at the rate of 20 ml of inoculum per litre of soil. Controls received only rinsed uninfested vermiculite/oat grain/V8 juice mixture at the same rate. Twelve, 2–3-mo-old *Q. robur* seedlings were planted into plastic tubs (150 × 300 × 250 mm) containing 9 l of infested soil mixture. The tubs (two per isolate) were kept in a greenhouse at 18–22° and 65 % r.h. and were flooded every 2 wk for 48 h. After 4 mo, above ground symptoms were recorded, and the amount of root rot on each seedling was estimated visually. Transverse and longitudinal sections of necrotic tissues stained with lactophenol blue were examined under the light microscope for the presence of oospores and non-septate, coralloid hyphae. Re-isolations of *Phytophthora* spp. from diseased tissues were made on PARPNH.

Apple. To test the pathogenicity of *P. quercina* and a series of other *Phytophthora* species to apple fruits, 5 mm diam. cores were cut aseptically from apple fruits of cv. Cox's Orange Pippin (2 inoculations per apple, 3 apples per isolate), and 0·5 cm V8A discs from the edge of 5-d-old cultures were placed in the holes. The cores were replaced, covered with moist cotton, and sealed with adhesive tape. After 7 d incubation at 20° in the dark, the apples were cut open longitudinally and the amount of rot observed.

RESULTS

Isolations

P. quercina was consistently isolated from necrotic fine roots by direct plating on to PARNPH and from rhizosphere soil by the oak-leaf baiting method. All isolates grew very slowly on the selective agar. All attempts to isolate *P. quercina* from necrotic fine roots or from soil using apples or pears as baits were unsuccessful. In contrast, *P. cambivora*, *P. citricola*, *P. gonapodyides* and *P. undulata* were isolated with all isolation methods used. *P. cactorum* and *Phytophthora* sp. 2 were isolated from soil using *Q. robur* leaves as baits only rarely (each twice from 33 stands).

Morphology and physiology

To avoid lengthy descriptions of many morphological and physiological features of all species belonging to Group I, a full description of *P. quercina* only is given, based on the 10 isolates examined. Individual measurements for the holotype are given when appropriate alongside the means for all 10

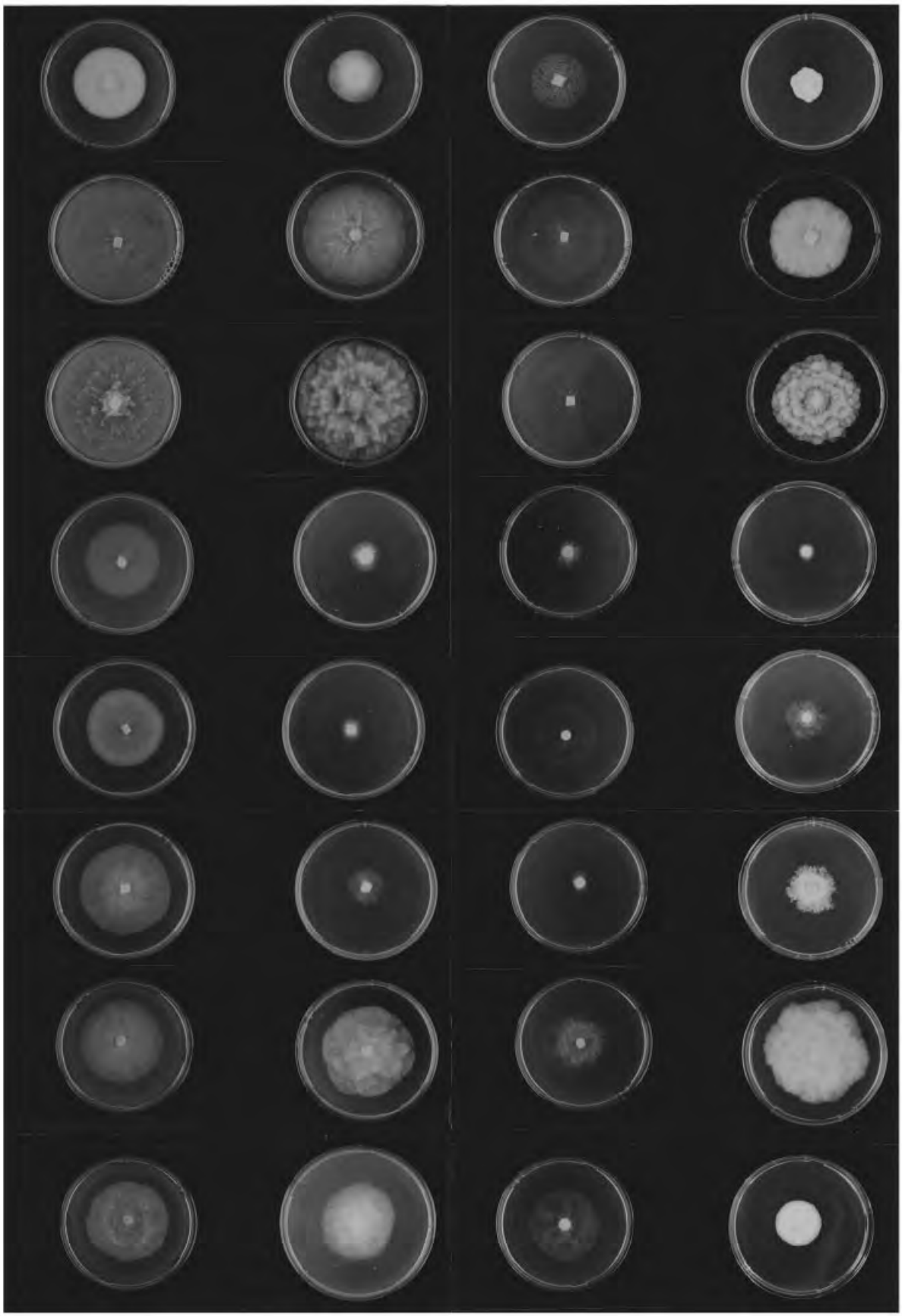
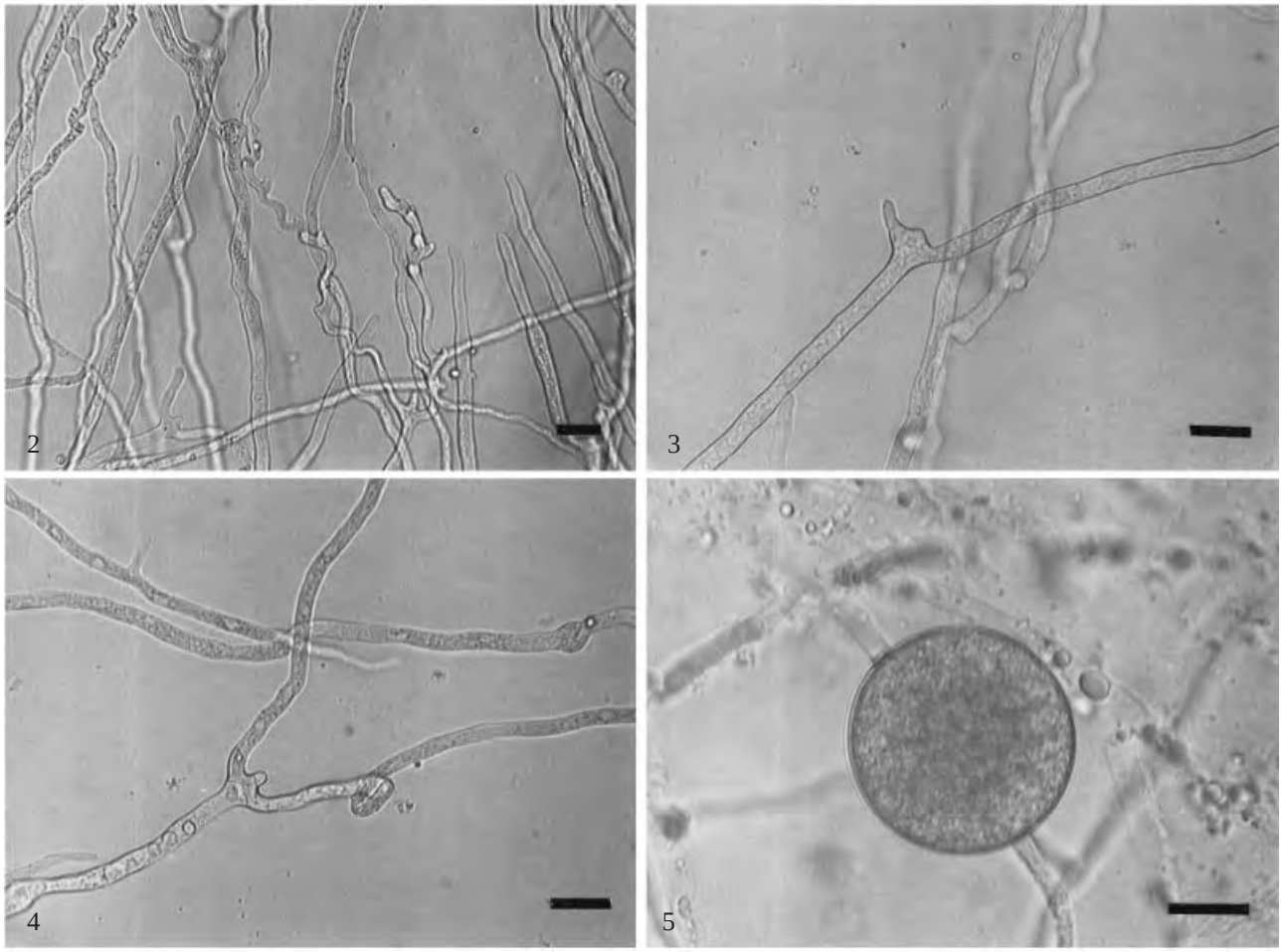


Fig. 1. Colony morphology of *Phytophthora* spp. at 20° on V8A, MEA and CMA after 7 d, and on PDA after 14 d (from left to right). From the top: *P. quercina, P. cactorum, P. citricola, P. clandestina, P. idaei, P. iranica, P. pseudotsugae, P. tentaculata.*



Figs 2–5. Hyphae and chlamydospores of *P. quercina* on V8A. Bars, 15 µm. **Fig. 2.** Primary hyphae at the hyphal fringe showing sympodial branching and undulating growth. **Figs 3–4.** Sympodial branching of primary hyphae. **Fig. 5.** Chlamydospore.

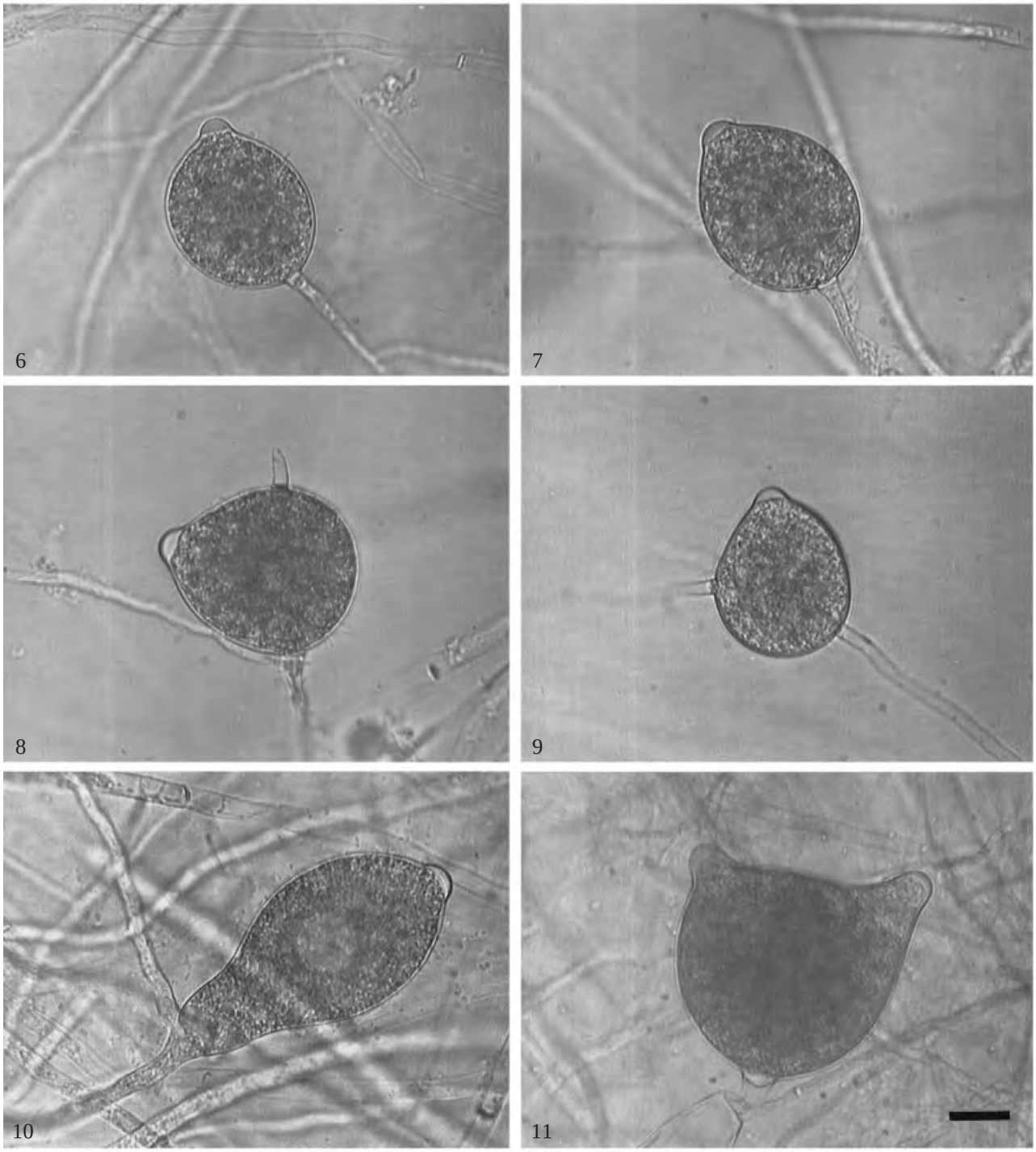
isolates. A formal Latin diagnosis, based on the holotype, is given later without English translation as all details pertaining to the holotype are in the following full description. For the other species, only the characteristics discriminating them from *P. quercina* are given. Colony growth patterns are shown in Fig. 1 and described in Table 4. All other data are presented in Tables 2–4.

Phytophthora quercina

Colony and hyphal morphology. Colonies of all 10 isolates, including holotype, were uniform without distinct growth patterns on all four test agar media, dome-shaped and fluffy on V8A and MEA, appressed dense-felty and dome-shaped on PDA and with sparse aerial mycelium on CMA (Fig. 1). Colonies on V8A and MEA became appressed as they aged, but remained dome-shaped. Primary hyphae on MEA were 3·8–9·2 µm wide (average 6·2 µm) (Table 2). Terminal, sympodial branching (monochasium or dichasium) with the mother hypha ending in a short protuberance (Figs 2–4) was common on all agar media. Hyphae were sometimes undulate (Fig. 2).

Sporangiophores. Simple or forming irregular lax sympodia, 1·5–5·8 µm diam. (av. 3·2 µm), sometimes wider near the point of attachment to the sporangium (Fig. 10). Length was variable and nodal swellings were infrequently observed. Lateral attachment of sporangia was noted in 22–80% (av. 49%) of cases (Figs 8, 9, 12, 13).

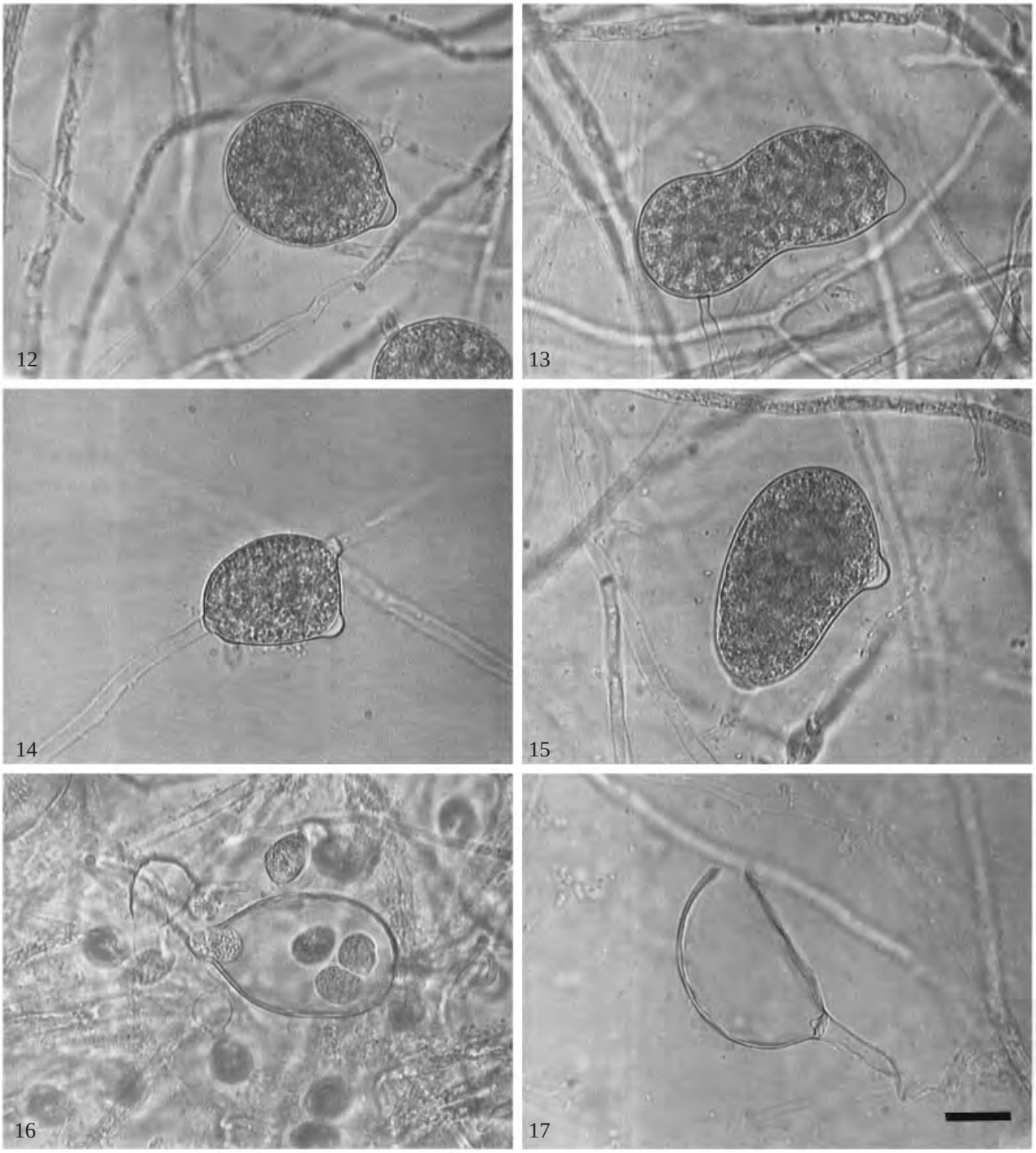
Sporangia. Produced in small numbers on solid agar and abundantly in liquid culture. Generally, they were terminal but occasionally also intercalary (Fig. 14). Sporangia were non-caducous, papillate or rarely bipapillate (Fig. 11) with a papillum depth of 2·3–6 µm (av. 3·4 µm) (holotype 2·7–5·6, av. 3·5 µm). Sometimes they had a conspicuous basal plug (Fig. 17). Sporangia were variable in size and shape, 18·8–112·5 × 13·8–47·5 µm (overall av. 42·4 × 29·3 µm) (holotype 21–70 × 20·4–40·8, av. 41 × 31·7 µm) with a range of averages for individual isolates from 36–49·2 (length) × 23·3–33·7 µm (breadth), and an average length to breadth ratio (l/b) of 1·45 (isolate avs 1·26–1·78, holotype 1·29) (Table 2). Sporangial shapes ranged from sub-globose, ovoid and obpyriform to ampulliform, banana- or peanut-like distorted shapes (Figs 6–17). Sporangia with markedly curved apices were common in all isolates (average occurrence 31%) (Figs 13, 17), and some sporangia had a lateral displacement of the papilla (Fig. 15). Occasionally sporangia produced short hyphal projections (Figs 8, 9). Zoospores were discharged through an exit pore 4·2–9·6 µm wide (av. 6·5 µm) (holotype 4·6–8·3, av. 7·1 µm).



Figs 6–11. Sporangia of *P. quercina* in liquid culture. Bar, 15 µm. **Figs 6–7.** Ovoid terminal sporangia. **Figs 8–9.** Ovoid terminal sporangia, laterally attached with a short hyphal projection. **Fig. 10.** Ampulliform sporangium, sporangiophore widening to the point of attachment. **Fig. 11.** Bipapillate sporangium.

They were limoniform to reniform (Fig. 16), 8·3–14·2 × 5·9–9·6 µm (av. 11·5 × 7·9 µm) (holotype 8·9–12·7 × 6·3–8·5, av. 11·1 × 7·4 µm) whilst motile, becoming spherical, 7·1–12·9 µm (av. 9·5 µm) diam. (holotype 7·1–11·7, av. 9·1 µm), on encystment. Direct germination of sporangia was only rarely observed.

Oogonia. Formed readily in single culture (Figs 18–28) and in the cortex of necrotic fine roots of oak (Fig. 29). In culture they were borne terminally. Oogonial shapes ranged from spherical (Figs 18–21) to ovoid and ellipsoid, 45 % (isolate avs 10–86 %) being markedly elongated as though assuming the shape of a host cell (Figs 22–28). On MEA they were 19–45 µm diam. (av. 29·4 µm) (holotype 23·5–41, av. 31·8 µm) with isolate averages ranging from 25·8 to 32 µm (Table 2). Some of the elongated oogonia reached a length of 52 µm. Oogonial walls were smooth and ranged in thickness from 0·5–2·1 µm (av. 1·4 µm).



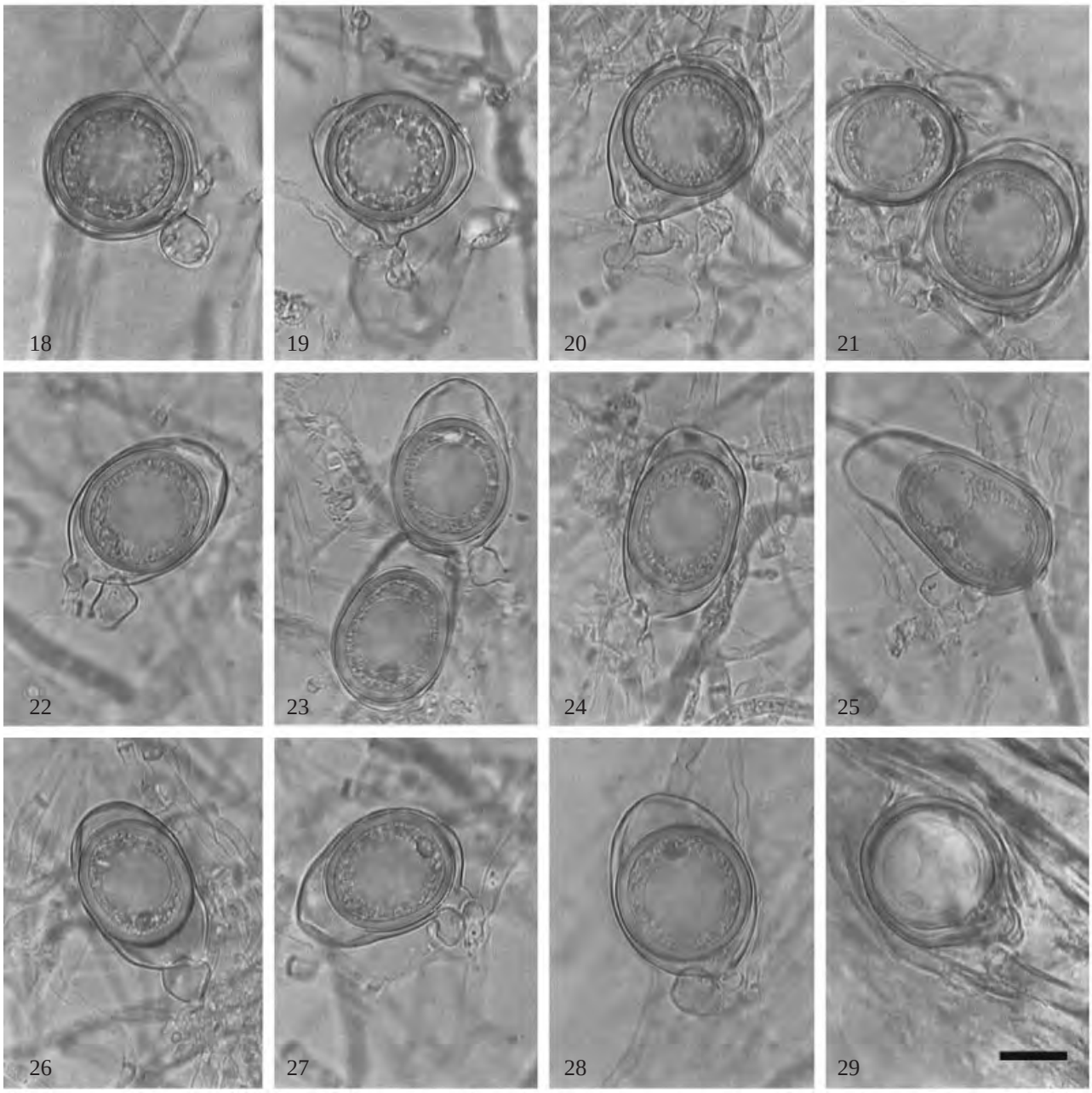
Figs 12–17. Sporangia of *P. quercina* in liquid culture. Bar, 15 µm. **Fig. 12.** Ovoid sporangium, laterally attached. **Fig. 13.** Peanut-like sporangium with a curved apex, laterally attached. **Fig. 14.** Intercalary sporangium. **Fig. 15.** Ellipsoid terminal sporangium, lateral displacement of the papilla. **Fig. 16.** Sporangium releasing zoospores. **Fig. 17.** Empty sporangium with a curved apex and a conspicuous basal plug.

Oospores. Spherical to ovoid, 17·9–38 µm (av. 26·6 µm) diam. (holotype 22·5–37, av. 29·4 µm), markedly aplerotic and thick-walled (0·8–5 µm; av. 2·5 µm) (holotype 1·5–5, av. 2·9 µm) (Table 2). Older oospore walls often turned golden-yellow.

Antheridia. Antheridia were hyaline, single, terminal, and spherical or club-shaped to irregular 8·3–26 × 6·3–15 µm (av. 14·8 × 9·5 µm) (holotype 10–19·1 × 7·5–12, av. 14 × 9·9 µm) (Table 2). They were always paragynous, usually inserted near the oogonial stalk.

Chlamydospores. Occasionally produced by some isolates on MEA, spherical, terminal or intercalary, 17–35 µm diam.

Growth rates and cardinal temperatures. Growth tests are summarized in Tables 3 and 4. Colonies grew moderately slowly on V8A, MEA and CMA with fastest growth on V8A



Figs 18–29. Oogonia and oospores of *P. quercina* with paragynous antheridia on MEA. Bar, 15 µm. **Figs 18–21.** Globose to subglobose oogonia. **Figs 22–28**. Ovoid to ellipsoid, elongated oogonia. **Fig. 29.** Oogonium in necrotic cortical tissue of a fine root of *Q. robur*.

and slowest on PDA. The growth rate on V8A at 20° was the slowest of all Group I species tested. Growth on V8A occurred at 5–27·5° with an optimum near 25° (radial growth rate 3·3 mm d^{-1}) (holotype 3·7 mm d^{-1}). No growth occurred at 30° and most isolates (including holotype) showed no re-growth when returned to 20° after 10 d at 30°.

Other characters. Malachite green reduced the growth rate on CMA to 71% compared to the unamended control (Table 4). *P. quercina* was slightly sensitive to HMI at 50 µg ml^{-1} and growth was reduced to 79% of the control. Nine isolates (including holotype) grew moderately well at 250 µg ml^{-1} and seven (including holotype) were able to grow at 500 µg ml^{-1} (Table 4). With increasing concentration of HMI, the duration of the lag phase also increased. All 10 isolates were able to use nitrate as sole nitrogen source (Table 4). They also produced a black pigment on CHT agar (Table 4).

Major differences from other Group I species

In the following short descriptions, only the differences between *P. quercina* and each species are mentioned and all comparatives apply to *P. quercina* and the relevant species. All except *P. tentaculata* had different growth patterns on all four media (Fig. 1, Table 4).

P. cactorum. Sympodial branching of primary hyphae was never observed. Sporangia were produced in higher numbers

Table 2. Morphological characters of *Phytophthora* species and isolates

Character	*P. quercina* (10 isolates)	*P. cactorum* (6 isolates)	*P. clandestina* (1 isolate)	*P. idaei* (2 isolates)	*P. iranica* (1 isolate)	*P. pseudotsugae* (1 isolate)	*P. tentaculata* (1 isolate)	*P. citricola* (5 isolates)
Primary hyphae								
Width (µm)	6·2	6·2	6·3	6·2	5·9	7·1	5·2	5·2
Range (µm)	3·8–9·2	4·2–8·8	3·8–11·7	3·8–8·8	4·2–8·8	5·4–10·0	4·2–6·9	2·5–7·5
Sympodial branching	+	−	−	−	−	(+)	−	−
Sporangia								
Average length (µm)	42·4	39·0	55·3	38·8	39·8	28·4	50·0	47·0
Average breadth (µm)	29·3	28·8	32·3	28·6	27·2	25·0	35·4	33·0
Range length (µm)	18·8–112·5	23·2–56·7	31·3–96·3	25·8–59·2	27·5–56·7	22·1–37·5	31·3–83·3	27·5–100·0
Range breadth (µm)	13·8–47·5	16·7–41·0	20·8–47·1	21·3–42·9	13·8–39·6	18·3–33·3	23·3–48·8	16·7–43·8
Isolate averages								
Isolate length (µm)	36·0–49·2	34·9–41·9		37·1–40·4				39·6–53·0
Isolate breadth (µm)	23·3–33·7	25·6–30·6		28·1–29·0				28·9–38·2
Length: breadth ratio	1·45 : 1	1·35 : 1	1·71 : 1	1·36 : 1	1 : 1	1·14 : 1	1·41 : 1	1·43 : 1
Isolate averages (µm)	1·26–1·78 : 1	1·30–1·40 : 1		1·32 : — 39 : 1				1·25–1·61 : 1
Shape	Ovoid-subglobose, obpyriform	Ovoid-subglobose	Obpyriform-ovoid	Ovoid-subglobose	Obpyriform-ovoid	Subglobose-globose	Ovoid-obturbinate, obpyriform	Ovoid-obpyriform
Distorted shapes	+	−	+	(+)	+	−	+	+
Curved apex (%)	31	10	32	28	11	2	36	12
Hyphal projections	+	(+)	+	(+)	+	(+)	(+)	(+)
Papilla	Papillate	Papillate	Papillate	Papillate	Papillate	Papillate	Papillate	Semipapillate
Average depth (µm)	3·4	3·8	3·4	3·9	3·7	3·2	4·7	2·1
Range (µm)	2·3–6·0	2·3–6·0	2·3–5·6	2·3–6·3	2·7–5·2	2·3–4·8	3·1–6·4	1·0–3·3
Isolate averages (µm)	3·1–3·8	3·4–4·2		3·6–4·3				1·8–2·4
Caducity	−	+	+	−	−	−	−	−
Sporangiophores	Irregular, lax, sympodium	Regular, compact sympodium	Irregular, lax sympodium	Irregular, lax, sympodium	Irregular, lax sympodium	Unbranched or lax sympodium	Irregular, lax sympodium	Irregular, lax sympodium
Lateral attachment (%)	49	13	18	16	26	34	22	19
Oogonia								
Average diam. (µm)	29·4	30·3	30·2	30·3	27·8	31·6	27·9	28·0
Range (µm)	19·0–45·0	18·0–41·3	25·8–35·0	28·3–50·0	23·8–31·3	25·4–37·1	22·5–33·3	15·0–37·5
Isolate averages (µm)	25·8–32·0	27·1–33·9		37·1–38·1				26·5–29·9
Markedly elongated oogonia	+	−	+	(+)	−	−	−	−
Oospores								
Average diam. (µm)	26·6	26·2	25·4	25·4	23·9	26·9	23·9	25·8
Range (µm)	17·9–38·0	17·5–33·3	22·1–30·0	18·3–32·5	20·8–27·5	22·5–31·3	19·2–28·3	14·0–35·8
Isolate averages (µm)	24·1–29·4	24·0–28·5		24·9–25·9				24·7–27·2
Oosporewall								
Average diam. (µm)	2·46	1·60	2·22	1·34	1·43	1·46	1·35	1·39
Range (µm)	0·8–5·0	0·8–2·5	0·8–4·2	0·6–2·1	0·6–2·5	0·4–2·9	0·8–1·9	0·8–2·5
Isolate averages (µm)	2·14–2·88	1·45–1·81		1·31–1·37				1·01–1·53
Antheridia	Paragynous	Mainly paragynous	Mainly amphigynous	Mainly paragynous	Mainly paragynous	Mainly paragynous	Mainly paragynous	Paragynous
Average length (µm)	14·8	12·9	15·8	14·3	12·8	15·2	15·5	12·2
Average breadth (µm)	9·5	10·1	15·6	12·2	11·4	11·6	12·1	9·1
Range length (µm)	8·3–26·0	8·8–22·0	11·3–20·0	7·9–20·4	10·0–17·5	9·6–21·3	10·0–21·7	7·5–25·0
Range breadth (µm)	6·3–15·0	7·1–15·0	12·1–19·6	6·7–17·9	8·3–15·0	7·5–18·8	8·3–19·2	6·3–16·0
Isolate averages								
Isolate length (µm)	11·2–17·3	10·5–16·2		13·9–14·6				10·9–14·8
Isolate breadth (µm)	8·5–10·3	8·3–11·4		11·7–12·6				8·2–10·3

+, feature occurring frequently; (+), feature occurring infrequently; −, feature not observed.

Table 3. Radial growth rate of *Phytophthora* isolates on V8-Agar at different temperatures (growth rate at optimum temperature shown in bold)

	Average radial growth rate (mm d^{-1}) at								
	5°	10°	15°	17·5°	20°	25°	27·5°	30°	35°
P. quercina	0·3	1·4	2·3	2·8	3·3	**3·7**	2·5	0	0
P. cactorum	1·3	3·1	4·8	5·7	6·4	7·4	**7·5**	6·8	0
P. clandestina	0·9	1·9	3·1	3·6	**4·3**	3·3	3·0	2·4	0
P. idaei	1·7	2·9	3·5	3·9	**4·5**	2·2	0	0	0
P. iranica	0	0	1·5	3·1	5·1	**5·3**	5·0	4·9	0
P. pseudotsugae	1·0	2·3	3·5	3·6	**4·9**	3·2	2·6	1·5	0
P. tentaculata	0·7	1·6	2·9	3·6	4·0	**4·9**	4·3	3·5	0
P. citricola	1·3	3·8	5·9	7·3	8·1	9·3	**9·5**	7·1	0

on agar, were borne on compact regular sympodia and were caducous. They were more uniform in shape and size and distorted sporangia with more than 1 papillum or inserted intercalary were lacking. Only 10% of sporangia had a curved apex or a lateral attachment of the sporangiophore (Table 2). *P. cactorum* did not produce markedly elongated oogonia. Oospore-walls were thinner and some amphigyny occurred in all 6 isolates (Table 2). Isolates grew markedly faster at all temperatures (Table 3) and also had higher optimum and maximum temperatures for growth. Furthermore, all produced a yellow pigmentation on CHT agar (Table 4).

P. clandestina. Sympodial branching of hyphae was rare and without protuberances but subspherical and deltoid hyphal swellings were formed at branching points and along the hyphae. Fewer sporangia were formed on agar with a lower proportion laterally attached. Distorted sporangial shapes were absent and sporangia had conspicuous and protruding

Table 4. Results of growth tests with *Phytophthora* isolates on various agar media at 20°.

No. of isolates		*P. quercina* 10	*P. cactorum* 6	*P. clandestina* 1	*P. idaei* 2	*P. iranica* 1	*P. pseudotsugae* 1	*P. tentaculata* 1	*P. citricola* 5
Agar medium									
					Form of mycelium (a) and colony growth pattern (b)				
V8A	a	Dome-shaped, fluffy	Appressed	Sparse aerial mycelium	Appressed	Limited aerial mycelium	Appressed little mycelium	Limited aerial mycelium	Limited aerial mycelium
	b	Uniform	Faintly stellate	Uniform	Uniform	Faintly stellate	Uniform		Chrysanthemum
MEA	a	Dome-shaped, fluffy	Limited aerial	Woolly mycelium on plug	Sparse aerial mycelium	Limited aerial mycelium	Limited aerial mycelium	Dome-shaped, fluffy	Limited aerial mycelium
	b	Uniform	Faintly stellate	Uniform	Uniform	Uniform	Petaloid	Uniform	Stellate
CMA	a	Sparse aerial mycelium	Sparse aerial mycelium	Sparse aerial mycelium	Appressed	Appressed	Appressed, little aerial mycelium	Sparse aerial mycelium	Limited aerial mycelium
	b	Uniform	Uniform	Uniform	Uniform	Stoloniferous	Uniform	Faintly stellate	Chrysanthemum
PDA	a	Appressed, dense felty	Felty	Appressed to submerged	Submerged	Felty submerged edge	Dense felty	Appressed dense felty	Appressed
	b	Uniform	Faintly petaloid	Stoloniferous	Stoloniferous	Stoloniferous	Slight petaloid	Uniform	Stellate
					Average growth rate (mm d^{-1})				
V8A		3·3	6·4	4·3	4·5	5·1	4·9	4·0	8·1
MEA		2·5	5·9	2·6	3·0	2·1	4·2	3·2	5·1
CMA		2·5	4·9	1·0	3·3	1·0	3·9	3·2	6·3
PDA		0·5	2·8	0·5	1·5	0·9	1·9	1·0	2·0
					Growth rate (% of unamended control), isolate ranges in parentheses				
CMA + malachite green		72 (59–85)	55 (35–86)	55	62 (58–66)	100	64	88	61 (56–0)
					Average growth rate (mm d^{-1})				
P3 (asparagine)		1·4	2·6	0·6	4·1	1·0	2·7	1·8	2·7
P4 (nitrate)		0·8	3·4	0·4	0	1·1	1·9	0·9	1·9
					Colour of pigment produced				
CHT-agar		Black	Yellow	None	Black	Orange	None	Black	Orange
					Growth rate on V8 agar with hymexazol added (% of unamended control), isolate ranges in parentheses				
10 µg ml^{-1}		85 (67–100)	94 (91–100)	94	84 (83–85)	93	90	98	96 (92–103)
50 µg ml^{-1}		79 (64–95)	89 (76–100)	91	55 (51–58)	83	83	98	75 (63–85)
100 µg ml^{-1}		70 (58–91)	72 (44–99)	82	46 (42–49)	71	60	85	64 (51–77)
250 µg ml^{-1}		49 (28–69)†	60 (38–93)	58	21 (19–24)	51	20	50	44 (39–49)
500 µg ml^{-1}		21 (16–28)†	40 (38–44)†	12	7*	0	15	33	25 (21–31)

* One isolate failed to grow.
† Three isolates failed to grow.

Table 5. Pathogenicity of *Phytophthora* isolates to *Q. robur* seedlings

	Root rot†‡ (%)	Seedlings with dieback of suberized long roots‡ (%)	Seedlings with necrotic lesions on suberized roots‡ (%)	Wilting of leaves and shoot dieback¶	Interveinal chlorosis of leaves¶
P. quercina (3)*	50 (37–60)	93 (83–100)	54 (17–75)	(+)	(+)
P. cactorum (2)	33 (31–35)	79 (75–83)	8 (8)	—	—
P. citricola (1)	19	58	33	—	—
Phytophthora sp. 2 (1)	< 10	17	17	—	—
P. cambivora (2)	37 (36–37)	88 (83–92)	13 (8–17)	(+)	(+)
P. gonapodyides (1)	16	17	8	—	—
P. undulata (1)	24	50	0	—	—
Control	< 10	8	0	—	—

* No. of isolates.
† Blackened roots with oospores or nonseptate, coralloid hyphae present in cortical cells.
‡ Random re-isolations performed. Isolate ranges in parentheses.
¶ (+), symptom occurring infrequently; —, symptom not occurring.

basal plugs. Some became detached after release of zoospores. As with *P. quercina*, many oogonia were markedly elongated, but there was a high proportion (86%) of amphigyny (Table 2). Optimum and maximum temperatures for growth were lower and higher respectively (Table 3). No pigment was produced on CHT agar (Table 4).

P. idaei. Primary hyphae never branched in a sympodium. Sporangia were markedly less variable in size and shape, and lateral attachment, intercalary insertion and hyphal projections less frequent. Lower numbers of elongated oogonia were produced and oospores had thinner walls. Although predominantly paragynous, there was some amphigyny (12–28%) in both isolates (Table 2). Growth was faster at all temperatures below 25° with lower optimum and higher maximum temperatures (Table 3). *P. idaei* was unable to use nitrate as sole nitrogen source and was the most sensitive to hymexazol of all Group I species (Table 4).

P. iranica. Primary hyphae never branched in a sympodium. Sporangia were more regular in size and shape and never distorted. Curved apices and lateral attachment were also less frequent. There were no markedly elongated oogonia and oospore walls were thinner. Low levels (8%) of amphigyny were observed (Table 2). Brown chlamydospores, previously reported (Ershad, 1971), were not produced by the sole isolate. The isolate died at 5°, survived but did not grow at 10° grew faster at 17·5° and had higher minimum and maximum temperatures for growth (Table 3). An orange pigment was produced on CHT agar (Table 4).

P. pseudotsugae. Primary hyphae sometimes branched sympodially but without protuberances. Sporangia were sparse in culture and mostly produced on unbranched long sporangiophores. They were more regular in size and shape without distortion: curved apices and hyphal projections were rare. Oogonia were not elongate and oospores had thinner walls. Amphigyny (22%) occurred. Growth was faster at all temperatures, except 25° with lower optimum and higher maximum temperatures for growth (Table 3). No pigment was formed on CHT agar (Table 4).

P. tentaculata. Growth patterns were different only on V8A and CMA (Fig. 1, Table 4). Primary hyphae never branched sympodially but had ovoid to globose to irregular hyphal swellings. Sporangia were not distorted. Oogonia were not elongated and oospore walls were thinner. Amphigyny (16%) and arachnoid antheridial stalks were observed. Growth was faster at all temperatures (Table 3) and on all media with a higher maximum temperature for growth (Table 4).

Major differences from P. citricola *(Group III)*

Growth patterns on all four media were different (Fig. 1, Table 4). Sympodially branched primary hyphae were never observed. Sporangia were semi-papillate, never intercalary nor produced on agar. Elongated oogonia were lacking and oospore walls were markedly thinner (Table 2). All isolates had markedly higher growth rates on all agar media and at all temperatures, higher optimum and maximum temperatures for growth (Table 3), and produced an orange pigment on CHT agar (Table 4).

Pathogenicity

Q. robur. All three isolates of *P. quercina* induced severe dieback of unsuberized and suberized long roots, and distinct, sometimes girdling, necroses on suberized roots which mostly developed from infection of lateral roots (Table 5). Analysis of thin sections of the necrotic tissues by light microscopy revealed the presence of non-septate, irregular to coralloid hyphae, and thick-walled, globose or elongated, ovoid to ellipsoid oospores (Fig. 29). Above-ground symptoms included necrotic spots and partial chlorosis of leaves. Seedlings with severe root rot showed wilting of leaves and die-back of the shoot. With an average root rot of 50% and dieback of suberized long roots in 93% of the seedlings, *P. quercina* was the most pathogenic *Phytophthora* species to oak *P. cambivora* caused average root rot of 37% with root symptoms similar to those caused by *P. quercina*. All isolates induced interveinal chlorosis of the leaves of severely infected seedlings. On some seedlings *P. cambivora* infected the root collar and grew up the stem to a height of *ca* 8 cm. *P. cactorum* and *P. citricola* caused dieback of long roots and some distinct cortical necroses on suberized roots. With average root rot of 33%, *P. cactorum* was more pathogenic than *P. citricola* (19%). Both species formed globose to subglobose oospores in the infected tissues. *P. undulata* caused 24% root rot, whereas *P. gonapodyides* and *Phytophthora* sp. 2 were only weakly pathogenic to *Q. robur*. All *Phytophthora* isolates could be re-isolated from most of the necrotic tissues. No *Phytophthora* was recovered from uninoculated control plants.

Apple. *P. quercina*, *P. pseudotsugae* and *P. tentaculata* caused only a slight rot on Cox's Orange Pippin fruits, whereas *P. clandestina*, *P. idaei* and *P. iranica* were non-pathogenic and *P. cactorum* and *P. citricola* caused severe rot.

DISCUSSION

Being homothallic with paragynous antheridia and distinctly papillate sporangia, *P. quercina* clearly belongs to Group I of the key to the species of *Phytophthora* in which Waterhouse (1963) only included *P. cactorum*. Since then, six new Group I species, *P. iranica* (Ershad, 1971), *P. pseudotsugae* (Hamm & Hansen, 1983), *P. clandestina* (Taylor, Pascoe & Greenhalgh, 1985), *P. tentaculata* (Kröber & Marwitz, 1993), *P. idaei* (Kennedy & Duncan, 1995) and *P. italica* (Cacciola *et al*., 1996) have been described. In this study, isolates of all *Phytophthora* spp. from Group I, except *P. italica*, have been compared with *P. quercina*. *P. citricola* from Group III was also included because it shows some similarities to *P. cactorum* (Waterhouse, 1957; Kröber, 1959) and, like *P. quercina*, was often isolated from declining oak stands (Jung, 1996; Jung *et al*., 1996).

P. quercina and the other Group I species are easily separated from Group III species such as *P. citricola* and *P. syringae* and Group IV species, of which *Phytophthora* sp. 2 is an example (Jung, unpublished), by the depth and shape of the

sporangial apex. Other Group IV differences include exclusively amphigynous antheridia, caducous sporangia and lower optimum and maximum temperatures for growth. There are some similarities in sporangial morphology of *P. quercina* with *P. nicotianae* from Group II, which was not included in the study, e.g. lateral displacement of the prominent papilla, lateral attachment of the sporangiophore, intercalary insertion, bipapillate sporangia and hyphal projections (Kröber, 1985; Hall, 1993, 1994). *P. nicotianae* is almost exclusively heterothallic, however, and has amphigynous antheridia, as well as many other morphological and physiological characters which mark it as clearly different from *P. quercina*.

Although lacking a single, unique diagnostic feature to distinguish it from all other Group I species, *P. quercina's* unique combination of vegetative, gametangial and physiological characters mark it as a different species. Generally, it differs from all other Group I species examined here by its uniform, dome-shaped and cottonwool-like colony growth pattern on V8A and MEA, the frequent occurrence of sympodially branched primary hyphae with the mother hypha ending in a short protuberance, the absence of amphigynous antheridia, the occurrence of oospore-wall thicknesses > 3 µm, and, with the exception of *P. clandestina*, by the high proportion of elongated, ellipsoid to ovoid oogonia. In addition, of all the species in this study, only *P. quercina* had such high variability in the size and shape of the sporangia. Many had a curved apex, hyphal projections, lateral displacement of the papilla and/or lateral attachment to the sporangiophore. Distinguishing characters between *P. quercina* and every Group I *Phytophthora* species, except *P. italica*, have been presented in detail in the results section and in Tables 2–5.

Waterhouse (1970) and Ho (1978) regarded colony morphology, as well as hyphal branching habit, as a useful character for identification of *Phytophthora* species. In some species, for instance the *P. cryptogea–P. drechsleri* complex, *P. medicaginis*, *P. palmivora* or *P. capsici* (Brasier & Griffin, 1979; Erwin & Ribeiro, 1996) colony morphology is highly variable but in others, e.g., *P. nicotianae* (Hall, 1993) and *P. citrophthora* (Erwin & Ribeiro, 1996), it has proved a useful macroscopic diagnostic character. In this study, only *Phytophthora* sp. 2 had a colony growth pattern and sympodial branching of primary hyphae similar to *P. quercina*. This species is, however, so different in many other ways from *P. quercina* that colony morphology might still be a good diagnostic character for distinguishing *P. quercina* from other Group I species.

Reported sizes for sporangia, oogonia and oospores vary considerably. In particular, the dimensions of sporangia are known to be highly variable and dependent on culture age and medium (Erwin, 1983; Hall, 1993) as well as the isolate examined. For example, reported average sporangial sizes of *P. cactorum* range from 30·4 × 22·8 µm (Tucker, 1931) to 49 × 36 µm (Kennedy & Duncan, 1995). The present study has confirmed such variation. Average sporangial dimensions were comparable with those reported in the original descriptions only for *P. cactorum*, *P. citricola* and *P. iranica*. They were markedly larger for *P. clandestina* (55·3 × 32·3 µm *v.* 41 × 32 µm) and *P. tentaculata* (50 × 35·4 µm *v.* 35·7 × 27·4 µm), and much smaller for *P. idaei* (38·8 × 28·6 µm *v.* 49 × 36 µm) and *P. pseudotsugae* (28·4 × 25 µm *v.* 38·7 × 31·6 µm) than previously reported (Hamm & Hansen, 1983; Taylor *et al.*, 1985; Kröber & Marwitz, 1993; Kennedy & Duncan, 1995).

Generally, *P. quercina* had sporangia larger than *P. cactorum*, *P. idaei* and *P. iranica* and markedly larger than *P. pseudotsugae* but much smaller than *P. clandestina* and *P. tentaculata*. Considering the overlap of isolate averages and the differences between sporangial dimensions of *P. clandestina*, *P. idaei*, *P. pseudotsugae* and *P. tentaculata* reported here and in the original descriptions, this feature does not appear to be very useful for distinguishing *P. quercina* and the other Group I species. This accords with Tucker (1931) who stated that 'dimensions of sporangia considered independently … cannot be accorded much importance taxonomically'. Erwin & Ribeiro (1996) also stated that 'size differences for diagnostic purposes should be treated with caution'. Interestingly, *P. quercina* had the broadest range of sporangial sizes (18·8–112·5 × 13·8–47·5 µm) of all Group I species examined. In itself, this might be of more diagnostic value than actual differences in average values. Except for *P. clandestina*, average sporangial l/b ratios for all species were relatively similar to those reported in the literature, but the range of isolate averages for *P. quercina* overlapped those of all other Group I species making l/b ratio unsuitable for distinguishing it from other species.

The dimensions of oogonia and oospores were generally somewhat smaller than in the original descriptions but again the averages were such that they also cannot be used to distinguish *P. quercina* from the other Group I species. Ratios calculated from direct measurements of oogonia and oospores have, however, proved useful in the taxonomy of *Pythium*, even when the measurements overlapped between species (Shahzad, Coe & Dick, 1992). A similar approach therefore could prove useful for discriminating among Group I species of *Phytophthora*.

Physiological characters which separated *P. quercina* from other Group I species were its slow growth on V8A and MEA; low maximum temperature of 27·5° for growth on V8A; and black pigmentation on CHT agar. Neither sensitivity to malachite green nor to hymexazol, which has been used to distinguish *Phytophthora* species (Kato *et al.*, 1990), were taxonomically useful. In both cases, variation among isolates of *P. quercina* overlapped nearly the entire range of averages of other species. Ability to use nitrate as a sole nitrogen source was likewise not useful, except for *P. idaei* (Kennedy & Duncan, 1995).

Pathogenicity to apple fruits has been used as an additional feature for species differentiation (Hamm & Hansen, 1983; Belisario *et al.*, 1993; Kennedy & Duncan, 1995). In this study, *P. quercina*, *P. pseudotsugae* and *P. tentaculata* caused a slight rot of fruit of Cox's Orange Pippin, thus distinguishing them from *P. clandestina*, *P. idaei* and *P. iranica* which were non-pathogenic, and from *P. cactorum* which caused a severe rot.

Although no isolate of the recently described Group I species *P. italica* was included in this study, reports (Belisario *et al.*, 1993; Cacciola *et al.* 1996) indicate that *P. italica* is clearly different from *P. quercina* in its faint stellate growth pattern on PDA and a slightly radiate growth pattern on

CMA, higher cardinal temperatures for growth (10–26–35°), faster growth rate on V8A and PDA, higher frequency of bi- or even tri-papillate sporangia, the absence of sporangia larger than 56 × 38 µm, considerably smaller dimensions of oogonia (15–29 µm, av. 22 µm) and antheridia (5–11 µm, av. 7·1 µm), thinner oospore walls (av. 1·8 µm, never > 2·5 µm), the absence of elongated, ovoid to ellipsoid oogonia, greater pathogenicity to apple fruit, and its natural host, myrtle *Myrtus communis*.

In addition to the differences in morphology and physiology, there are also clear differences in the host ranges of *P. quercina* and the other Group I Phytophthoras. With the exception of the plurivorous *P. cactorum* (Nienhaus, 1960) which has also been isolated rarely from rhizosphere soil samples of declining oaks, Group I species have only been recorded from one or a few host species (Ershad, 1971; Hamm & Hansen, 1983; Taylor *et al*., 1985; Kröber & Marwitz, 1993; Kennedy & Duncan, 1995; Cacciola *et al*., 1996), and never from declining oaks. *P. quercina* also appears to have a limited host range since it was consistently isolated from five European oak species but never recovered from any other tree species (Jung & Blaschke, 1996; Jung *et al*., 1996). *P. quercina* is the most common *Phytophthora* in declining Central European oak forests, in particular where trees die rapidly and in groups (unpublished results). Its frequent association with, and high aggressiveness to roots of *Q. robur* (this study) and *Q. ilex* (Jung & Paoletti, unpublished) as shown by soil infestation tests, probably make *P. quercina* the most damaging *Phytophthora* of oaks in Central Europe.

RAPD analysis has been shown to be a sensitive method for differentiation between *Phytophthora* isolates of Group I species and even at the subspecies level: apple, raspberry and strawberry isolates of *P. cactorum* were distinguished using RAPDs (Cooke *et al*., 1996). There were sufficient shared bands to show affinities between *P. cactorum*, *P. idaei* and *P. pseudotsugae*, and to distinguish them from the less closely related *P. iranica* and *P. clandestina* (Cooke *et al*., 1996). RAPD banding patterns obtained with four different primer sets gave almost identical fingerprints for 10 *P. quercina* isolates. Cluster analysis of the patterns grouped all *P. quercina* isolates very closely with similarities > 95% and with no close affinity to any other Group I species. ITS1 and ITS2 sequence data from the ribosomal RNA gene repeat confirms this result (Cooke *et al*., 1999).

Considering its unique combination of cultural, morphological and physiological characters, its pathogenicity to the genus *Quercus*, and its unique fingerprint, it is clear that the papillate *Phytophthora* isolates from oak belong to an undescribed and unrecorded species which is hereby designated *Phytophthora quercina* sp. nov. A formal description of a type specimen is presented below.

Phytophthora quercina T. Jung. sp. nov.

Etym.: *quercina* is derived from its original host, *Quercus* spp.

Coloniae modice lente crescentes in agaris 'V8-juice agar (V8A)', 'malt-extract agar (MEA)' et 'cornmeal agar (CMA)' et lentissime crescentes in agaro 'potato-dextrose agar (PDA)'. Crescunt in agaro 'V8A' a 5–27·5° optime a 25° (incrementum radiatum 3·7 mm d^{-1}). Coloniae uniformes, sine ordinatione proprio in omnibus agaris expertis, cum mycelio aerio restricto in agaro 'CMA', umbonatae et pubescentes in agaris 'V8A' et 'MEA', adpresse tomentosae et umbonatae in agaro 'PDA'; coloniae maturae adprementes, etiam nunc manentes umbonatae. Incrementum radiatum minutum in agaris 'CMA' cum 'malachite green' et 'V8A' cum 'hymexazol'. Producit pigmentum atrum in agaro 'casein hydrolysate tyrosin'. Hyphae hyalinae, non septate, maturitate septatae. Hyphae primariae in medio 6·9 µm diam. (4·6–8·3 µm), terminale frequenter ramosae monochasiis aut dichasiis cum hypha remanescens cuspide curta, laterale ramosae ad angulam ca 90°. Inflationes hypharum et chlamydosporae raro observatae. Chlamydospora intercalares aut terminales, globosae, hyalinae; paucae cum appendicibus brevibus. Sporangiophora in medio 3 µm diam. (2·1–4·6 µm), interdum latior prope basim sporangii, in longitudine variabilia; simplicia aut ramosa sympodiis laxis irregularibus; inflationes ad nodos rarae. Sporangiophora saepe inserta lateralia ad sporangia. Sporangia pauca in agaris solidis, sed abundantia in cultura liquida; terminalia aut interdum intercalaria, persistentia, interdum cum obturamento conspicuo basale, papillata aut interdum bipapillata. Papillae in alto 1·7–5·6 µm (in medio 3·5 µm). Sporangia in medio 41 × 31·7 µm (21–70 × 20 ± 4–40·8 µm), ratio longitudo ad latitudinem in medio 1·29:1, in forma variabilia: subglobosa, ovoidea, obpyriformia, bananiformia, fabiformia et irregularia. Apex sporangiorum saepe arcuatus; sporangia interdum cum appendicibus brevibus. Zoosporae limoniformes ad reninformes, in medio 11·1 × 7·4 µm (8·9–12·7 × 6·3–8·5 µm), emissae per porum, in medio 7·1 µm (4·6–8·3 µm); zoosporae incystatae globosae, in medio 9·1 µm (7·1–11·7 µm). Germinatio directa sporangiorum raro observata. Oogonia numerosa in cortice radicorum gracilium *Quercorum* et in cultura signulari in agaro 'V8A' et 'MEA'; in cultura terminalia, globosa ad ovoidea, saepe perspicue elongata quasi assumentes forma cellulae hospitalis, diameter in agaro 'V8A' in medio 31·8 µm (23·5–41 µm); paries levis, in medio 1·3 µm (0·5–2 µm) crassus. Oosporae globosae ad ovoidea, perspicue apleroticae, diameter in medio 29·4 µm (22·5–37 µm), crasse tunicatae, paries in medio 2·9 µm (1·5–5 µm) crassus, maturitate frequenter pigmentatus aureo-fuscus. Antheridia singularia, terminalia, hyalina, globosa ad claviformia aut irregularia, in medio 14 × 9·9 µm (10–19·1 × 7·5–12 µm), semper paragynosa.

Habitat. In cortice radicorum gracilium et in solo rhizosphaerae, *Quercus robur* in Germania. Nomen habet ab hospite originali, *Quercus* spp. *Holotypus*: In collectione fungorum apud CBS, Baarn, The Netherlands, CBS 784.95.

The details in the Latin diagnosis of the holotype are included in the species description given in the Results section and are not repeated here.

Holotype: Isolated in May 1995 (Isolate QUE 3 in this paper) from rhizosphere soil containing necrotic fine roots of mature declining *Quercus robur* L. near Munich, Germany, deposited in CBS, Baarn, The Netherlands, as CBS 784.95.

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