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Characteristics of some *Phytophthora* species isolated from oak forest soils in central and northern Italy

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Summary. Four *Phytophthora* species, *P. citricola*, *P. megasperma*, *P. quercina* and *P. syringae*, were isolated during a systematic survey of oak forests (*Quercus cerris* and *Q. robur*) in Tuscany (central Italy), and in the Po Valley and the Venetian Plain (northern Italy) from 22 out of 54 soil samples. The main morphological characteristics of the isolates and their growth rates on different substrates and at different temperatures are reported.

Key words: *Phytophthora*, *Quercus*, morphology.

Introduction

Oak decline has been repeatedly reported over the last two decades, on many of the most common *Quercus* species in Europe [*Quercus cerris* L., *Q. frainetto* Ten., *Q. ilex* L., *Q. petraea* (Matt.) Liebl., *Q. pubescens* Willd., *Q. robur* L. and *Q. suber* L.] (Delatour, 1983; Siwecki and Liese, 1991; Luisi *et al.,* 1993; Ragazzi *et al.,* 1995). Among the possible biotic factors associated with declining stands, *Phytophthora* spp. were frequently recorded. Declining stands of *Quercus suber* in Spain and *Q. rubra* L. in France were found associated with root infections of *Phytophthora cinnamomi* Rands (Brasier *et al*., 1993; Desprez-Loustau and Dupuis, 1994; Marçais *et al.*, 1996). In Germany *P. cactorum* (Lebert & Cohn) J. Schröt, *P. cambivora* (Petri) Buism., *P. citricola* Sawada, *P. gonapodyides* (Petersen) Buism., *P. quercina* Jung and *P. undulata* (Petersen) Dick have been recovered from declining oaks (Jung *et al.,* 1996; Jung *et al.,* 1999). In Italy a recent study revealed the presence of *P. citricola* and *P. cactorum* near Cornuda (Treviso, northern Italy), while *P. quercina* was isolated from the rhizosphere of *Q. ilex* and *Q. pubescens* in Tuscany (central Italy) (Blaschke *et al.,* 1995, Jung *et al.*, 1996). More recently, *P. cactorum*, *P. cambivora*, *P. cinnamomi*, *P. citricola*, *P. cryptogea* Pethybridge and Lafferty and *P. gonapodyides* have all been reported from both declining and non-declining *Q. cerris* forests in central and southern Italy (Bianco, 1999; Anselmi *et al.,* 2000).

The present note gives a brief description of the main characteristics of four *Phytophthora* species isolated from oak forests in some stands in central and northern Italy.

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Materials and methods

Sampling sites

Eight oak sites, distributed as follows, were visited for sampling material: site 1 to 3 in Tuscany [1, Vivo d'Orcia (Siena); 2, Ulignano (Pisa); 3, S. Rossore (Pisa)] and site 4 to 8 in the Po Valley

and the Venetian Plain [4, Trino (Vercelli); 5, Vigevano (Pavia); 6, Carrega (Parma); 7, Cornuda (Treviso) and 8, Castions di Strada (Udine)]. Most of these areas were included in the survey programmes from the "Controllo ecosistemi forestali" (CON.ECO.FOR.) and the "Monitoraggio Intensivo foreste Toscane" (MON.I.TO.), supported respectively by the Ministero per le Politiche Agricole and the Regione Toscana (Bartolozzi *et al*., 1996; Ministero per le Politiche Agricole e Corpo Forestale dello Stato, 1999). Two areas (1 and 2) were planted with *Q. cerris,* one (No. 6) with *Q. petraea*, and the remaining (3, 4, 5, 7 and 8) with *Q. robur*. Detailed description of sampled areas are reported by Barzanti (1999) and by Vettraino *et al*. (2001).

Phytophthora isolation

At each site, 6–8 trees, half of which were declining and half healthy-looking were selected, and one representative soil sample per tree was collected at a distance of about 50 cm from their base. A total of 54 soil samples were tested for *Phytophthora* spp. using the baiting technique (Erwin and Ribeiro, 1996; Jung *et al.,* 1996). For isolation, about 200 ml of soil was flooded in a plastic container with 500 ml distilled water. Some freshly picked leaves of *Q. robur,* a few days old, were placed directly on the water surface, incubated at 20°C and inspected daily until spots appeared on the leaves or the leaves became discoloured. The leaves were then blotted on filter paper, cut into small pieces (0.5 cm^2) and placed on a selective medium [PARPNH V8 Agar (Tsao, 1983, modified by Jung *et al*., 1996)] containing 100 ml V8 juice (Campbell Grocery Products Ltd., Norfolk, England), 3 g $CaCO_3$, 10 mg pimaricin, 200 mg ampicillin, 10 mg rifampicin, 25 mg pentachloronitrobenzene (PCNB), 50 mg nystatin and 50 mg hymexazol l^{-1}. All Petri dishes were examined daily under a dissecting microscope in order to isolate any fungus belonging to Pythiaceae before the development of saprophytic microflora. When no isolates were found the soil was allowed to air-dry at ambient temperature before being again subjected to baiting (Jeffers and Aldwinckle, 1987).

The developing *Phytophthora* isolates were transferred to V8 Agar (10% V8 juice, v:v) and maintained at 20°C, or they were stored in tubes containing sterile distilled water at room temperature (Boesewinkel, 1976; Ann and Ko, 1990; Erwin and Ribeiro, 1996).

Species identification

Isolates were identified by comparing colony growth patterns and morphological features of sporangia, oogonia, antheridia, chlamydospores and hyphal swellings with the species descriptions reported in the literature (Stamps *et al.,* 1990; Erwin and Ribeiro, 1996; Jung *et al.*, 1999).

Colony morphology was observed on 7-day-old cultures (14-day-old cultures if growth was slow) grown on cornmeal agar (CMA) (Difco, Sparks, MD, USA), malt extract agar (MEA) (Difco), potato dextrose agar (PDA) (Difco) and V8 juice agar (V8A) in 90-mm Petri dishes at 20°C in darkness (3 replications per substrate).

Sporangia were produced after 24–48 hours of incubation at 20°C by placing a disk of mycelium from a 10-day-old culture grown on V8A in soil extract prepared according to Jung *et al.* (1996). Morphology was assessed under the light microscope and for each isolate the length and breadth of 30 sporangia were measured (200x or 400x).

In vitro growth

A selection of isolates collected was used for *in vitro* growth tests (seven isolates of *P. citricola*: Ph38, Ph45, Ph46, Ph47, Ph49, Ph50, Ph58; two of *P. megasperma*: Ph37, Ph43; six of *P. quercina*: Ph39, Ph40, Ph41, Ph42, Ph53, Ph54; and one of *P. syringae*: Ph24).

For each isolate the following parameters were determined:

a) colony size, by measuring two diameters after 5 days of growth on V8A at temperatures of 2, 5, 10, 15, 17.5, 20, 25, 27.5, 30 and 35°C. There were three replications for each isolate;

b) the mean daily growth rate after 5 days of incubation at 20°C on the same substrates used for colony morphology description.

Results

Phytophthora isolation

Phytophthora spp. were detected from soil collected at the base of 22 of the 54 selected trees (40.6%) in 6 of the 8 areas sampled (Table 1). In general more species were isolated from the soil sampled from Tuscany (4 species) than from sam-

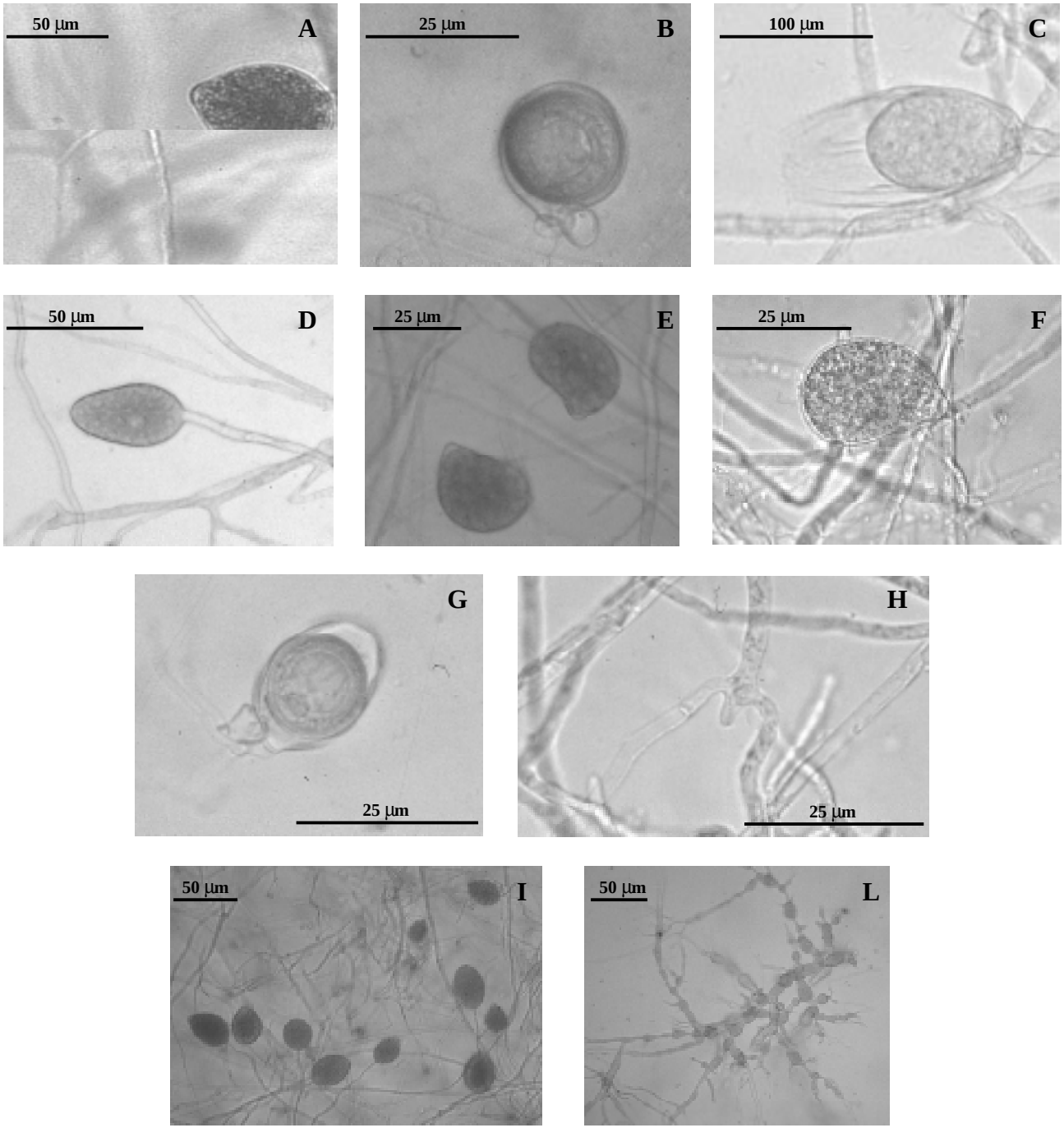

Fig. 1. A, B) *Phytophthora citricola*: A) semipapillate sporangia of various shapes; B) spherical oogonium with paragynous antheridium and plerotic oospore. C, D) *Phytophthora megasperma*: C) internal proliferation; D) non papillate sporangium. E-H) *Phytophthora quercina*: E) distorted, papillate sporangia; F) hyphal projection; G) elongated oogonium with aplerotic oospore; H) dichasium. I, L) *Phytophthora syringae*: I) ovoid, semipapillate sporangia; L) hyphal-swellings.

ples taken in the Po Valley and the Venetian Plain (2 species) (Table 1).

Species identification

The isolates collected were assigned to four species: *P. citricola* and *P. quercina* (9 isolates for each species), *P. megasperma* and *P. syringae* (2 isolates for each species). The main morphological features and the growth patterns of the species identified are shown in Table 2 and 3.

***In vitro* growth**

a) The optimal growth temperature on V8A ranged from 17.5°C for *P. syringae* to 27.5°C for *P. citricola* (Table 4, Fig. 2). Mycelium from all species except *P. megasperma* managed to grow from 2°C, and as regards the maximum temperature tolerated, *P. citricola* still grew at 30°C, whereas *P. quercina* died if maintained at this temperature for three days. At 20 and 25°C plate colonisation of *P. quercina* isolates from Vivo d'Orcia (site n. 1, 3 isolates tested) was higher than that of the other isolates of the same species (data not shown).

b) At 20°C, all the growth rates of the four *Phytophthora* species on the four substrates differed widely (from 0.2 to 11.1 mm/day) except those of *P. megasperma* and *P. quercina* on MEA, which were fairly similar. The mean daily growth rates are shown in Table 4.

Discussion

The objective of this paper was to describe the main characteristics of the *Phytophthora* species isolated from oak forests soils in central and northern Italy, since the Italian literature on this subject is relatively poor, especially when compared with that from other European countries (Brasier *et al*., 1993; Jung *et al*., 1996; Robin and Desprez-Loustau, 1998; Robin *et al*., 1998; Hansen and Delatour, 1999; Jung *et al*., 1999).

P. megasperma and *P. syringae*, found in Tuscany, are distributed world-wide but have only recently been reported from oak forests soils in Italy (Barzanti, 1999; Vettraino *et al.*, 2001). *P. megasperma,* mainly pathogenic on herbaceous and fruit plants, also infects forest plants (*Picea abies* L. Karst, *Pinus* spp., *Pseudotsuga menziesii* Mirb. Franco) (Erwin and Ribeiro, 1996). It has sporadically been recovered from oak forests soils in France and Germany (Hansen and Delatour, 1999; Jung *et al.*, 2000). However, while Jung *et al.* (2000), isolated *P. megasperma* from soils with a pH range (measured in $CaCl_2$) of 5.6–7.0, in Italy it was found in more acid pH (3.8 in $CaCl_2$) soil samples (Barzanti, 1999; Vettraino *et al.*, 2001). This difference underlines the plasticity of this species.

Likewise, *P. syringae* was usually recorded from herbaceous plants, rarely from forest trees, although it has been noted in a few cases on *Aesculus hippocastanum* L., *Alnus glutinosa* (L.) Gaertn., *Quercus* sp. and *Fagus* sp. (Erwin and Ribeiro, 1996). The identification of the isolates from Tuscany was based on the morphological characters which corresponded to those reported in the literature. Recent molecular studies suggest however that these isolates could belong to a new and as yet undescribed species for which the name *Phytophthora pseudosyringae* will probably be proposed (T. Jung, personal communication). This species is the least thermophilous of the four species tested as it has its maximum growth at 17.5°C, but it can still grow even at 30°C.

During the survey the most common species found both in Tuscany and in the Po valley and Venetian plain were *P. citricola* and *P. quercina*.

Morphological characteristics and temperature requirements for the growth of the *P. citricola* isolates tested in this study fitted those from the literature very well (Erwin and Ribeiro, 1995; Jung *et al.*, 1999). However their growth rate on all the four substrates at 20°C was higher than that reported by Jung (6.3, 5.1, 2 and 8.1 mm/day on CMA, MEA, PDA and V8A respectively, Jung *et al.*, 1999). In the growth test this species was the most thermophilous, with its maximum growth at 25–27.5°C. *P. citricola* is widespread throughout the world with a wide host range on which it causes root rot and stem cankers. In the USA it is reported to cause root rot on seedlings of *Q. alba* L. and *Q. rubra* (Erwin and Ribeiro, 1996), and in Europe it has been found in oak forests in France (Hansen and Delatour, 1999), Switzerland, Hungary and Germany (Jung *et al.,* 1996). The occurrence of *P. citricola* in oak forests soils in Tuscany and Veneto, characterised by different climatic situations, confirms the wide ecological plasticity of this species, which has also been reported from oaks and chestnut in central and southern Italy (Anselmi *et al.*, 2000; Vettraino *et al.*, 2001).

As regards *P. quercina*, the isolates tested showed no micro- or macroscopic morphological

Table 1. Isolates of four *Phytophthora* species collected from oak forest soils.

Phytophthora species	Waterhouse group[a]	No. of soil samples with *Phytophthora*	Soil pH range ($CaCl_2$)	Host species	Location of sampling areas[b]
P. citricola	III	9 (16.6)[c]	4.2–7.5	*Q. robur*	Tuscany (3) Venetian plain (4, 6)
P. megasperma	V	2 (3.7)	3.8	*Q. cerris*	Tuscany (1)
P. quercina	I	9 (16.6)	3.8–5.7	*Q. cerris* *Q. robur*	Tuscany (1, 2); Po valley (8); Venetian plain (6)
P. syringae	III	2 (3.7)	3.8–5.7	*Q. cerris*	Tuscany (1, 2)
Total		22 (40.6)	3.8–7.5		

[a] According to Stamps *et al.*, 1990.
[b] In brackets is given the number of the sampling area where the fungus was isolated (see text).
[c] In brackets percentage over 54 soil samples examined.

Table 2. Main morphological characteristics of four *Phytophthora* species collected from *Quercus cerris* and *Quercus robur* forests soils in northern and central Italy.

Characteristics	*P. citricola*	*P. megasperma*	*P. quercina*	*P. syringae*
Sporangia				
Range length (µm)	30–50	32–79	20–55	40–75
Range breadth (µm)	20–35	16–40	15–45	30–40
Papilla	Semipapillate	Non papillate	Papillate	Semipapillate
Shape	Ovoid, obpyriform	Ovoid, obpyriform	Ovoid, distorted	Ovoid, obpyriform
Occasional observations	2 apices		Lateral papilla, ifal hyphal projection, 2 apices	
Oogonia				
Shape	Spherical	Spherical	Elongated	Spherical
Range diam. (µm)	20–30	30–50	d1 30–52 d2 20–32	25–40
Oospores	Plerotic	Plerotic	Markedly aplerotic	Plerotic
Antheridia	Paragynous	Paragynous	Paragynous	Paragynous
Other observations		Zoospores direct germination from within the sporangia	Sympodial ramification of the mycelium	

Table 3. Colony morphology on four different substrates of four *Phytophthora* species collected from *Quercus cerris* and *Quercus robur* forests soils in northern and central Italy.

Substrate[a]	Colony characteristic	*P. citricola*	*P. megasperma*	*P. quercina*	*P. syringae*
CMA	Mycelium	Limited aerial mycelium	Scarce aerial mycelium, tufted surface, not very distinct margin	Sparse aerial mycelium	No aerial mycelium, very uniform, with clear, regular margin
	Growth pattern	Chrysanthemum	Uniform	Uniform	Uniform
MEA	Mycelium	Scarce aerial mycelium	Very sparse aerial mycelium, irregular margin	Cottony, dome-shaped	Smooth mycelium
	Growth pattern	Stellate	Uniform	Uniform	Vaguely radiate
PDA	Mycelium	Appressed mycelium	Abundant aerial mycelium, cottony appearance, not very distinct margin	Felty, appressed, dome-shaped	Cottony aerial mycelium, wavy margin
	Growth pattern	Stellate	Uniform	Uniform	Uniform
V8A	Mycelium	Limited aerial mycelium	Cottony, dome-shaped, clear margin	Cottony, dome-shaped	Little aerial mycelium, clear margin
	Growth pattern	Chrysanthemum	Uniform	Uniform	Roughly stellate

[a] CMA, cornmeal agar; MEA, malt extract agar; PDA, potato dextrose agar; V8A, V8 juice agar.

Table 4. Growth rate of four *Phytophthora* species on different media at 20°C and temperature requirements for growth on V8A (in mm).

Phytophthora species	Growth rate (mm/day, 20°C)				Temperature requirements for growth on V8A		
	CMA[a]	MEA[a]	PDA[a]	V8A[a]	Min.	Optimum	Max.
P. citricola	11.1±2.0	8.3±1.5	6.2±0.7	10.9±1.7	2.0	27.5	>30.0
P. megasperma	1.8±0.2	0.2±0.1	0.6±0.3	1.6±0.5	5.0	20.0	27.5
P. quercina	3.5±1.0	0.3±0.2	1.5±0.4	3.5±0.5	2.0	20.0	<30.0
P. syringae	6.1±0.1	1.7±0.5	2.4±0.0	5.8±0.6	2.0	17.5	30.0

[a] CMA, cornmeal agar; MEA, malt extract agar; PDA, potato dextrose agar; V8A, V8 juice agar.

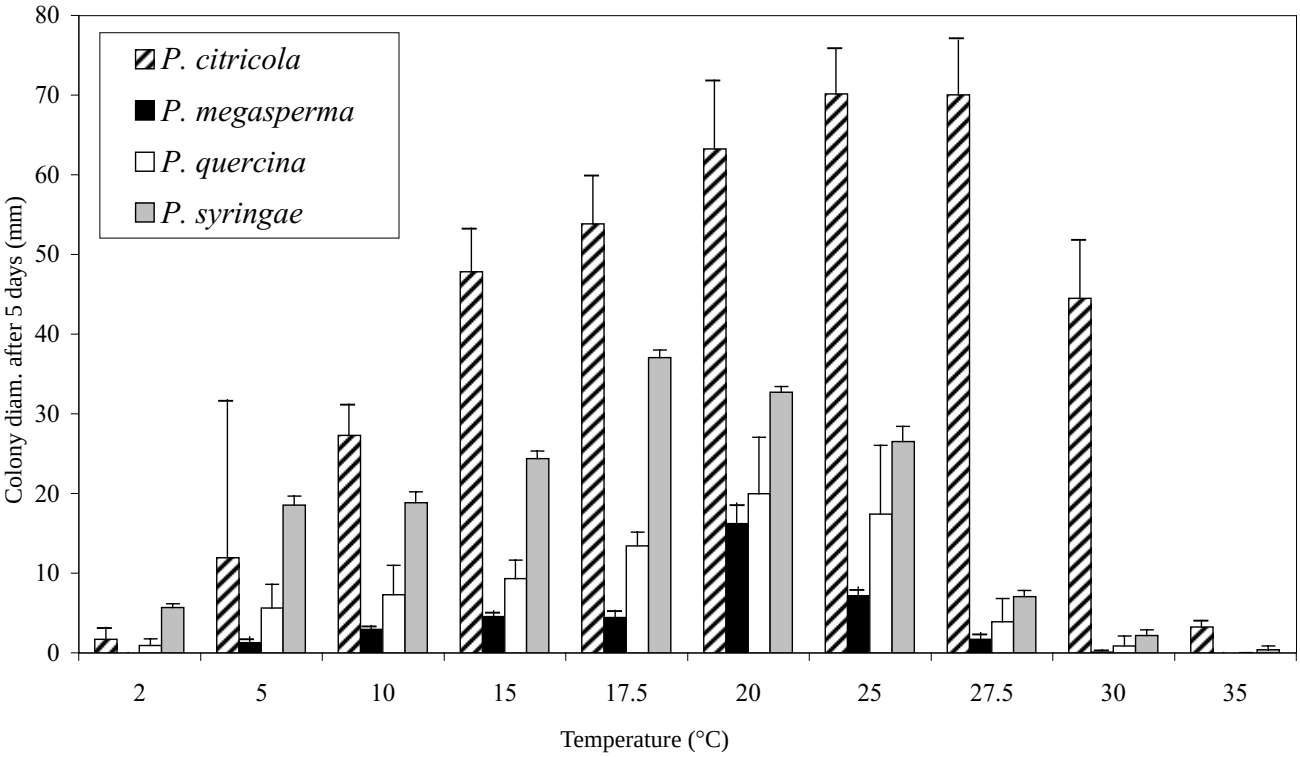

Fig. 2. Growth of *Phytophthora* isolates at various temperatures on V8 agar.

differences with the species description (Jung *et al.*, 1999), but the mean growth rate at 20°C was higher on PDA (1.5 vs. 0.5 mm/day) and lower on MEA (0.3 vs. 2.5 mm/day) than those reported by Jung *et al.* (1999). Another difference was the optimum growth, which in our study occurred at 20 instead of at 25°C. The high standard deviation registered at these two temperatures (20 and 25°C, Fig. 2) was due to a different behaviour of the three isolates from site 1 (Vivo d'Orcia, Tuscany) which showed a much higher plate colonisation than the other isolates from Tuscany and north Italy (data not shown). It should be noted that the isolates from site 1 were collected from soil samples with a pH ($CaCl_2$) ranging from 3.8 to 4.0, while all the other *P. quercina* isolates tested came from soil samples whose pH ($CaCl_2$) was never lower than 4.4 (data not shown). The different environment in which these isolates grew may have influenced their *in vitro* growth.

Concerning the temperature, *P. quercina*, just like *P. syringae*, is still able to grow at 27.5°C and this can explain its occurrence in Tuscany, a Mediterranean area with a generally mild climate, on *Q. ilex*, *Q. cerris* and *Q. pubescens*.

P. quercina was also found on *Q. robur* in two areas of northern Italy (Lombardy and Friuli) that are ecologically more similar to those parts of central Europe where it has hitherto been most widespread (Jung *et al.,* 1996).

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