Identity and Pathogenicity of Two *Phytophthora* Taxa Associated with a New Root Disease of Olive Trees

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ABSTRACT

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Fifty-two *Phytophthora* isolates from necrotic roots of olives were characterized. Colony morphologies on carrot-agar medium led us to separate them into two groups: A (36 isolates) and B (16 isolates). The optimum growth temperature for Group A was about 21°C, with slow growth at 30°C. In contrast, Group B isolates had an optimum temperature for growth of 26°C, and grew rapidly at 30°C. Growth rates, sporangial and oogonial characteristics of the Group A isolates conformed to *P. megasperma* "BHR-type" *sensu stricto*. This designation was supported by a sequence analysis of their ITS rDNA regions. Colony patterns, sporangial characteristics and temperature-growth relationships of the Group B isolates conformed closely to those of the 'O-group' taxon of *Phytophthora*. They also conformed to this unusual taxon in their ITS sequence. In addition, Group B isolates were either entirely self-sterile, self-sterile A1s or weakly self-fertile. Pathogenicity tests showed that both taxa were highly aggressive on roots of olive trees. The association of flooding with *Phytophthora* infection indicates that the previously reported high sensitivity of olive to root asphyxiation may be more properly regarded as root-rot caused by *Phytophthora* spp.

In recent years a wilt and death of young olive trees of unknown etiology has been observed on new olive (*Olea europaea*) plantations in southern Spain. Death of trees occurs rapidly, with or without previous yellowing or defoliation. The syndrome has been called "seca" (drying) to distinguish it from other diseases, such as Verticillium wilt or insect damage, that can induce similar symptoms (19).

In field surveys carried out in 1996, most of the affected plantations had waterlogged or very wet soils. In all of these plantations, death was associated with root rot. Fungal isolations from the affected roots consistently yielded *Phytophthora* spp. (94% of the plantations showing root rot). The same problem was observed in older plantations, but trees were not severely affected (19).

Several species of *Phytophthora*, including *P. citricola* and *P. drechsleri*, have been associated with root and crown rot of olive trees in California (22). In Greece, Kouyeas and Chitzanidis (15) reported *P. megasperma* as an olive tree pathogen.

Other non-identified *Phytophthora* spp. have been associated with root rot of olive trees (11,25). High soil water content and low oxygen content favor root rots caused by *Phytophthora* species on woody hosts such as orange trees, avocado, apple trees, and others (6,10,21). Traditionally, olive trees have been considered very susceptible to "root asphyxiation" associated with waterlogging. The aim of the present work was to clarify the role of the *Phytophthora* spp. in olive "seca" through the characterization of isolates obtained from symptomatic roots of olive.

MATERIALS AND METHODS

Fifty-two *Phytophthora* isolates were obtained from necrotic roots of young olive trees sampled in 1996 in southern Spain (Provinces of Córdoba, Jaén, and Sevilla) (Table 1).

Morphology of the colonies and growth-rate tests. Two different culture media were used to characterize colony morphology: Corn-meal Agar (CMA), a general purpose medium (8); and Carrot Agar (CA) for assessing colony characteristics and oogonial production (4,10). Agar plugs 7-mm in diameter were cut from edges of actively growing colonies, placed in the center of Petri dishes (9-cm diameter, 20-ml media/plate), and incubated in darkness. For each isolate, three replicates per culture medium and temperature were prepared. Temperatures used were 5, 10,

15, 20, 25, and 30°C. Colony diameters were measured after 4, 6, 10, and 15 days.

Colony morphology was assessed after 4 or 6 days growth at 20°C. Growth-rates per day were calculated for the different media and temperatures and maximum average data were adjusted to a regression curve. Adjustment was made using Statistix for Windows (Analytical Software, Tallahassee, FL). The best polynomial model was chosen from several combinations of terms, based on the significance of the estimated parameters ($P \leq 0.05$), coefficients of determination (R^2), coefficients of determination adjusted for degrees of freedom (R_a^2), and pattern of residuals (9). The optimum growth temperature was estimated for each isolate tested.

Sporangial morphology. Twenty-five isolates from different location were selected and grown individually on CA medium for 7 days in darkness at 20°C. Nine-mm-diameter agar plugs from these plates were placed in 5-cm-diameter Petri dishes and soil extract was added to just cover the plugs. Soil extract was prepared by mixing 10 g of soil from olive tree rhizosphere and 1 liter distilled water, standing for 24 h at 20°C and filtering through filter paper. Petri dishes then were incubated in darkness at 15°C. Every 24 h, the soil extract was removed and replaced with fresh extract that had been stored at 4°C. Sporangial production was assessed by direct observation using an inverted microscope. Sporangial development occurred between 12 and 120 h. Mature sporangia were removed and placed on glass microscope slides, then stained with acid fuchsin in lactophenol. The length and width of 25 sporangia per isolate were measured. Analysis of variance was performed and mean values were compared by Fisher's protected least significant difference (LSD) test (20). Other sporangial characteristics such as shape, presence or absence of papilla, persistence, proliferation, and branching of sporangiophores, were also recorded.

Sexual behavior. The 25 *Phytophthora* isolates (Table 1) were grown on CA medium at 20°C in darkness. In some cases, after 7 to 10 days of growth, sexual reproductive structures were present. Samples of these were moved to slides and stained with acid fuchsin in lactophenol, and oogonial and oospore diameter, antheridial

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length, and oospore cell wall thickness were measured for 25 randomly selected mature oogonia.

When no oogonia were present in single culture after 15 days growth, pairing tests were carried out with A1 and A2 compatibility type isolates of *P. drechsleri* (P537 and P540; *Phytophthora* Culture Collection, Forestry Commission Research Agency, Farnham, UK). *P. drechsleri* has proved an effective test organism for assaying sterility and unusual compatibility reactions in other *Phytophthora* species, such as *P. gonapodyides* (5). Pairings were

replicated three times on CA medium and incubated for 15 days at 20°C in the dark. When oogonia formed, scrapings were taken from the colony interface and mounted and stained as above.

Pathogenicity tests. Eight *Phytophthora* isolates from olive, representing groups A and B established here, were used; three *Phytophthora* isolates from other hosts in southern Spain and in California (Table 1): *P. megasperma sensu stricto* (isolate PM007 from peach tree); *P. medicaginis* sp. nov. (isolate PM004 from alfalfa); and *P. ciceris* sp. nov. (isolate PM033 from

chickpea) (18) were used for comparison. These last three isolates were previously assigned to *P. megasperma sensu lato*.

Plant material was obtained from a commercial nursery, and consisted of young rooted cuttings (6 to 12 months old, cv. Picual). Inocula were prepared by modifying the method of Wilcox and Mircetich (24). For each isolate, one inoculum flask was prepared by mixing 250 ml vermiculite and 20 ml autoclaved oat seeds, with 150 ml carrot broth. Each flask was inoculated with one isolate and incubated at 20°C in darkness for 30 days, stirring every 3 to 4 days. To prepare infested soil, the contents of each flask were mixed separately with 12 liters of previously autoclaved soil (sand:lime:peat, 1:1:1) (23), resulting in a concentration of 35 ml inoculum per liter of soil.

Roots were carefully cleaned under tap water before the plants were transferred into plastic pots containing 2 liters of infested soil. Six replicates of each isolate were gathered in one plastic tray to avoid cross contamination and placed in a growth chamber (228 $\mu\text{E}/\text{cm}^2 \text{ s}$ light, 14 h/day, 24°C maximum mean temperature, 19°C minimum mean temperature, 87% maximum RH, 49% minimum RH). Every tray, including the control tray, was partially filled with tap water as described previously (19).

After 4 weeks in the growth chamber, the severity of aerial and root symptoms was assessed for each plant on a 0 to 4 scale, according to the percentage of foliage with yellowing or necrosis or root necrosis (0 = 0%, 1 = 1-33%, 2 = 34-66%, 3 = 67-99%, 4 = dead plant) (19). Analysis of variance was performed for aerial and root symptom severity, and mean values were compared by the Fisher's protected LSD test (20). In addition, orthogonal comparisons were made between the mean severity values of the olive isolates and isolates from other hosts.

A second pathogenicity test was carried out using chickpea (*Cicer arietinum*) as a host. Cultivar ILC-3279 was used because of its high susceptibility to *P. ciceris* (*P. megasperma sl*) root rot (18). Three *Phytophthora* isolates were chosen: two from olive trees (PO4 and PO8) and one from chickpea (PM033). Inoculum preparation, climatic conditions, waterlogging, and statistical analysis of the data were as described above. Two-liter plastic pots were filled with infested or control soil (three pots per isolate tested plus three control pots), and 10 pregerminated chickpea seeds were placed in each pot. When the seedlings emerged, the plastic trays were partially filled with tap water.

At the end of each test, segments from inoculated and control roots were plated on PARPH (Cornmeal agar + Pimaricin, Ampicillin, Rifampicin, Pentachloronitrobenzene, Hymexazol) medium (14) for reisolation of the pathogen. Both tests were conducted twice.

Table 1. Origin and colony patterns of the isolates of *Phytophthora* studied

Isolate	Location	Date of isolation	Host/cultivar	Colony pattern ^y
PO3 ^z	La Conchuela, Córdoba	March 1996	Olive 'Arbequino'	A
PO4*	La Conchuela, Córdoba	March 1996	Olive 'Arbequino'	A
PO5	El Saucejo, Sevilla	March 1996	Olive 'Arbequino'	A
PO6	El Saucejo, Sevilla	March 1996	Olive 'Picual'	A
PO8*	El Saucejo, Sevilla	March 1996	Olive 'Picual'	B
PO10*	El Saucejo, Sevilla	March 1996	Olive 'Hojiblanco'	A
PO11	Lucena, Córdoba	April 1996	Olive 'Picual'	A
PO12*	Lucena, Córdoba	April 1996	Olive 'Picual'	A
PO13	Lucena, Córdoba	April 1996	Olive 'Picual'	A
PO15*	Ecija, Sevilla	April 1996	Olive 'Picual'	B
PO16*	Guillena, Sevilla	April 1996	Olive 'Picual'	B
PO18	Porcuna, Jaén	May 1996	Olive 'Picual'	A
PO19	Ecija, Sevilla	April 1996	Olive 'Picual'	A
PO20*	La Carlota, Córdoba	April 1996	Olive 'Arbequino'	A
PO23	Castro del Río, Córdoba	April 1996	Olive 'Picual'	A
PO24	Castro del Río, Córdoba	April 1996	Olive 'Picual'	A
PO25	Castro del Río, Córdoba	April 1996	Olive 'Picual'	A
PO26*	Castro del Río, Córdoba	April 1996	Olive 'Picual'	A
PO27*	Castro del Río, Córdoba	April 1996	Olive 'Picual'	B
PO28*	Castro del Río, Córdoba	April 1996	Olive 'Picual'	B
PO29	Castro del Río, Córdoba	April 1996	Olive 'Picual'	A
PO30	Castro del Río, Córdoba	April 1996	Olive 'Picual'	A
PO34*	Vilches, Jaén	June 1996	Olive 'Picual'	B
PO35*	Vilches, Jaén	June 1996	Olive 'Picual'	B
PO36	Vilches, Jaén	June 1996	Olive 'Picual'	B
PO37*	Vilches, Jaén	June 1996	Olive 'Picual'	A
PO38*	Vilches, Jaén	June 1996	Olive 'Picual'	B
PO39*	Vilches, Jaén	June 1996	Olive 'Picual'	B
PO40*	Vilches, Jaén	June 1996	Olive 'Picual'	A
PO41	El Mármol, Jaén	June 1996	Olive 'Picual'	A
PO42*	Espejo, Córdoba	June 1996	Olive 'Arbequino'	A
PO43	Espejo, Córdoba	June 1996	Olive 'Arbequino'	A
PO45	Espejo, Córdoba	June 1996	Olive 'Arbequino'	A
PO47*	Castro del Río, Córdoba	June 1996	Olive 'Picual'	B
PO48	Castro del Río, Córdoba	June 1996	Olive 'Picual'	B
PO49	Ubeda, Jaén	June 1996	Olive 'Picual'	A
PO50*	Fuente del Rey, Jaén	June 1996	Olive 'Picual'	B
PO51	Castro del Río, Córdoba	June 1996	Olive 'Picual'	A
PO52	Beas de Segura, Jaén	June 1996	Olive 'Picual'	A
PO53*	Torres de Albánchez, Jaén	June 1996	Olive 'Picual'	A
PO54	La Puerta de Segura, Jaén	June 1996	Olive 'Picual'	B
PO55*	La Puerta de Segura, Jaén	June 1996	Olive 'Picual'	B
PO56*	Morón, Sevilla	June 1996	Olive 'Manzanillo'	A
PO59	La Luisiana, Sevilla	June 1996	Olive 'Arbequino'	A
PO61	Cañada del Rosal, Sevilla	June 1996	Olive 'Picual'	A
PO62*	La Luisiana, Sevilla	June 1996	Olive 'Arbequino'	B
PO63*	La Luisiana, Sevilla	June 1996	Olive 'Arbequino'	A
PO64	Doña Mencía, Córdoba	July 1996	Olive 'Picual'	A
PO65	Doña Mencía, Córdoba	July 1996	Olive 'Picual'	A
PO66	Cabra, Córdoba	July 1996	Olive 'Picual'	A
PO67	Priego, Córdoba	July 1996	Olive 'Picual'	A
PO68	Priego, Córdoba	July 1996	Olive 'Picual'	A
PM004	California, USA	1989	Alfalfa	-
PM007	Sevilla	1989	Peach tree	-
PM033	Castro-Cañete, Córdoba	1990	Chickpea	-

^y Colony pattern for isolates from olive trees on carrot agar medium: A = flat to slightly petaloid, B = "rosette."

^z * = Isolates used for growth tests and morphological characterization.

RESULTS

Colony morphologies and growth rates. The colony morphology assessments on CA medium separated the isolates into two groups, A and B (Table 1). Group A comprised 36 of the 52 isolates examined. These isolates produced white, flat colonies, with a powdery appearance when oospores were abundant. Some isolates developed slight petaloid patterns. The 16 isolates of group B produced white colonies with a clear petaloid (rosette) pattern. On CMA medium, none of the isolates or groups showed differences in colony morphology.

Growth rates taken at 4 to 6 days were the most representative for all isolates. No differences were observed in growth rates of the isolates on CA or CMA. To express growth rate versus temperature, a third degree polynomial model was selected: $y = a*T^3 + b*T^2 + c*T$, with y being the growth rate, T = temperature and a , b , c , the regression constants. Two different

growth patterns, corresponding to groups A and B were obtained (Fig. 1). The optimum growth temperature estimated for the group A isolates was approximately 21°C (Table 2). This group grew slowly at 30°C. The optimum growth temperature for group B isolates was approximately 26°C (Table 2). This group exhibited fast growth at 30°C. No growth was recorded for any isolate at 35°C.

Morphological characterization. All 12 group A isolates produced gametangia in single culture. Five of the 13 group B isolates examined produced gametangia slowly and sporadically on CA (Table 4). Isolate PO34 did not produce gametangia on CA, but did do so in single culture in liquid non-sterile soil extract. (In this exceptional case, measurements of gametangial structures were made from this medium). Two other group B isolates, PO8 and PO39, mated with the A2 tester of *P. drechsleri*. The other five isolates remained sterile (Table 4).

The mean dimensions of the oogonia, oospores, and antheridia varied significantly ($P \leq 0.05$) among all the isolates, and among the isolates of each group (Tables 3 and 4). Asexual structures were morphologically similar in groups A and B, but those of group B isolates were larger than those of group A (Tables 5 and 6). Table 7 summarizes the morphological characteristics of each group of isolates. Characteristics of Group A are in a good agreement with the description of *P. megasperma* "BHR-type" or *sensu stricto* (12). Isolate PO3 was confirmed to be *P. megasperma sensu stricto* in a sequence analysis of its ITS rDNA region (7, C. M. Brasier and D. L. Cooke, unpublished data). In terms of colony pattern, sporangial type and temperature-growth relationships, group B isolates conformed closely to the "O-group" taxon of Brasier et al. (5). Furthermore, the sequences of the ITS rDNA regions of isolates PO15 and PO8 confirmed their alignment with this taxon (7, C. M. Brasier and D. L. Cooke, unpublished data).

Pathogenicity tests. All plants inoculated with A and B group isolates from olive showed extensive root necrosis accompanied by moderate to severe crown symptoms (Fig. 2). In contrast, those inoculated with *Phytophthora* isolates from other hosts had only a low level of root rot, limited crown symptoms, and exhibited growth of new rootlets. Orthogonal comparisons showed these isolates were significantly less aggressive than the olive isolates on olive cuttings (Fig. 2). No differences in aggressiveness were detected between isolates from morphological

Fig. 1. Estimated growth rate curves for the Group A isolate PO37 and the Group B isolate PO39. Points represent actual measurements. Arrows mark the estimated optimal temperatures (21.4 and 26.7°C, respectively). Within each group, the isolates exhibited similar growth patterns.

Table 2. Optimum growth temperature estimated for *Phytophthora* isolates in carrot agar medium

Isolate group	Isolate	Optimum growth temperature (°C)
A	PO3	21.3
A	PO4	20.5
A	PO10	21.7
A	PO12	21.4
A	PO20	20.1
A	PO26	21.5
A	PO37	21.4
A	PO40	21.8
A	PO42	23.7
A	PO53	22.7
A	PO56	20.6
A	PO63	20.3
B	PO8	>30
B	PO15	25.8
B	PO16	25.8
B	PO27	25.3
B	PO28	25.3
B	PO34	26.1
B	PO35	24.9
B	PO38	25.4
B	PO39	26.7
B	PO47	25.3
B	PO50	24.2
B	PO55	27.0
B	PO62	24.7

groups A and B. In the control plants, there was a low level of root necrosis, likely due to root asphyxiation, abundant production of new rootlets and no crown symptoms.

In the pathogenicity tests on chickpea, only the *P. ciceris* isolate from chickpea (PM033) produced extensive root necrosis and a total collapse of the aerial part of the plant. Control plants and plants inoculated with other isolates of *Phytophthora*, including the isolates from olive, did not show any symptoms of disease.

In all cases, the inoculated *Phytophthora* species were consistently recovered from olive or chickpea necrotic roots.

DISCUSSION

Fifty-two *Phytophthora* isolates associated with root rot and sudden death of young olive trees in southern Spain (19) were characterized. On the basis of their morphological and physiological properties, they were assigned to two distinct

groups, A and B, representing 66% and 33% of the isolates respectively.

All the group A isolates conformed to *P. megasperma sensu stricto* (13); i.e., the

broad host range (BHR) biological species group of Hansen et al. (12). They were also confirmed to be *P. megasperma* based on molecular analysis of the ITS regions.

Table 5. Characteristics of sporangia of Group A *Phytophthora* isolates in soil extract

Isolate	Sporangial length (L) (µm)	Sporangial breadth (B) (µm)	Pore width (µm)	L/B
PO-3	50.0 ± 4.9 ^y	36.8 ± 3.5	11.2 ± 1.5	1.4 ± 0.2
PO-4	36.0 ± 4.9	23.7 ± 3.8	8.5 ± 1.4	1.5 ± 0.2
PO-10	43.3 ± 4.9	29.8 ± 3.7	8.5 ± 1.7	1.5 ± 0.2
PO-12	34.7 ± 12.7	22.5 ± 10	8.7 ± 1.9	1.6 ± 0.3
PO-20	46.3 ± 8.0	28.6 ± 4.4	10.1 ± 0.6	1.6 ± 0.2
PO-26	59.8 ± 4.8	43.8 ± 4.6	11.5 ± 1.6	1.4 ± 0.1
PO-37	44.0 ± 4.5	30.7 ± 4.2	9.8 ± 0.7	1.4 ± 0.1
PO-40	38.0 ± 4.6	24.5 ± 3.4	7.6 ± 1.9	1.7 ± 0.2
PO-42	28.2 ± 7.2	19.1 ± 5.3	7.5 ± 2.1	1.5 ± 0.2
PO-53	34.0 ± 5.3	22.8 ± 6.8	5.9 ± 1.3	1.6 ± 0.3
PO-56	36.4 ± 4.7	22.3 ± 2.6	8.8 ± 2.1	1.6 ± 0.3
PO-63	40.7 ± 6.2	24.9 ± 5.4	9.6 ± 1.2	1.7 ± 0.2
LSD ^z	3.90	2.97	1.01	0.12

^y Mean values (in µm) of 25 structures measured ± standard error.

^z Least significant difference according to Fisher's protected test ($P = 0.05$) (20).

Table 3. Characteristics of sexual structures of Group A *Phytophthora* isolates in single culture

Isolate	Mean and SE (µm)					Amphigynous antheridia (%)
	Oogonium diameter	Oospore diameter	Oospore wall thickness	Antheridium length		
PO-3	44.4 ± 4.0 ^y	40.0 ± 3.8	7.8 ± 1.5	13.9 ± 1.9		6
PO-4	37.3 ± 3.9	30.0 ± 4.6	4.3 ± 1.4	12.4 ± 2.6		12
PO-10	41.4 ± 2.2	36.5 ± 2.3	6.9 ± 1.7	15.3 ± 3.3		65
PO-12	44.7 ± 4.2	39.2 ± 5.1	3.7 ± 1.1	13.4 ± 1.4		20
PO-20	41.5 ± 3.0	36.6 ± 3.0	4.9 ± 0.9	10.4 ± 1.9		17
PO-26	39.1 ± 4.4	33.7 ± 4.5	4.2 ± 1.1	12.0 ± 1.8		40
PO-37	42.0 ± 3.7	36.6 ± 3.3	4.7 ± 0.8	12.5 ± 2.4		12
PO-40	39.5 ± 2.9	34.6 ± 2.9	4.2 ± 1.1	12.8 ± 2.6		44
PO-42	40.9 ± 3.0	36.2 ± 1.5	4.9 ± 0.4	13.2 ± 2.0		16
PO-53	39.4 ± 2.9	35.5 ± 3.0	4.6 ± 0.9	9.50 ± 1.9		0
PO-56	40.9 ± 2.7	35.2 ± 2.5	4.8 ± 0.7	14.0 ± 2.0		20
PO-63	45.2 ± 3.3	41.7 ± 3.2	4.7 ± 0.8	14.7 ± 2.6		20
LSD ^z	1.90	1.92	0.60	0.88		

^y Mean values (in µm) of 25 structures measured ± standard error.

^z Least significant difference according to Fisher's protected test ($P = 0.05$) (20).

Table 4. Characteristics of sexual structures of Group B *Phytophthora* isolates in single culture

Isolate	Mean and SE (µm)				Amphigynous antheridia (%)	Sexual compatibility type in pairings with A1 or A2 <i>P. drechsleri</i>
	Oogonium diameter	Oospore diameter	Oospore wall thickness	Antheridium length		
Weakly self fertile						
PO-15	34.7 ± 7.5 ^u	31.4 ± 7.4	7.2 ± 3.0	15.8 ± 2.6	100	nt ^x
PO-27	41.4 ± 4.6	37.3 ± 3.7	5.0 ± 1.3	17.3 ± 3.6	100	nt
PO-28	43.2 ± 5.2	38.1 ± 4.7	5.1 ± 1.3	15.1 ± 2.5	100	nt
PO-47	40.5 ± 4.0	35.9 ± 3.2	5.0 ± 0.0	18.4 ± 2.2	100	nt
PO-50	43.4 ± 4.7	38.2 ± 4.1	5.3 ± 1.4	17.0 ± 3.3	100	nt
PO-34 ^v	37.1 ± 3.0	33.1 ± 3.1	4.9 ± 0.5	15.6 ± 2.3	73	A1
Self sterile						
PO-8	... ^z	...	...	...	...	A1
PO-16	...	...	...	...	...	neutral ^y
PO-35	...	...	...	...	...	neutral
PO-38	...	...	...	...	...	neutral
PO-39	...	...	...	...	...	A1
PO-55	...	...	...	...	...	neutral
PO-62	...	...	...	...	...	neutral
LSD ^w	2.80	2.57	0.86	1.57		

^u Mean values (in µm) of 25 structures measured ± standard error.

^v Sexual structures in single cultures of PO34 observed only in soil extract.

^w Least significant difference according to Fisher's protected test ($P = 0.05$) (20).

^x nt = not tested.

^y No oogonia produced in pairings with *P. drechsleri*.

^z Gametangia not produced in single culture.

Therefore, *P. megasperma* appears to be the most common species associated with olive root rot in Spain. Kouyeas and Chit-zanidis (15) described a single *Phytophthora* isolate from roots of an olive tree in Greece with similar morphological characteristics to our group A isolates. They also attributed this isolate to *P. megasperma*. Optimum growth temperatures reported by various authors for *P. megasperma* ss isolates from different hosts (excluding the 'AC' and 'DF' taxa of Hansen et al. (12), which are probably

unique *Phytophthora* species) range from 20 to 25°C (10,13). The optimum temperature for growth of all 32 olive isolates of *P. megasperma* in the present study was also 20 to 25°C, with a rapid decline in growth rate above 25°C.

Group B isolates were distinct from the *P. megasperma* isolates. Their colonies exhibited a distinctive rosette growth pattern on CA, whereas those of *P. megasperma* were uniform and flat. They had a higher optimum temperature for growth and were still able to grow rapidly at 30°C.

They produced almost exclusively amphigynous antheridia. In addition, group B isolates were either entirely self-sterile, self-sterile A1s or weakly self-fertile. Therefore, they have a different breeding system from the homothallic *P. megasperma*. A separate study indicates they are fundamentally outcrossing, heterothallic but with a degree of sporadic self-fertility (C. M. Brasier and M. E. Sánchez-Hernández, unpublished data). Their self-fertility may be due to the presence of an extra chromosome carrying the opposite mating type (2,16).

The colony types, sporangial types and temperature-growth relationships of the B-group isolates, including their rapid growth at 30°C, conform closely to those of the unusual 'O-group' taxon described by Brasier et al. (5). They also conformed to the 'O-group' in their ITS sequence (7). In a separate study, involving O-group isolates from a wide range of hosts, both A1 and A2 mating types have been identified (C. M. Brasier and M. E. Sánchez-Hernández, unpublished data). Because of its unique morphogenetic and behavioural characteristics, including a unique gametangial morphology, the 'O-group' taxon will be designated elsewhere as a new species.

Pathogenicity tests showed both the *P. megasperma* and 'O-group' isolates were highly aggressive pathogens on roots of young olive trees. In contrast, control

Table 6. Characteristics of sporangia of Group B *Phytophthora* isolates in soil extract

Isolate	Sporangial length (L) (µm)	Sporangial breadth (B) (µm)	Pore width (µm)	L/B
PO-8	78.2 ± 4.6 ^x	62.8 ± 5.4	16.9 ± 2.8	1.2 ± 0.1
PO-15	...	...	...	...
PO-16	55.0 ± 5.8	41.1 ± 5.1	10.2 ± 1.1	1.3 ± 0.1
PO-27	61.1 ± 7.8	44.1 ± 7.9	12.7 ± 1.9	1.4 ± 0.1
PO-28	...	...	...	...
PO-34	54.7 ± 6.5	40.0 ± 5.5	11.2 ± 1.7	1.4 ± 0.1
PO-35	55.8 ± 6.7	40.5 ± 6.7	9.9 ± 0.6	1.4 ± 0.2
PO-38	85.6 ± 7.5	66.5 ± 5.1	12.6 ± 2.0	1.3 ± 0.1
PO-39	62.9 ± 7.5	42.4 ± 7.3	14.5 ± 2.1	1.5 ± 0.1
PO-47	...	...	...	...
PO-50	...	...	...	...
PO-55	62.6 ± 9.1	41.9 ± 7.4	11.0 ± 1.3	1.5 ± 0.1
PO-62	64.9 ± 7.9	50.3 ± 8.2	10.4 ± 1.0	1.3 ± 0.1
LSD ^z	4.48	4.12	1.09	0.08

^x Mean values (in µm) of 25 structures measured ± standard error.

^y No sporangial production.

^z Least significant difference according to Fisher's protected test ($P = 0.05$) (20).

Fig. 2. Pathogenicity of *Phytophthora* isolates in olive tree. Scale of disease severity: 0 = no symptoms, 4 = 100% necrotic tissue. Asterisks mark the mean values not significantly different from the control (least significant difference, $P \leq 0.05$).

Table 7. Morphological characteristics of *Phytophthora* spp. Group A and B

Breeding system		Group A	Group B
		Homothallic ^x	Sterile or A1/A2 heterothallic ^y
Oogonia	Smooth	+	+
Oospores	Mean diam (µm)	41.7 ± 4.1 ^z	40.1 ± 5.7
	Aplerotic	+	+
Antheridia	Thick-walled	+	+
	Mean diam (µm)	36.9 ± 4.4	35.5 ± 5.0
	Paragynous	+	(+)
Sporangiophores	Amphigynous	(+)	+
	Mean length (µm)	12.9 ± 2.6	16.8 ± 3.3
Sporangia	Sympodial branching	+	+
	Non papillate	+	+
	Persistent	+	+
	Proliferating	+	+
	Shape	ovoid-obpyriform	ovoid
	Mean length (µm)	41.6 ± 10.2	64.2 ± 12.3
	Mean breadth (µm)	27.9 ± 8.3	47.4 ± 11.5

^x + = Character always present; + = Character usually present; (+) = Character sometimes present

^y Some heterothallic isolates weakly self-fertile.

^z Mean values (in µm) ± standard error.

Phytophthora species from other hosts, including a *P. megasperma* isolate from peach, were either weakly or non-pathogenic to olive. Moreover, the two olive isolate types were non-pathogenic to chickpea plants, while the chickpea isolate, obtained from the same area and soil type that olive isolates (19), was highly aggressive to chickpea. *P. megasperma* is viewed as having a broad host range, including an ability to attack many tree genera (e.g. 3, 10, 12). Whether the olive isolates of this species exhibit a degree of specificity towards their host needs to be investigated through additional pathogenicity tests using isolates from a wider range of hosts.

The temperature-growth requirements of *P. megasperma* and the O-group are distinct, the former being a moderate and the latter a high temperature organism. Some O-group isolates grow well even at 37°C (5). Provided the soil has a suitably high water content for infection, the two species may be at their most active in different seasons. This might represent a low level of competition between the two taxa for infection of olive roots.

Traditionally, olive trees are considered highly sensitive to "root asphyxiation" following flooding (1). However, we have shown previously that higher soil moisture levels result in higher levels of root rot (19). Based on this study, what was previously considered as "root asphyxiation" should more properly be regarded as *Phytophthora* root-rot. Such new perspectives, together with a growing understanding of the ecology and variability of the patho-

gens involved, should help to establish better management strategies for the control of the disease. It seems reasonable to propose that susceptibility to *Phytophthora* root rot may have limited the natural ecological distribution of olive trees (17). Certainly, it is clear that they should not be planted in conditions where flooding occurs or in areas with poor drainage.

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