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Occurrence and etiology of death of young olive trees in southern Spain

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Abstract

New plantations of olive tree in southern Spain are being severely affected by wilt or dieback and death, which has been locally called 'Drying Syndrome'. To determine the etiology of this problem, a study was carried out in samples of affected young trees collected during a seven year period (1989–1995), and in two field surveys in 1994–95 and 1996. Besides some insect damage and agronomic problems, the 'Drying Syndrome' was associated with Verticillium wilt, winter frost and root rot fungi. Although 'Drying Syndrome' can be distinguished from Verticillium wilt, the latter was included in this study, since, frequently, Verticillium wilt symptoms were unspecific and *Verticillium dahliae* could not be always isolated in the diagnostic work that preceded this study. Early winter frost caused a vascular necrosis and wilt of the young olive trees. This unusual and severe damage was related with the lack of frost hardiness due to warm temperatures during the previous autumn. Root rot fungi were very frequent in the samples of diseased olive trees of field or nursery origin, and they were the main cause of 'Drying Syndrome' in the second field survey, when a heavy rainfall level occurred during winter. Pathogenicity tests showed that five fungal species (*Cylindrocarpon destructans, Phytophthora megasperma, P. palmivora, Pythium irregulare* and *Sclerotium rolfsii*) were pathogenic to olive trees and reproduced symptoms of 'Drying syndrome' in rooted cuttings of cultivar Picual. Other fungal species associated with root rot of olive trees in the field or in the nurseries, including *Fusarium acuminatum, F. eumartii, F. oxysporum, F. solani, Macrophomina phaseolina* and *Rhizoctonia solani*, were weakly or not pathogenic. Pathogenicity of *P. megasperma, P. palmivora* and *P. irregulare* depended on soil water content, since isolates tested caused extensive root rot and sudden plant death only when the soil was continuously waterlogged. The high frequency of *P. megasperma* in waterlogged field soils and its pathogenicity dependence on soil water content suggest that this pathogen may play an important role in the well known sensitivity of young olive trees to 'root asphyxiation'.

Abbreviations: DS – drying syndrome of young olive trees; PHSC – plant health service in Córdoba (Spain).

Introduction

Olive tree is the most important oil crop in Andalucía, southern Spain, covering more than 1.2×10^6 ha. This distribution area has steadily increased during the last decade (Anonymous, 1983–93). Together with the establishment of new plantations, the incidence of wilting, dieback and death of young trees has also increased (Sánchez Hernández et al., 1996), alarming olive farmers. This problem, called 'Seca' in Spanish or Drying Syndrome (DS) by farmers and pest management technicians, has been diagnosed at the Plant Health Service in Córdoba (PHSC) separately from Verticillium wilt, the most important disease affecting young olive trees in southern Spain (Rodríguez-Jurado et al., 1993), mainly because *Verticillium dahliae* Kleb.

has not been isolated from affected plant tissues. The great increment of inquiries about DS received by the PHSC during the last few years led us to study the occurrence and etiology of this problem that causes important losses in new plantations.

A number of fungi have been reported to be associated with olive tree branch wilting and dieback or tree death. Notable among them are soilborne root rot fungi, such as *Armillaria* spp., *Rosellinia necatrix* Prill., *Macrophomina phaseolina* (Tassi) Goid., *Sclerotium rolfsii* Sacc. and *Omphalotus olearius* (DC.:Fr.) Singer (Aycock, 1966; Ciccarone, 1976; Andrés, 1991; Teviotdale, 1994), and some stem decay fungi such as species of *Fomes* and *Stereum* (Ciccarone, 1976). More recently the root rot fungi *Cylindrocarpon destructans* (Zins.) Scholten (Zazzerini and Marte, 1976) and *Phytophthora* spp. (Teviotdale, 1994), as well as some stem canker fungi, including *Phoma incompta* Sacc. et Mart. (Malathrakis, 1979), *Diplodia* sp. (Teviotdale, 1994) and *Eutypa lata* (Pers.:Fr.) Tul. et C. Tul. (Rumbos, 1993), also have been described as agents inducing root and crown rot and/or dieback of twigs on olive trees.

In a preliminary survey, some root rot fungal species of the genera *Armillaria, Dematophora, Macrophomina, Rhizoctonia, Thielaviopsis* and *Phoma* were isolated from young olive trees affected by DS, but it was suspected that under the unspecific symptoms of DS (wilting or dieback and death) different diseases, abiotic disorders and even pest injuries were included.

The objective of this study was to determine the nature and pathogenicity of the biotic agents, together with the abiotic factors associated with the drying syndrome of olive trees in southern Spain. Preliminary results of this work have already been reported (Sánchez-Hernández et al., 1995; Sánchez-Hernández et al., 1996).

Materials and methods

Field and nursery surveys

During 7 years (1989–1995), 372 samples of diseased olive trees were sent by farmers from all olive production areas of Andalucía to the PHSC. DS diagnosis was made by the PHSC on the basis of symptomatology of the plant samples and, if Verticillium wilt was suspected, successful isolation of the pathogen from affected tissues. When root rots were detected, isolation of fungi from the affected tissue was made. In both cases potato-dextrose agar medium (PDA) was used for isolation. No field observations were carried out.

From the enquiries received at the PHSC and direct requests addressed to the Plant Pathology Laboratory in the Department of Agronomy at Córdoba, sixty two olive tree plantations affected by DS were chosen to be surveyed during the autumn-summer period of 1994–95. A second survey was carried out during the spring–summer period of 1996 in seventy six affected new plantations. In both surveys, after a preliminary field diagnosis based on cultural and phytopathological information of the orchards (previous crops, soil type, watering, unusual weather conditions, herbicides, pest treatments and previous diagnosis), a number of DS-affected and DS-non-affected young trees per field (3–6) were sampled in order to isolate potential pathogens from diseased tissues.

Tissues from branches and stems were washed in running tap water, cut into small wedged pieces, surface disinfested in 1% sodium hypochlorite for 30 s to 2 min, blotted dry on sterile filter paper, and plated on 2% PDA and 2% water-agar (WA) media (Dhingra and Sinclair, 1985). PDA was used to allow most fungi present to grow, and WA for easy morphological recognition of sclerotia-forming fungi. Surface sterilised chips from roots and collar were plated on 2% PDA, 2% MBS (malt extract-benomyl-streptomycin) for isolation of Basidiomycetes (Singleton et al., 1992), PARP (pimaricin-ampicillin-rifampicin-PCNB), a selective medium for species of *Pythium* and *Phytophthora*, and PARPH (PARP + hymexazol), a selective medium for species of *Phytophthora* (Jeffers and Martin, 1986). The plates were incubated at 20 °C in the dark for 5–7 days and then they were placed under 14 h light period for another 5–7 days, except for PARP and PARPH plates that were incubated continuously in the dark. Fungal colonies isolated from the tissues were classified on the basis of cultural characteristics and identified by the morphology of vegetative and reproductive structures produced on different culture media. For each fungal genus, the references used to identify the species were: *Fusarium* (Booth, 1971; Toussoun and Nelson, 1976), *Cylindrocarpon* (Booth, 1966), *Rhizoctonia* (Sneh et al., 1991), *Macrophomina* (Dhingra and Sinclair, 1978), *Sclerotium* (Mordue, 1974; Punja and Rahe, 1992), *Phytophthora* (Erwin and Ribeiro, 1996) and *Pythium* (Plaats-Niterink, 1981).

In addition to field surveys, plant material affected by damping-off from three commercial olive tree

nurseries and one experimental greenhouse were also analysed. Rooted cuttings and young trees showing wilting or dieback and death were used to isolate potential pathogens from collar and roots. Isolation methods and culture media used were the same as described for field samples.

Pathogenicity tests

Fungi consistently isolated from diseased tissues and not previously identified as olive tree pathogens in Spain were tested for pathogenicity. Pure cultures of each fungus growing on corn meal agar medium (CMA) for pythiaceous fungi, and PDA medium for the rest, were used as inoculum. Plates were incubated for 10–15 days at different temperature conditions to allow each fungal isolate to produce abundant infective structures: 20-22 °C for species of *Fusarium, Cylindrocarpon, Phytophthora* and *Pythium*, and 29-31 °C for species of *Rhizoctonia, Macrophomina* and *Sclerotium* (Aycock, 1966; Dhingra and Sinclair, 1978; 1985). Inoculum suspensions were prepared by mixing the contents of six agar plates (9-cm diameter) with 600 ml of sterile deionized water in a blender for 3 min at high speed. Plant material for pathogenicity tests was obtained from a commercial nursery. Young rooted cuttings (6 months old, cv. Picual) were inoculated as follows: roots were carefully cleaned under tap water and submerged for five minutes into the inoculum suspension. Then, they were placed in plastic pots (12 cm diameter × 9 cm high, one plant per pot) containing 600 ml of previously autoclaved soil (sand:lime:peat, 1:1:1) plus 50 ml of inoculum. Twelve inoculated plants per fungal isolate plus twelve control ones were placed in the greenhouse (10–30 °C, 40–95% RH). Plants were watered once a week.

A second pathogenicity test was carried out in growth chambers, with light, temperature and humidity conditions partially controlled, trying to reproduce as close as possible the optimum conditions for infection and disease development described for each fungus tested (Aycock, 1966; Dhingra and Sinclair, 1978; 1985). Growth chamber conditions for plants inoculated with isolates of species of *Cylindrocarpon, Fusarium, Phytophthora* and *Pythium* were 18–22 °C, 50–86% RH, 14 h light period, watering once a week. Plants inoculated with *Macrophomina, Rhizoctonia* and *Sclerotium* isolates were incubated at 25–30 °C and watered once every two weeks. Plant material, inocula preparation and inoculation procedure were similar to the first experiment. For pythiaceous isolates the experiment was carried out as described for the other fungi, except that plants grew under two different soil water conditions: in soil watered once a week, and in continuously water saturated soil. In the last case, pots with inoculated and control plants were placed separately in trays filled with tap water. The water level in the trays was maintained between 4–6 cm below the pot soil surface by adding tap water periodically. Pathogenicity tests for pythiaceous fungi were carried out both under greenhouse and growth chamber conditions (10–30 °C, 40–95% RH for the greenhouse and 18–22 °C, 50–90% RH and 14 h light period for the growth chamber). Similarly to the other experiments, twelve pots (one plant per pot) for each fungal isolate or control in both soil water conditions were used.

Severity of aerial symptoms was periodically assessed for each plant on a 0–4 scale, according to the percentage of foliage with yellowing or necrosis (0 = 0%, 1 = 1–33%, 2 = 34–66%, 3 = 67–100%, 4 = dead plant). At the end of each experiment, root rot was assessed by using the same 0–4 scale. Added to that, chips from inoculated and control roots were plated on PDA, PARP and PARPH to reisolate each inoculated fungus and other fungi present in the root tissues. Incubation conditions of plates and methods for identification of reisolated fungi were the same as described above. Analysis of variance was performed for the aerial and root symptom severity and mean values were compared with those from control plants by the Dunnett's test (Steel and Torrie, 1985).

Results

Field and nursery surveys

Results of the diseased olive samples received by the Plant Health Service in Córdoba for the period 1989–1995 are summarized in Table 1. Every sample originated from a different field. From a total of 372 samples, *V. dahliae* was isolated from affected tissues of 69 samples. Root rots were frequent, and various fungi were consistently associated with diseased roots, including *Cylindrocarpon* sp. (23 samples), *Rhizoctonia* or *Sclerotium* sp. (22 samples), *Fusarium* spp. (18 samples), *R. necatrix* (14 samples) and *Armillaria* spp. (13 samples). No fungi were isolated from 24 samples exhibiting root rot symptoms. Most of the root rots associated with the last two plant pathogenic fungal genera occurred in olive trees older than 50 years. Two insect borers, *Euzophera pinguis* Haw (Lepi-

Table 1. Factors associated with Drying Syndrome in samples of olive trees received by the Plant Health Service at Córdoba during 1989–1995[a]

Tree age (years)	Verticillium wilt	Root Rots[b]	Frost	Pests[c]	Others[d]	Unknown[e]	Number of fields
0–3	9	9	5	14	20	3	49
4–10	50	42	18	4	31	29	184
>10	10	63	3	3	14	45	139
Total	69	114	26	21	65	77	372

[a] Every sample came from a different field in Andalucía, southern Spain.
[b] Fungi consistently associated were unidentified species of the genera *Cylindrocarpon, Rhizoctonia, Fusarium, Dematophora* and *Armillaria.*
[c] The insect pests *Euzophera pinguis* and *Resseliella oleisuga* were identified for any age tree in 18 and 3 fields, respectively.
[d] Several injuries: nutrient deficiencies or toxicities, high salt content in soil or in irrigation water, excess of water and rodent damage.
[e] No biotic or abiotic agent was identified.

doptera: Pyralidae) and *Resseliella oleisuga* (Targ.) Coutin (Diptera: Cecidodymae), were associated with DS for trees of all ages in 18 and 3 fields, respectively.

Severe symptoms caused by frost were also frequent (26 samples). Other abiotic disorders observed were associated with nutrient deficiencies, high salt content in the irrigation water and poor drainage. Rodent damage was also recorded. No biotic or abiotic agent was associated with DS in 77 samples.

Results from the surveyed fields in 1994–95 are shown in Table 2. Plant pathogenic fungi were consistently isolated from tissues of DS-affected trees from 36 of the 62 fields surveyed. Vascular wilt due to *V. dahliae* infection was the most common disease present in new olive tree plantations, being the main cause of death of young trees between 4 and 10 years old.

Trees less than 4 years old were the main age-group affected by DS and also showed a high incidence of Verticillium wilt. Surprisingly, root rots were infrequently associated with DS. No *Pythiaceae* organisms were isolated, but *C. destructans* and *M. phaseolina* were consistently isolated from tree roots in two of three fields in groups of trees with 0–3 or 10–20 years old. *Rhizoctonia solani* Kühn and *Dematophora necatrix* Hartig, the anamorph of *Rosellinia necatrix*, were consistently associated with root rot-affected trees in the rest of the field in the 0–3 and 10–20 years old groups, respectively. *Fusarium oxysporum* Schlechtend.: Fr. and *F. solani* (Mart.) Sacc. also were isolated frequently from rotten roots in 5 and 4 fields, respectively.

Injury due to early winter frost was the main cause of death in the youngest trees (Table 2). Affected trees showed uncommon frost damage: wilting of the aerial part of the tree associated with an extensive xylem necrosis of the main stem and lateral branches. This vascular necrosis was present from the ground line to the top of the tree. The underground stem and roots were not affected. In most cases stem cankers observed were associated with pruning wounds, but no fungi were isolated either from the necrotic tissue or the healthy surrounding tissue. Likewise, no fungal isolations were obtained from the necrotic vascular tissue.

Other abiotic disorders observed were some injuries due to inappropriate culture practices: scald by unproper use of protective black plastic bags around the stem, even during the hot summer, excessive fertilisation with fresh manure, collar girdling below the ground level produced by plastic ropes which were used to tie the young trees to a stake, and damage by animals (rabbits and moles).

In six cases DS symptomatology was at first confused with a quick dieback of the whole tree due to insects, including stem borers (*Euzophera pinguis* in one field and *Resseliella oleisuga* in three fields) and root feeding by *Melolontha* sp. in two fields.

Results from the 1996 surveys are shown in Table 3. In 69 out of 76 fields surveyed, potentially plant pathogenic fungi were consistently isolated from diseased tissues. Again vascular wilt associated with *V. dahliae* was usually found in trees less than 10 years old.

Similar to the 1994–95 results, the youngest trees were the most affected by DS. But, in contrast with

Table 2. Factors associated with Drying Syndrome in 62 olive fields surveyed in southern Spain during 1994–95[a]

Tree age (years)	Verticillium wilt	Root Rots[b]	Frost	Pests[c]	Others[d]	Unknown[e]	Number of fields
0–3	9	3	12	5	4	0	33
4–10	18	0	0	1	0	2	21
11–20	3	3	0	0	1	1	8
———	—-	–	—-	–	–	–	—-
Total	30	6	12	6	5	3	62

[a] Every sample came from a different field in Andalucía, southern Spain.
[b] Fungi consistently associated were *Cylindrocarpon destructans*, *Macrophomina phaseolina*, *Rhizoctonia solani* and *Dematophora necatrix*.
[c] The insect pests *Euzophera pinguis* and *Resseliella oleisuga* were identified in 5 and 1 field, respectively.
[d] Several injuries: mechanical girdling of the main stems, excess of manure and rodent damage.
[e] No biotic or abiotic agent was identified.

Table 3. Factors associated with Drying Syndrome in 76 olive fields surveyed in southern Spain during 1996[a]

Tree age (years)	Verticillium wilt	Root Rots associated with		Frost	Others[b]	Unknown[c]	Number of fields
		Phytophthora megasperma	*Cylindrocarpon destructans*				
0–3	10	22	0	2	1	0	35
4–10	7	20	2	0	0	4	33
11–20	3	4	1	0	0	0	8
———	—-	—-	–	–	–	–	—-
Total	20	46	3	2	1	4	76

[a] Every sample came from a different field in Andalucía, southern Spain.
[b] Herbicide damage.
[c] No biotic or abiotic agent was identified.

the situation found in the former survey, occurrence of root rots was high, being the main cause of death for trees between 0 to 10 years old. In this case, diseased trees were found mainly in waterlogged plantations. Roots from those trees showed extensive necrosis with almost total loss of rootlets, occasionally affecting the collar, and even causing partial or total loss of the underground stem bark. Only two fungal species were consistently isolated from rotten roots*: Phytophthora megasperma* Drechs. in 46 fields and *Cylindrocarpon destructans* in another three fields. In contrast with the preceding year, DS cases associated with abiotic disorders were not as frequent, since frost injury was found only in two fields.

It is worth pointing out that there were important differences between climatic conditions during both field surveys. Figure 1A shows temperature and rainfall during the first survey period: autumn 1994–summer 1995. It deserves special attention because of the low level of rainfall registered, and by the fact that 1995 was preceded by 4 years of severe drought that affected southern Spain. Another remarkable point is the unusual temperature conditions, which were high during the autumn of 1994 followed by a sudden drop in temperature at the end of December.

By contrast, autumn temperatures were normal for southern Spain during the second survey period (Figure 1B), ending the long drought period. Winter rainfall was unusually high for that zone, resulting in extensive flooding of the olive orchards planted in areas with poor drainage.

Results from nursery surveys revealed that some fungi isolated from rotten roots in the field were also present in nursery plants affected by damping-off (*C. destructans*, *F. solani*, *R. solani*). Other fungi found only in nurseries were: *Fusarium eumartii* Carpenter, *F. acuminatum* Ellis & Everh., *Pythium irregulare* Buisman and one species of the genus *Phytophthora* provisionally identified as *P. palmivora* (Butler) Butler on the basis of its asexual structures. Only the last two species were consistently associated with diseased plants.

Table 4. Origin and identification of fungal isolates tested for pathogenicity on olive trees

Species	Field isolation level (%)[a]	Isolate	Origin	Date of isolation
Cylindrocarpon destructans	6.0	CD1	Nursery	Aug1995
		CD3	Field	Jun 1996
		CD4	Field	Jun 1996
Fusarium oxysporum	83.0	FO1	Field	Feb 1995
		FO2	Field	Jun 1995
Fusarium solani	66.0	FS1	Field	Jun 1995
		FS2	Nursery	Aug 1995
Fusarium eumartii	–	FE1	Greenhouse	Apr 1996
Fusarium acuminatum	–	FA1	Nursery	Jul 1996
		FA2	Nursery	Jul 1996
Macrophomina phaseolina	33.0	MP1	Field	May 1995
		MP2	Field	May 1995
Phytophthora megasperma	94.0	PM1	Field	Mar 1996
		PM2	Field	May 1996
Phytophthora palmivora	–	PP1	Nursery	Jul 1996
Pythium irregulare	–	PI1	Greenhouse	Jun 1996
Rhizoctonia solani	33.0	RS1	Field	Feb 1995
		RS2	Greenhouse	Apr 1996
Sclerotium rolfsii	0.8	SR1	Field	Mar 1995

[a] Number of orchards with positive isolation/Number of orchards affected by root rot (114 olive samples diagnosed by PHSC, or 6 and 49 orchards in the first and second survey, respectively) x 100.

Pathogenicity tests

A number of isolates were chosen for pathogenicity tests: those consistently associated with root rots in field and nursery surveys and others not consistently isolated from diseased plants, but very frequently found in most fields associated with rotten roots. None of the selected fungal species were previously described as olive tree pathogens in Spain. Table 4 shows the origin and identification of these isolates.

Results of the first pathogenicity test carried out under greenhouse conditions are summarized in Figure 2. Control plants were affected by some root necrosis and they showed a very low level of aerial symptoms. For this reason, symptom severity of the inoculated plants was compared with those in the control. Only the plants inoculated with isolates SR1 and FO1 showed symptom incidence and severity significantly higher than control plants. The isolate of *S. rolfsii* (SR1) caused an extensive necrosis on the base of the cuttings, affecting the collar and root insertion, while the isolate FO1 of *F. oxysporum* caused necrosis of the rootlets. *S. rolfsii* was reisolated from all pieces of collar tissue and *F. oxysporum* was reisolated from 83% of the root pieces. Plants inoculated with other isolates only showed some root necrosis and aerial symptoms which did not differ from the control plants. However it should be noted the high reisolation level (100%) of *F. solani* and *R. solani* from their respective inoculated roots. In the control plants, only *F. solani* and *R. solani* were isolated occasionally from necrotic roots.

The second pathogenicity test was carried out under more favourable conditions for disease development. Results from this experiment (Figure 3) corroborate the former, and demonstrate the pathogenicity of *C. destructans* isolates from the 1996 survey (CD3, CD4). Plants inoculated with CD3 and CD4 isolates showed a severe root rot, particularly affecting to the rootlets. Values of symptom incidence and severity were highly significant, with the reisolation level (100%) of each inoculated organism being also high. For isolate SR1, besides the extensive necrosis on the base of the olive cuttings, it was even possible to see the white fungal mycelium growing around the collar of inoculated plants. As in the first test, *F. solani* showed the maximum level of reisolation from roots inoculated with

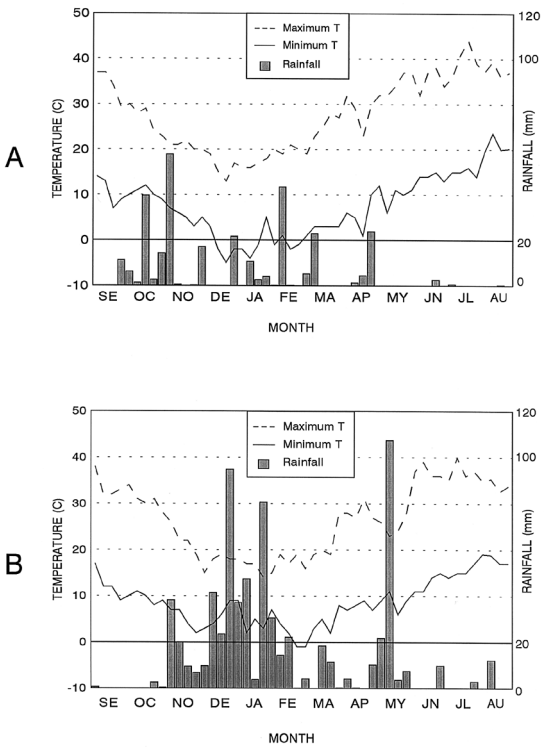

Figure 1. Temperature fluctuations and weekly rainfall levels in Córdoba, southern Spain. (A) September 1994–August 1995. (B) September 1995–August 1996.

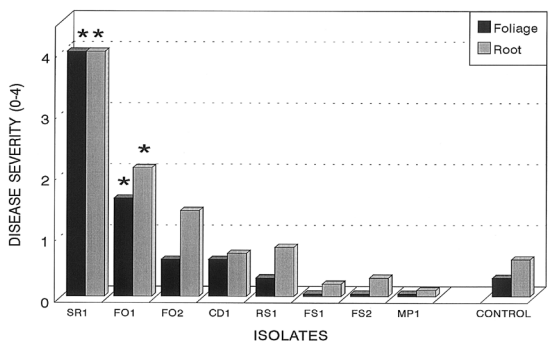

Figure 2. Pathogenicity of soilborne fungal isolates to olive tree cuttings under greenhouse conditions. Six months old plants were grown for one month in sterile soil infested with isolates of *Sclerotium rolfsii* (SR1), *Fusarium oxysporum* (FO1, FO2), *Cylindrocarpon destructans* (CD1), *Rhizoctonia solani* (RS1), *Fusarium solani* (FS1, FS2) and *Macrophomina phaseolina* (MP1). Symptom severity was assessed on a 0–4 scale according to percentage of foliage or root with yellowing, wilting or necrosis (0=0%, 1=1–33%, 2=34–66%, 3=67–100%, 4=dead plant). Asterisk denotes mean values significantly different from the control according to Dunnett's test (P=0.05).

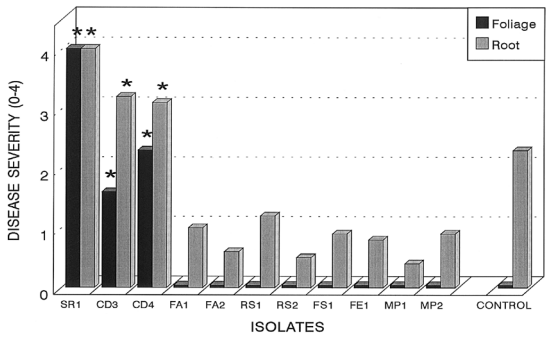

Figure 3. Pathogenicity of soilborne fungal isolates to olive tree cuttings under growth chamber conditions. Six months old plants were grown during one month in sterile soil infested with isolates of *Sclerotium rolfsii* (SR1), *Cylindrocarpon destructans* (CD3, CD4), *Fusarium acuminatum* (FA1, FA2), *Rhizoctonia solani* (RS1, RS2), *Fusarium solani* (FS1), *Fusarium eumartii* (FE1) *and Macrophomina phaseolina* (MP1, MP2). Symptom severity was assessed on a 0–4 scale (as in Figure 2). Asterisk denotes mean values significantly different from the control according to Dunnett's test (P=0.05).

isolate FS1 (100%), even when plants showed a very low level of root necrosis without foliar symptoms. A similar high level of reisolation was recorded for plants inoculated with *F. acuminatum* (75% for plants inoculated with FA1 and 100% for plants inoculated with FA2, *M. phaseolina* (maximum reisolation level for plants inoculated with both isolates MP1 and MP2) and *R. solani* (66% for plants inoculated with isolate RS1 and 100% for plants inoculated with the isolate RS2). Control plants remained free of foliar symptoms, but some root rot was observed. *C. destructans* and, at a lower level, *F. solani* and *P. palmivora* were isolated from necrotic roots of control plants.

Results of pathogenicity tests with pythiaceous isolates under greenhouse conditions are shown in Figure 4. Almost no foliar symptoms were recorded under weekly watering conditions for inoculated and control plants. In contrast, under waterlogging conditions, all plants, controls included, showed some development of aerial symptoms (Figure 4A). The severity values of foliar symptoms were significantly higher for plants inoculated with isolates PM1 and PM2 in comparison with control plants, under waterlogged (C1) or non waterlogged (C2) conditions. Statistical analysis also showed no significant differences between foliar severity values of non-inoculated plants, independently of soil water conditions. Concerning root rot development (Figure 4B), severity values were significantly higher for plants inoculated with all isolates under waterlogged conditions in comparison with data from their corresponding control plants (C1). Moreover, under

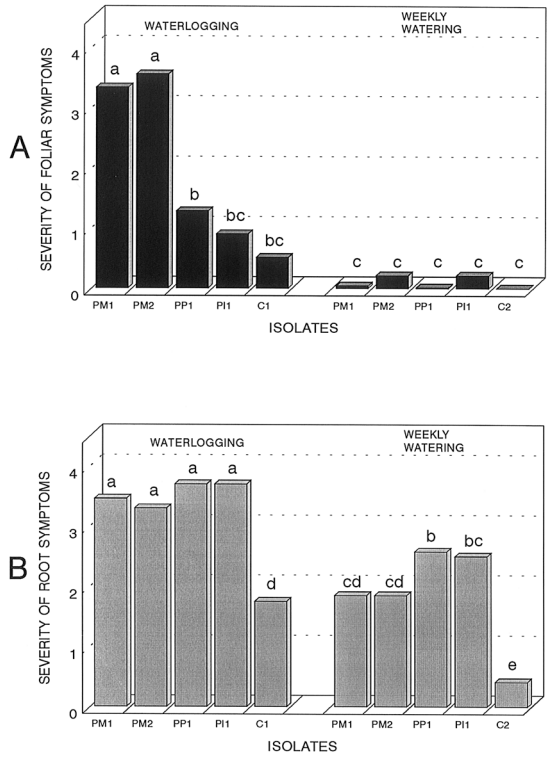

Figure 4. Pathogenicity of some pythiaceous isolates to olive tree cuttings: Foliar (A) and root (B) symptoms. Plants were planted in sterile soil (C1, C2) or in soil infested with two isolates of *Phytophthora megasperma* (PM1, PM2), one isolate of *Phytophthora* sp., provisionally identified as *P. palmivora* (PP1), or one isolate of *Pythium irregulare* (PI1). Symptom severity was assessed on a 0–4 scale (as in Figure 2). Bars with different letters are mean values significantly different according to Fisher's protected LSD test (P=0.05).

weekly watering conditions, root rot severity was also higher than the values from their corresponding control plants (C2). On the other hand, root rot severity was significantly higher for C1 control plants compared to C2 control plants. No differences in pathogenicity were recorded for both *P. megasperma* isolates. Reisolation percentage of inoculated fungi from necrotic roots was higher for plants maintained under waterlogged conditions. None of the inoculated species was recovered from control plants, in treatments C1 or C2. *R. solani* and *F. solani* were isolated occasionally from the roots of C2 control plants, and only *F. solani* from C1 roots.

The experiment was repeated under growth chamber conditions. In this case, all the isolates tested, even PI1, caused the death of the waterlogged plants in a short period of time (2 weeks). Waterlogged control plants remained free of aerial symptoms, exhibiting some degree of root rot which was significantly less than in inoculated plants. In this short period of time, no foliar or root symptoms were recorded under weekly watering conditions, neither for inoculated nor for control plants.

Discussion

A number of different causes of death of young olive trees, after their successful establishment in the field, have been included under the name of DS. Common symptomatology observed includes a sudden wilting and death of young trees or a slower wilting, dieback and death that is usually accompanied by defoliation (Sánchez-Hernández et al., 1996).

Frequently, the observed sudden wilting of twigs and branches without loss of leaves (apoplexy) was the result of infection by *V. dahliae* (Blanco-López et al., 1984). The high incidence of Verticillium wilt observed is likely due to the establishment of new olive tree plantations on land previously cropped to plants susceptible to *V. dahliae*, and the probable use of infested soil or infected planting material in olive nurseries (Rodríguez-Jurado et al., 1993; Thanassoulopoulos, 1993). This disease can be easily diagnosed in trees older than 10 years, because of the presence of a characteristic brown vascular discoloration (Ciccarone, 1976). However, *V. dahliae* infections in younger trees often resulted in death of the whole tree without any vascular discoloration (Wilhelm and Taylor, 1965). This fact, together with the unsuccessful pathogen isolation from affected olive trees should be the reason for the negative diagnosis of Verticillium wilt in these affected fields made by Pest Management technicians and the PHSC, and therefore the inclusion of these fields in the DS surveys. Nevertheless, the lack of isolation of *V. dahliae* seems common for this disease (Wilhelm and Taylor, 1965).

At the first field survey period (1994–95), an uncommon winter frost injury that severely affected the youngest trees (0–4 years old) was observed. These trees showed an extensive wilting of leaves and branches without defoliation, together with a dark brown xylem necrosis. It is remarkable that there was a lack of cracks on the outer bark which are typical symptoms described on olive tree branches and main stems affected by frost injury (Graniti, 1993; Roselli and Verona, 1989). This atypical symptomatology and its manifestation from January to June has likely been the reason why farmers and Pest Management technicians have

not been able to identify the causes of wilting in the affected fields. In some cases the deep brown colour of the xylem was usually confused with a vascular wilt disease.

Most of young trees showing this unusual symptoms were growing in valleys where winter frosts were exceptionally sudden and severe after the very warm autumn of 1994. It has been reported that olive tree cultivars tolerate winter frosts relatively well (Graniti, 1993), but in the winter of 1994 these very young trees were still actively growing when several days of temperatures below 0 °C occurred in southern Spain. The lack of hardiness exhibited by these trees would be the reason for the unusual winter frost damage observed. A similar situation has been observed for some forest trees (Livingston, 1994). This author reported that warm autumn temperatures reduced freezing tolerance in shoots of spruce and pine seedlings. In our case, after a very warm autumn, active green tissues of young olive trees (branches and stem) were suddenly submitted to very low temperatures (−5 to −7 °C), and this fact could have resulted in a lack of frost tolerance and, as a consequence, the unusual frost symptoms observed.

The relatively low incidence of root rots observed in the first survey period, in spite of the preliminary data, could be a consequence of the drought that occurred during the previous four years in southern Spain. In fact, no pythiaceous organisms were isolated, but fungi such as *M. phaseolina* that are usually found in dry soils under high temperature conditions (Dhingra and Sinclair, 1978) were present.

In contrast with the described situation, the incidence of the different causes of DS changed during the second field survey period (1996). Despite the high incidence of Verticillium wilt recorded again, the very different climatic conditions determined the nature and relative importance of diseases and abiotic injuries that resulted in the wilting and death of young trees observed in the fields. In fact, the usual behaviour of temperatures during the autumn-winter period of 1995–96 resulted in a drop of the incidence of frost injuries. Nevertheless, the unusual frost symptoms described above were again observed in two fields, corresponding with areas where temperature locally dropped under the average level for this period of time.

On the other hand, root rots were very frequent, being the main cause of wilting and death of the youngest plantations surveyed (0–10 years old) and also being the main cause of dieback of the oldest ones (10–20 years old). The high level of rainfall recorded during this period of surveys, not only in absolute values but also in frequency, could explain this change in the incidence of root rot, since the young olive plantations that remained waterlogged during long periods of time were the most affected by DS. In fact, severely affected fields appeared in valley zones and/or zones submitted to poor drainage. In spite of the general consideration about the high sensitivity of olive trees to root asphyxiation by an excess of soil water (Barranco et al., 1997), it has been possible to isolate *P. megasperma* and, at lower level *C. destructans*, from rotten roots in every waterlogged plantation surveyed, suggesting a heavy implication of these fungi, particularly *P. megasperma*, in the development of root necrosis and death of trees.

For nursery/greenhouse surveys, it is interesting to note that fungi consistently isolated from roots of plants affected by damping-off were never found associated with root rots in the field. However, most of these fungal species are very frequent in the field soils of the area surveyed (Trapero-Casas and Jiménez-Díaz, 1985; Trapero-Casas et al., 1990).

All fungal species tested for pathogenicity were soilborne fungi causing root rot in many different hosts. Frequently, a mixture of these fungi has been associated with diseased olive tree roots (Boulila et al., 1995; Ciccarone, 1976; Wilhelm et al., 1962). With the exception of *P. irregulare* and the fungus provisionally identified as *P. palmivora*, the other fungi that were pathogenic in our experiments have been previously isolated from olive trees. In Greece, Georgopoulos and Thanasoulopoulos (Aycock, 1966) mentioned *S. rolfsii* as an olive tree pathogen and Kouyeas and Chitzanidis (1968) reported the same about *P. megasperma*. In Italy, Zazzerini and Marte (1976) demonstrated the pathogenicity of *C. destructans* to olive trees. Several species of *Phytophthora*, including *P. citricola* and *P. drechsleri*, have been associated with root and crown rot of olive trees in California (Teviotdale, 1994). *Pythium ultimum* and other non-identified species of *Pythium* and *Phytophthora* have also been associated with root rots of olive tree (Farr et al., 1989; Wilhelm et al., 1962).

Moreover, our pathogenicity tests demonstrated that isolates of five fungal species (*C. destructans, P. megasperma, P. palmivora, P. irregulare, S. rolfsii*) were clearly pathogenic to olive trees and reproduced typical symptoms of DS in rooted cuttings of cv. Picual. Pathogenicity *of P. megasperma, P. palmivora* and *P. irregulare* depended on soil water content, since the isolates tested caused extensive root rot and sud-

den plant death only when the soil was continuously waterlogged. Under the usual watering rate required by olive plants, only *S. rolfsii* and some field isolates of *C. destructans* clearly caused a severe root rot leading to aerial symptoms. Both isolates of *P. megasperma* caused some degree of root rot under weekly watering conditions without development of aerial symptoms. By contrast, isolates of pythiaceous fungi from nurseries caused a root rot significantly higher under these conditions. However, no aerial symptoms were observed in these plants. It is also noteworthy that the nursery isolate of *C. destructans* was weakly pathogenic, and this situation was similar to the rest of the non-pythiaceous isolates tested. As *P. palmivora* and *P. irregulare* have never been found associated with diseased trees in the field, we can conclude that root rot fungi present in the field do not seem to come from nurseries, at least from those located in the zones surveyed. Damping-off observed in olive nurseries should be due to the influence of two factors: presence of the damping-off fungi found, together with poor drainage conditions.

As plant material used for the pathogenicity tests came from a commercial nursery, that could be the reason why it was not possible to have plants totally free of root fungi. This fact could determine the appearance of some level of root rot in control plants and could interfere with the experimental evaluations, since fungi present in plant roots were similar to some isolates tested, such as *C. destructans*, *F. solani* or *F. oxysporum*. For this reason, it was difficult to evaluate the real importance of weakly pathogenic fungi found associated with rotten roots of olive trees in the field. The possibility that these fungi could play a role as secondary pathogens under stress situations should be taken into account. Concerning the role played by *P. megasperma* in the death of young olive trees subjected to flooding conditions, it is necessary to continue experimental work under field and controlled conditions to characterise pathogen population, its pathogenicity to olive cultivars and its dependence on soil water content. Clarification of that role could be a matter of special relevance, since the cv. Picual is being planted extensively in southern Spain because of its good productivity and agronomic characteristics, even in zones with poor drainage conditions.

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