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Phytophthora palmivora: A New Pathogen of Olive Trees in Morocco

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Abstract

In spring of 2012, olive-trees with crown dieback, root rot and defoliation were observed in two years old olive tree in commercial plantations of tree nurseries in Sidi Taibi and in twenty to fifty years old field trees in Souk El Arbaa olive crops in Northwest of Morocco (Gharb area). The objective of this study was to isolate the responsible pathogen of the observed symptoms to the olive trees, to demonstrate its pathogenicity and fulfill the Koch´s postulate. Phytophthora palmivora was consistently isolated from roots (56%) and stems (73.6%) of the young olive trees and 85% from stems of field trees. Koch's postulate was completed using two isolates of Phytophthora palmivora on 2-year old plants of Dahbia and Haouzia varieties grafted onto wild olive-trees. The affected branches percentages (Pab%) of the inoculated olive plants with the isolate 1 were higher (81.8% for Dahbia and 68% for Haouzia) than those what were inoculated with the isolate 2 (43% for Dahbia and 32% for Haouzia). The reisolation percentages (Pr%) of isolate 1 (84%) and isolate 2 (76%) in the roots of Dahbia variety were higher than isolate 1 (48%) and isolate 2 (55%) in roots of Haouzia variety. The reisolation percentage of isolate 1 in the stem of Dahbia (64%) was higher than that in the stem of Haouzia (41.33%). No significant difference was observed between the Reisolation percentages of isolate 2 in stem of Dahbia olive plants (38%) and in stem of Haouzia olive plants (33%). The pathogenicity of *P. palmivora* was demonstrated in the olive plants and this was the first report of this pathogen in Moroccan olive trees.

Key words: Olive tree crown dieback, root rot, defoliation, Phytophthora palmivora, Koch's postulate, pathogenicity.

Introduction

Olive-tree (*Olea europaea* L.) is one of the most ancient domesticated fruit trees and the most extensively cultivated fruit crop in the world (Fabbri, 2009). In Morocco, the olive plantation is estimated at about 650 000 ha accounting for 50% of the national surface dedicated to arboreal crops (Ministry of Agricultural and Marine Fischeries, 2009).

This cultivar is susceptible to major fungal diseases particularly to leaf-spot disease caused by Spilocaea oleagina, the most widespread fungal disease of olive tree in the world (Anton & Laborda, 1989), Verticillium dahliae responsible to defoliation and wilting of olive trees and death of young trees (Vossen et al., 2008), and Fusarium solani that provokes the root rots to the olive trees (Perez et al., 2011). In 2012, a survey of nurseries in Sidi Taibi and in olive trees field in Souk El Arbaa, allowed us

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Figure 1. Leaf chlorosis, defoliation, wilting and twig in some olive trees collected from Sidi Taibi nurseries (A) and Souk El Arbaa fields in the Gharb area (B).

to remark the presence of leaf chlorosis, defoliation, wilting and twig dieback in some olive plants (Figure 1).

The objective of this study was to isolate the responsible pathogen of the observed symptoms to the olive trees, to demonstrate its pathogenicity and to fulfill the Koch´s postulate.

Material and Methods

Pathogen Isolation

One hundred stem segments and one hundred root segments of olive trees were taken from commercial plantations of tree nurseries in Sidi Taibi and one hundred stem segments from the olive crops in Souk El Arbaa, washed with water, disinfected with alcohol for five minutes, put on sterile distilled water and then dried with sterile filter paper. Then they were put on PSA agar plates (Potato Sucrose Agar: 200 g potato, 15 g sucrose, 20 g Agar-Agar, and 1,000 ml distilled water) and incubated on darkness at 28°C. The developing colonies were then observed for species determination.

Isolation percentage (Pi %) was obtained by applying the following formula:

$$Pi = N_{sX} / N_T \times 100$$

N_{SX}: Number of segments containing the fungal species X.
N_T: Total number of used segments.

Nine mm diameter agar plugs from these plates were placed in a 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 and 1 litre of distilled water. After 24 h at 20°C, the mixture was filtered and Petri dishes were incubated in darkness at 15°C. After each 24 h incubation, the soil extract was removed and replaced with fresh extract previously stored at 4 oC. Sporangial production and development, occurring between 12 and 120 h, was assessed by direct observation using an optical microscope. Mature sporangia were removed and placed on glass microscope slides and stained with blue cotton.

Inoculum Production

Zoospores of *P. palmivora* were produced by growing cultures on oatmeal agar at 28°C in the dark for 14-21 days. The mycelium was transferred to a sterile Petri dish, covered with sterile distilled water (SDW) and incubated overnight at 28°C, under lights. The mycelia plates were chilled for 5 min at -20°C to induce zoospores release. The concentration of the inoculum was adjusted at 105 zoospores/ml by SDW.

Inoculation Test

The Koch's postulate was verified by inoculating twelve olive plants of Haouzia and Dahbia varieties (six plants per variety) with two isolates of *Phytophthora palmivora* (Isolate 1: isolated from the nursery olive trees and Isolate 2 : isolated from the Souk El Arbaa olive trees field). Three plants from each variety were none inoculated and used as a control.

Plants were inoculated according to the method described by Olbricht et al. (2006). The roots of investigated plants were washed under running water to discard soil remnants, trimmed to 2/3 of their length, and subsequently dipped in the prepared inoculum during 6 hours. The olive trees plants were potted into universal soil substrate, watered with the remaining fungal suspension (about 20 ml per plant), and cultivated in a greenhouse. For control plants, sterile water was used instead of fungal suspension.

Pathogen Reisolation

At the end of each test, twenty five roots and stems segments were taken from inoculated plants and controls, washed with water, disinfected with alcohol for five minutes, put on SDW and then dried with sterile filter paper. After, they were plated on PSA and incubated on obscurity at 28°C

The symptoms were evaluated after five weeks of inoculation, the number of new formed branches (N_{nb}) and the number of affected branches (N_{ab}) were estimated on the inoculated olive plants and the percentages of the affected branches (P_{ab}%) were calculated using the formula :

$$P_{ab}(\%) = N_{ab} / N_{nb} \times 100$$

Reisolation percentage (Pr %) was obtained by applying the following formula:

$$Pr = N_{sPp} / N_T \times 100$$

NSPp: Number of segments containing *Phytophthora palmivora*.
NT: Total number of segments used in the reisolation.

Analysis of the variance and of the mean comparisons using the LSD test (p = 5%) were performed using the software STATISTICA program. Statistical analyses focused on the results of five plants, for the affected branches and five repetitions for the reisolation percentage of the inoculated and non inoculated plants.

Results and Discussion

Fungi morphological characterization showed that all isolates produced papillate sporangia on the soil extract medium (Figure 2a), which were ellipsoid to ovoid with a length of 18.31 µm and a larger of 13 µm. Some isolates produced subglobose, non-papillate sporangia (Figure 2b) and abundant chlamydospores (Figure 2c), sexual forms were absents (heterothallic species).

The pathogen causing disease on the olive trees was identified as *Phytophthora palmivora* on the basis of morphological and cultural characters especially on sexual and asexual reproduction forms (Ho et al., 1992. Cacciola et al., 2000. Lucero et al., 2006. Gallegly and Hong, 2008).

On the PSA plates, some colonies appeared four days later on the segments of stems and roots of the diseased olive trees (Figure 3). The microscopic observations leaded us to identify simultaneously the isolated species as *Phytophthora palmivora* (Pi=73.6%) and *Alternaria alternata* (Pi=26.4%) in the stem fragments of the young olive trees from the nurseries. In the field olive-trees, the isolated species from the stem fragments were *P. palmivora* (85%) and *A. alternata* (15%). The root segments of the young olive trees showed the presence of *P. palmivora* and *Fusarium solani* with 56 and 44% frequencies respectively.

After five weeks of inoculation, all the plants showed defoliation (Figure 4-A2, A3, B2 and B3), wilting, degradation and reduction of root system (Figure 4- C2, C3, D2 and D4) necrosis and dropping of the new branches and vascular browning primarily of the xylem tissue (Figure 4).

Table 1 showed that Dahbia olive trees inoculated with isolate 1 formed on average of 12.8 branches (Figure 5A) significantly fewer at p = 5% than those inoculated with isolate 2 (21.6). Haouzia olive plants inoculated with isolate 1 and isolate 2 formed the same number of new branches (17). The obtained results were statistically significant at the 5% level for the isolate 2 of *Phytophthora palmivora*, this isolate affected more new branches in Haouzia (5.6) and Dahbia (9) varieties than isolate 1 in the same varieties (11.8 and 10.8, respectively) (Figure 5B).

The affected branches Percentages of the inoculated olive plants with the isolate 1 were significantly higher (81.8% for Dahbia and 68 % for Haouzia) than those of plants varieties inoculated with isolate 2 (43% for Dahbia and 32% for Haouzia) (Table 1).

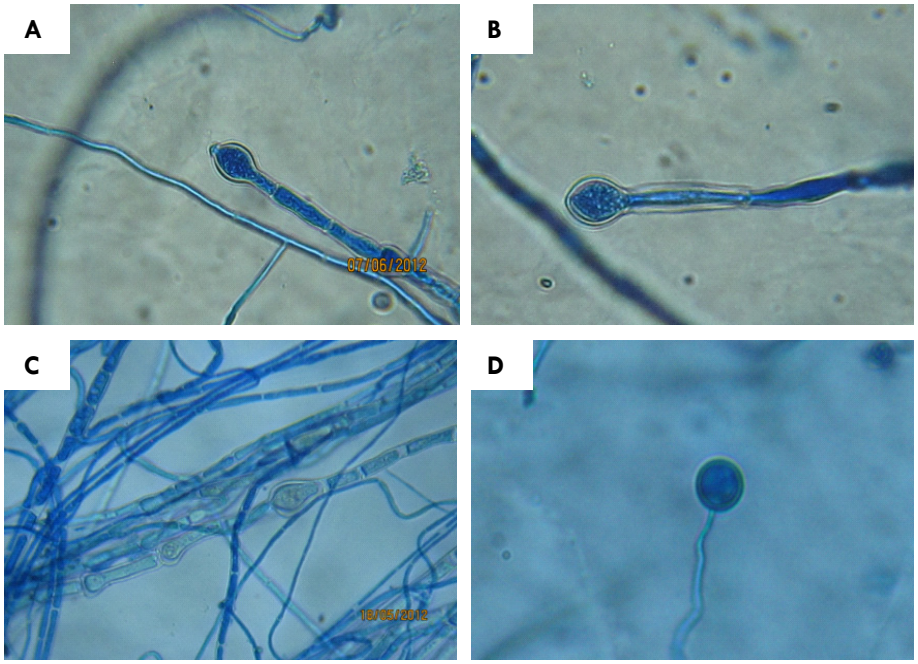

Figure 2. Morphological characterization of *Phytophthora palmivora*. Papillate sporangia (A), Non semi-papillate sporangia (B), Hyphal swellings (C), Terminal chlamydospore (D) on cotton blue (×400).

Table 1. Percentage of the affected branches relative to the new formed branches on the inoculated olive trees of Dahbia and Haouzia varieties with *Phytophthora palmivora* after five weeks of inoculation.

Variety	*Phytophthora palmivora*	New formed branches	Affected branches	Percentage of the affected branches (%)
Dahbia	**Isolate 1**	12.8 c	10.8 ab	81.8
	Isolate 2	21.6	9 b	43 c
Haouzia	**Isolate 1**	17 c	11.8	68 b
	Isolate 2	17 c	5.6 c	32 d

The results of the same column followed by different letters differ significantly at 5%.

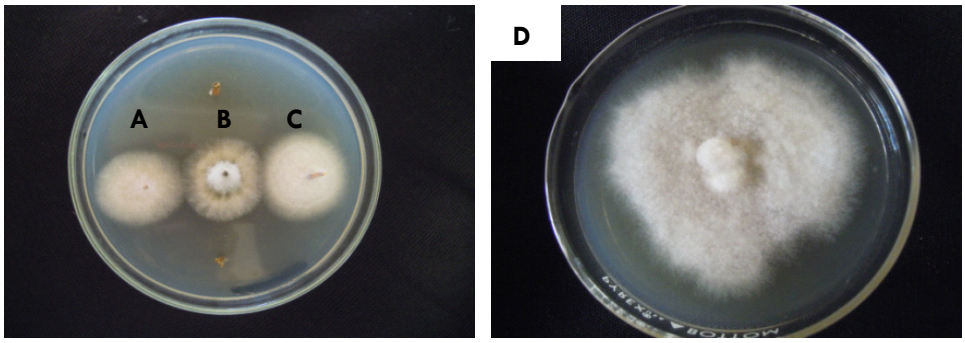

Figure 3. *Phytophthora palmivora* (A , C), *Alternaria alternata* (B) isolated from the olives stem segments on PSA agar and Phytophthora palmivora in the age of 12 days on the PSA agar plate (D).

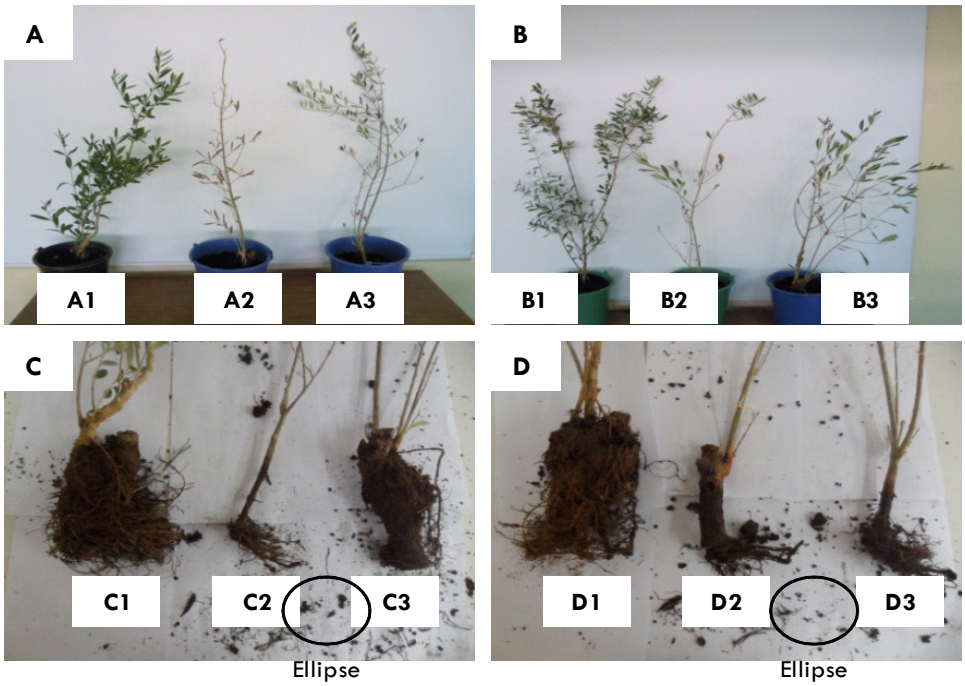

Figure 4. Symptoms on different organs of olive trees after inoculation with two isolates of *Phytophthora palmivora*: Olive trees of Dahbia variety(A), Non inoculated plant (A1), Inoculated plant with isolate 1 (A2), Inoculated plant with isolate 2 (A3), Olive trees of Haouzia variety (B), Non inoculated plant (B1), Inoculated plant with isolate 1 (B2), Inoculated plant with isolate 2 (B3), Olive trees root system of Dahbia variety (C), non inoculated plant (C1), Inoculated plant with isolate 1 (C2), Inoculated plant with isolate 2 (C3), Olive trees root system of Haouzia variety (D), non inoculated plant (D1), Inoculated plant with isolate 1 (D2), Inoculated plant with isolate 2 (D3), roots degradation due to the inoculation with the isolates of *Phytophthora palmivora* (Ellipse).

Table 2. Reisolation percentage of *Phytophthora palmivora* from roots and stems of the inoculated olive trees of Haouzia and Dahbia varieties after five weeks of inoculation.

Variety	*Phytophthora palmivora*	Reisolation percentage (%)	
		Root	Stem
Dahbia	Isolate 1	84 a	64 a
	Isolate 2	76 b	38 bc
Haouzia	Isolate 1	48 c	41,33 b
	Isolate 2	55 d	33 c

The results of the same column followed by different letters differ significantly at 5%.

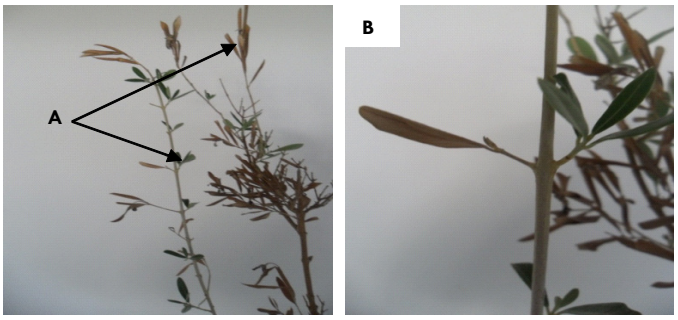

Figure 5. Inoculated olive plant with *Phytophthora palmivora*. Affected branch (A), New formed branch (B).

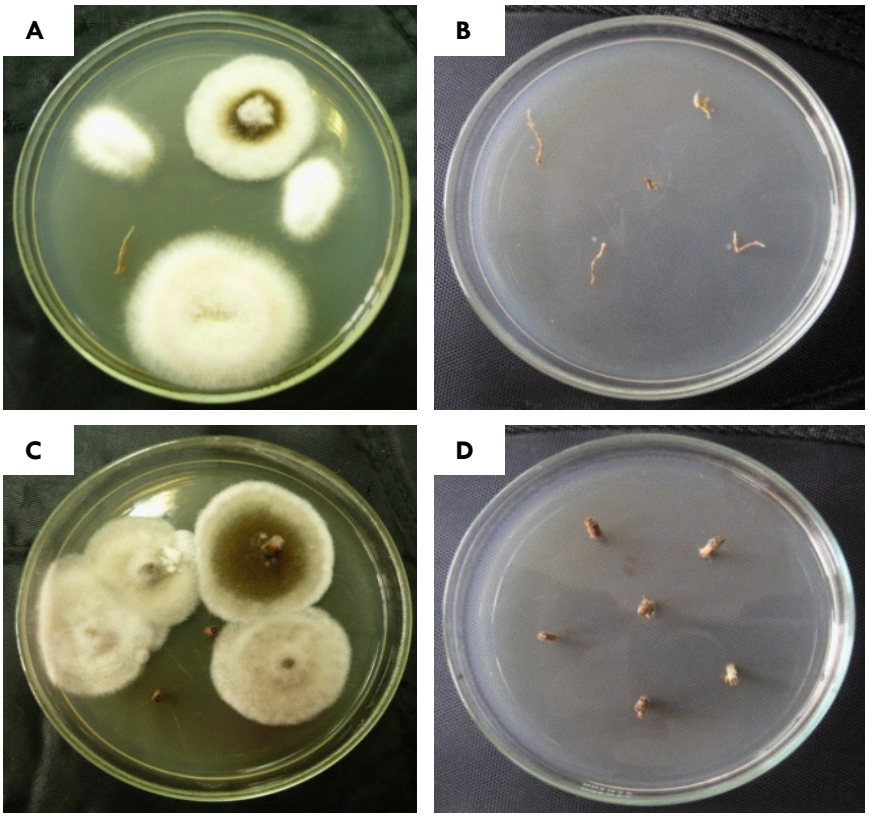

Figure 6. Reisolation of *Phytophthora palmivora* from the inoculated olive tree on PSA agar. *Phytophthora palmivora* reisolated from root segments (A), the control root segments (B), *Phytophthora palmivora* reisolated from stem segments (C), the control stem segments (D).

The two isolates of *P. palmivora* were reisolated from the root and stem with *A. alternata* (Figure 6). As shown in Table 2, the reisolation percentage of isolate 1 (84%) was significantly higher at 5% than that of isolate 2 (76%) in the root of Dahbia trees. The reisolation percentage of isolate 1 (48%) was lower than that of isolate 2 (55%) in the root of Haouzia trees.

In the stems of Dahbia, isolate 1 was higherly reisolated (64%) than isolate 2 (38%). The reisolation percentage of isolate 1 (41.33%) was higher than isolate 2 (33%) in the stem of Haouzia.

The Koch's postulate was verified by inoculating olive trees of Haouzia and Dahbia varieties with two isolates of *Phytophthora palmivora* isolated from the nursery olive tree and the olive trees field.

Sanchez Hernandez et al. (1998) described the effect of *Phytophthora palmivora* in southern Spain on young olive trees as wilt or dieback and death, and they announced that its pathogenicity depends on soil water content.

Cacciola et al. (2000) showed decline symptoms in new plantations of 1 to 2 years old plants in Catanzaro Province (Calabria) and considered *Phytophthora palmivora* as a pathogen of olive tree in Italy that provoked leaf chlorosis, defoliation, wilting, twig dieback and eventual plant collapse associating the symptoms with the root rot.

Olive trees infected by *P. palmivora* in Argentina were indicated by Lucero et al (2006). Similarly to this current report, all

isolates produced papillate sporangia . Olive plants could have been contaminated by *P. palmivora* from other host species as suggested by these authors. *P. palmivora* infects more than 200 species of ornamental, shade and hedge plants, mostly from tropical areas. In Argentina, for instance, *P. palmivora* was first recorded in Citrus spp in 1937. The pathogen may have been introduced through rooted olive plants of Mediterranean varieties currently used in Argentina (Lucero et al., 2006).

As regards the ability of *Phytophthora palmivora* to reduce and to degrade the olive tree roots, we could find the same symptoms that Lucero announced in 2006.

Lo Giudice et al. (2010) outlined that leaf chlorosis, defoliation, rot of fine roots, twig dieback and wilt were observed in 4-year-old olive trees cv. Tonda Iblea in drip-irrigated orchard in eastern Sicily. Trees declined slowly or collapsed suddenly with withered leaves still attached. Incidence of affected trees was 10 %. A fungus identified as *V. dahliae* Kleb. was isolated from the xylem of main roots and basal stem. An oomycete was isolated and identified from roots and basal trunk bark. Both pathogens were recovered from symptomatic trees with means frequencies of positive isolations per tree of 80 and 30% for *V. dahliae* and *P. palmivora*, respectively.

The pathogenicity of *P. palmivora* was demonstrated in the olive plants. This was the first report of this pathogen in Moroccan olive trees. In the long-term, it may constitute a real danger to this culture in the nurseries as in the fields. So, due to the severe symptoms and the increasing incidence recorded; *P. palmivora* should be considered a potential threat to olive cultivation in Morocco.

Acknowledgments

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References

Anton FA and E Laborda (1989) Estudio de la susceptibilidad/resistancia de variedades del olivo (*Olea europaea* L.) al patogeno *Cycloconium oleaginum* (Cast.) (*Spilocaea oleaginae* Hugh.). Bol. San. Veg. Plagas, 4: 385–403.

Cacciola SO, Agosteo GE, and A Pane (2000) First Report of *Phytophthora palmivora* as a Pathogen of Olive in Italy. Plant disease 84 (10): 1153.

Fabbri A, Lambaradi M, and YO Tokatli (2009) Olive breeding in breeding plantation tree crops : Tropical species. Spinger New York pp. 423-465.

Ho HH (1992) Keys to the species of Phytophthora in Taiwan. Plant Pathol. Bull. 1: 104-109.

Lo Giudice V, Raudino F, Magnano Di San Lio R, Cacciola SO, Faedda R, and A Pane (2010) First report of a decline and wilt of young olive trees caused by simultaneous infections of *Verticillium dahliae* and *Phytophthora palmivora* in Sicily. Plant Disease 94 (11): 1372.

Lucero G, Vettraino AM, Pizzuolo P, Di Stefano C, and A Vannini (2006) First report of *Phytophthora palmivora* on olive trees in Argentina. New Disease Reports 14: 32.

Ministry of Agriculture and marine Fischeries (2009) Plan vert du Maroc: de la stratégie à l'action du Maroc.

Gallegly ME and CX Hong (2008) *Phytophthora*: Identifying species by morphology and DNA fingerprints. American Phytopathology Society Press 158pp.

Olbricht K, Ulrich D, and B Dathe (2006) Cross-breeding with accessions of Fragaria chiloensis resulting in selections with outstanding disease resistance and fruit quality characteristics. Acta Hortic. 708: 507–509.

Perez AB, Farinon OM, and MF Berretta (2011) First report of Fusarium solani causing root rot of olive in southeastern Argentina. Plant Disease 95 (11): 1476.

Sanchez Hernandez ME, Ruiz Davila A, Perez De Algaba A, Blanco Lopez MA, and A Trapero Casas (1998) Occurrence and etiology of death of young olive trees in southern Spain. European Journal of Plant Pathology 104 (4): 347-357.

Vossen P, Gubler D, and MA Blanco (2008) Verticillium Wilt of Olive. Newsletter of Olive Oil production and Evaluation. Univ. of California Cooperative Extension. Vol. 3 (4): 1-4.