

NECTRIA HAEMATOCOCCA CAUSING ROOT ROT IN OLIVE GREENHOUSE PLANTS

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SUMMARY

This is the first report of *Nectria haematococca* Berk. & Br. in Argentina. It was isolated from 8 to 12 month greenhouse olive *Olea europaea* L. plants. The anamorph, *Fusarium solani* (Mart.) Sacc., was frequently isolated while the teleomorph was rare in culture. Perithecia developed on plated olive root segments and around the PDA medium. Two isolates of *F. solani* produced orange perithecia, which remained immature for several months. A third isolate developed perithecia containing asci with 2-cell light brown and longitudinally striated ascospores, at maturity. Young olive plants inoculated with *F. solani* showed leaf browning, root rot, wilting and plant death. Controls remained healthy. The presence of the sexual stage is responsible for the genetic variability of this pathogen.

Keywords: *Olea europaea*, *Fusarium solani*, soil-borne, wilt.

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INTRODUCTION

The fungus *Fusarium solani* has been reported on a wide variety of crops, soils, and plant debris. It has *formae speciales* pathogenic on specific hosts (Booth, 1971). The host range includes species of the genera *Allium*, *Chrysanthemum*, *Citrus*, *Cucumis*, *Cupressus*, *Glycine*, *Gossypium*, *Helianthus*, *Lycopersicon*, *Medicago*, *Pinus*, *Populus*, *Prunus*, *Solanum*, *Zea*, among others. The reported symptoms were wilting, rotting of seeds, seedlings (damping-off), roots, lower stems, crowns, corms, bulbs, and tubers (Farr *et al.*, 1989). Usually, asexual spores are produced, but under certain conditions a perithecial stage identified as *Nectria haematococca* has been found (Booth, 1971). The first report on the pathogenicity of *F. solani* on olive was from India. The described symptoms included the leaf drooping, drying up of branches tip downwards, and the death of the entire plant. Partial wilting was the characteristic feature of the disease. The small roots of diseased field plants rotted early and it took 3 to 4 months for the pathogen to kill infected plants (Munjal *et al.*, 1982). In Argentina, olive grown area increased from 70,000 to 13,500,000 plants during the last decade. New pathologies have appeared in greenhouse plants and established plantations. Lately, the drying syndrome, partial wilting and sudden death increased in northwestern Argentina (Babbitt *et al.*, 2000; 2002; Pérez *et al.*, 2001; Roca *et al.*, 2001; 2002).

The objectives of our research were to report the presence of *Nectria haematococca*, teleomorph of *Fusarium solani* in greenhouse olive plants, and to determine its pathogenicity.

MATERIALS AND METHODS

Pathogen isolation and identification. Since 1997, 171 diseased olive plants (8-24 month-old), grown under greenhouse conditions in northwestern Argentina (La Rioja province), were sent to the laboratories of IMYZA-INTA-Castelar, and Agronomy Faculty of Buenos Aires University, to identify the organism causing the disease. Selected root segments of diseased plants were washed by running water for 3 hours (Streets, 1979), surface disinfected by immersion in a 0,5 % NaOCl solution for 2 min, rinsed with sterilized water, dried under a laminar airflow hood, and cut into pieces (3-5 mm). The root pieces were placed on plates containing potato-

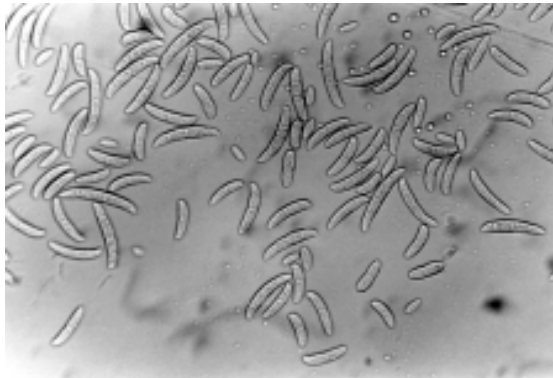
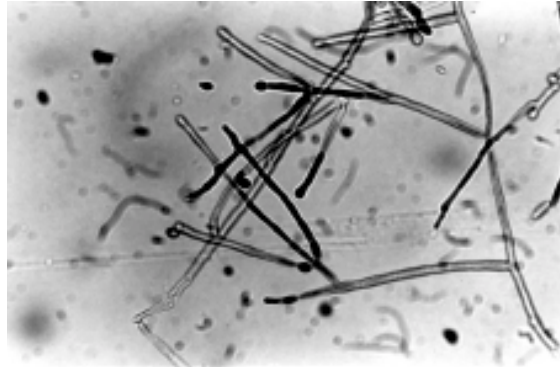
dextrose agar (PDA, Merck) at 2 %, pH 5.6 + 0.2, transferred to a controlled-environment chamber at a temperature of 22 ± 2 °C, and 12 hr of near-ultraviolet light (Philips Black Light lamps TL 40W/08) on/off cycle to stimulate sporulation. The identification of the fungi were based on morphology of their fructifications, and cultural characteristics (Booth, 1971; 1977; Gerlach and Nirenberg, 1982; Hanlin, 1990). The isolates are kept in the IMYZA fungal collection.

Pathogenicity tests. Autoclaved oat grains (100 g oat grains/70 ml distilled water) were infected with plugs of a 10 day-old pure culture of *F. solani*, and incubated for 2-3 weeks until grains were covered with the fungus. Sterilized oat grains, without *F. solani*, were used as control. Young olive plants (6-12 month-old) were placed in 9 x 10.5 cm plastic pots, one plant in each of 20 pots. A mixture of *F. solani* infected oat grains and a commercial potted mixture (1:10 v/v, Grow Mix S1-organic substrate) was added to ten inoculated pot. For the control, sterilized substrate was placed in ten pots. The plants were maintained at 25-28 °C with saturated relative humidity for 48 hr. After uncovering them, the plants remained in the growth chamber at 26 ± 2 °C, and 12 hr of light on/off cycle (CAB International, 1983). Disease symptoms were registered daily. When foliar symptoms appeared, rotted root segments were surface disinfected, as described previously, and placed on PDA plates for reisolation of the pathogen.

RESULTS AND DISCUSSION

Pathogen isolation and identification. The anamorph *F. solani* was consistently isolated in the 90% of the analyzed greenhouse plants. Other *Fusarium* species associated were *F. acuminatum*, *F. equiseti*, *F. moniliforme* and *F. oxysporum*. On roots pieces placed onto PDA culture, *F. solani* developed colonies after 2-3 days of incubation that were characterized by typical dirty-white or white blue pigmentation, long and branched phialides (Photo 1), hyaline to milky-white microconidium droplets, and pionnotes with macroconidia (Photo 2). The size of the microconidia averaged 11.8 x 3.9 mm (0-septated) and 18.1 x 4.0 mm (1-septated); the macroconidia were only 3-septated and averaged 29.4 x 3.9 mm. Chlamydo-spore were present.

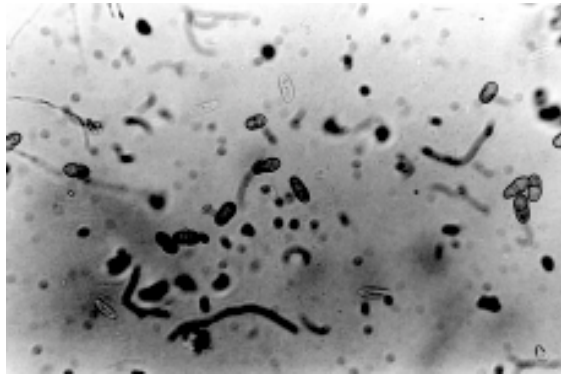
1. Long and branched phialides of *Fusarium solani* from culture (x 500)



2. Macroconidia and microconidia of *F. solani* from culture (x 500).

After 15 days of incubation, sparse to 2-3 gregarious orange perithecia developed readily on the diseased root segments and around the roots on the culture medium.

The pomiform perithecia and ellipsoid, striated and light brown ascospores (Photo 3) measured 266 μm and 14.7 x 6.6 μm , respectively. These measurements agreed with those indicated by Gerlach and Nirenberg (1982) and Booth (1971) for *Nectria haematococca*. It has been reported that the teleomorph stage occurs frequently in culture with homothallic strains of *F. solani* under suitable conditions (Booth and Waterston, 1964; Booth, 1971). Also, it is known that vegetal samples from humid tropics yield perithecia abundantly while collections from temperate zones produce the teleomorph only in culture (Booth, 1971).



3. Ascospores of *N. haematococca* with constriction at the central septum and long longitudinal striations (x 500).

Pathogenicity tests. The symptoms of inoculated plants involved apex bending, leaves browning and drooping, wilting tip downwards, and finally, death of plants. Apex bending began after 5 days. From 20 inoculated plants, 13 showed apex bending on the 5th day. Later, the remaining plants showed symptoms. Most of inoculated plants showed changes in the leaf color which turned brownish. The wilting of the plants started 9 days after inoculation. The fungus *F. solani* was reisolated from inoculated plants after wilting or death. Root rot was clear in all infected plants when pulled-out for reisolation of the pathogen. Controls remains healthy.

Although, the soil-borne transmission of *Fusarium* is predominant in cultivated crops, we have now evidence for the occurrence of *F. solani* as a serious root rot in greenhouse plants that could be an important source of inoculum (Babbitt *et al.*, 2002).

Several important fungal pathogens are involved in the root rot of olive plants. In southern Spain, the pathogenicity tests carried out in cuttings (6-24 month old) gave 5 pathogenic species including *Cylindrocarpon destructans*, *Phytophthora megasperma*, *P. palmivora*, *Pythium irregulare* and *Sclerotium rolfsii* that reproduced symptoms of root rot, wilting and death of plants. Other fungi including *F. solani*, were either weakly or non-pathogenic (Blanco López *et al.*, 1998; Sánchez Hernández *et al.*, 1998). However, isolates of *F. solani* from olive in India were pathogenic (Munjal *et al.*, 1982). As in India, our study confirmed that some *F. solani* isolates can be pathogenic indicating that this fungus

has an important role in the root rot, wilting and death of olive plants in Argentina (Babbitt *et al.*, 1999, 2002; Pérez *et al.* 2001; Roca *et al.* 2001, 2002). The transfer of *F. solani* infected plants from greenhouses to field could influence disease development. The presence of the teleomorph stage is responsible for the genetic variability of this pathogen.

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