Susceptibility of *Phytophthora palmivora* Associated with Root Disease of Some Olive Trees Cultivars

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Abstract

Nowadays more than 60 species of *Phytophthora* have been described all over the world as causal agents of diseases in vegetables, fruit trees, shrubs, ornamentals and forest trees. Those pathogens are worldwide distributed and, some of them have several hosts like *Phytophthora palmivora*, causing severe epidemics in different crops. In Argentina, this organism has been described as one of the causal agents of the "dry branch" of olive trees. The previous disease develops mainly in young olive trees managed with modern technologies. Canopy symptoms are strongly related with the death of rootlets. The aim of the study was to evaluate the susceptibility of some olive trees cultivars to *P. palmivora*. Assays were performed inoculating ten potted olive trees of one year old of cultivars Coratina, Arbequina, Arauco, Picual, Manzanilla and the rootstock FS-17 with a *P. palmivora* strain. The disease symptoms and the number of death plants were recorded weekly. Susceptibility tests have shown that Picual was the most affected cultivar followed by Arbequina and Arauco. It is interesting to note that the rootstock SF-17 was the less susceptible. These results confirm as we have been observing in the field that Picual behaves as a very susceptible cultivar in the Argentinean olive growing area. It was concluded that all cultivars tested were susceptible to *P. palmivora* in varying degrees.

INTRODUCTION

Species of *Phytophthora* (meaning ‘plant-destroyer’ in Greek) are an extremely broad host-range group of plant pathogenic organisms. Nowadays more than 60 species have been described all over the world and affecting several hundred plant species worldwide, including vegetable and fruit crops, ornamental plants and forest trees (Erwin and Ribeiro, 1996; Agrios, 1997). Many of the diseases they cause are of great economic importance due to their devastating effects. Some species attack only one or two species of plants, but others may cause similar symptoms on many different kinds of plants whereas more than one species may be involved in a given disease. Infections caused by *Phytophthora* species on roots and stems, especially in trees and shrubs, are frequently unnoticed because the affected plants at first show only non-specific symptoms similar to those of drought and lack of nutrients, and then plants quickly become weak and susceptible to other pathogens or other abiotic stress factors that are mistakenly considered as the causes of the death of the plants (Agrios, 1997).

These pathogens are worldwide distributed and, some of them have several hosts like *P. palmivora,* infects more than 200 species of ornamental, shade and hedge plants, mostly from tropical areas which has caused severe epidemics. The last one has recently

been described as the causal agent of root rot of olive tres in Italy, where it was isolated from collapsed trees (Cacciola *et al* ., 2000). In Spain it has been also confirmed as a pathogen of olive tres regardless, *P. megasperma* is more commonly associated with field symptoms (Hernández *et al*., 1998). In Argentina, *P. palmivora* was first reported in 1937 causing disease on *Citrus* spp (Frezzi, 1950). Recently, in Argentina, the former organism together with *P. nicotianae* and *P. citrophthora* have been described as causal agents of the "dry branch" of olive trees (Lucero *et al*., 2007; Vettraino *et al*., 2009; Lucero *et al*., 2011). The pathogens may have been introduced through olive plants imported from the Mediterranean growth regions during the decade of nine thousand nine hundred ninety.

The Argentine`s olive growing area has increased greatly since last years. This fact has also contributed to an enhancement of the incidence of a syndrome so-called "Rama seca" (dry branch). The disease causes wilt and death of young olive trees and has been observed on new olive (*Olea europea* L.) plantation in Catamarca, La Rioja, and San Juan provinces. It is responsible of important economic losses at those provinces. The name "Dry Branch" has been used to distinguish it from other diseases, such as *Verticillium dahliae*, that can induce similar symptoms (Lucero *et al*., 2005).

Host resistance is probably the most valuable control measure in agriculture. Its success is the result of extensive research in many state experiment stations. It is usually successful as a sanitation measure. Several methods for testing the resistance of fruit trees to *Phytophthora* spp. have been used (Jeffers et al. *et al.*, 1981; Kim et al.*et al.*, 1985; Stylianides et al., 1985; Matheron and Porchas, 1996). A widespread and reliable method consists on the artificial inoculation of young trees in pots or in the field.

The aim of this research was to evaluate the relative resistance or susceptibility of some olive trees cultivars to *P. palmivora* that are important to the olive industry in Argentina, using an inoculation method of trees growing in pots in a glasshouse.

MATERIALS AND METHODS

Isolates of *Phytophthora*

One isolate of *P. palmivora* was used in this study. It was obtained originally from naturally infected trees in commercial olive orchards in Argentina in 2005. The pathogen was isolated from rotted rootlets on selective V8 medium (PARPNH) (Jung *et al*., 1996). Isolates were maintained in sterile water. The isolate was identified by cultural, morphological and molecular characteristics (Lucero *et al.*, 2007). *P. palmivora* isolate was then transferred to V8 agar (Robin *et al*., 1992, 1998) for resistance studies.

Inoculum preparation

Infected millet seeds which were used as inoculum were obtained as follows: the millet grains moistened with V8 juice were sterilized in 250 mL flasks by autoclaving twice, 24 h apart and inoculated by adding *P. palmivora* cultures on V8 agar (10 discs of 8 mm diameter per flask) and incubated for 1 month in the dark at 25°C.

Plant material

Susceptibility test were performed according to Lucero (2000) and conducted at the Phytopathological Institute of National University of Cuyo Mendoza, Argentina. For the flooding experiment, uniform one year old self-rooted olive trees (*Olea europea* L. 'Coratina', 'Arbequina', 'Arauco', 'Picual', 'Manzanilla' and the rootstock 'FS-17') were obtained from a commercial nursery. Plant were transferred into 2,5 L plastic pots filled

with mixture of sand, peat and perlite on a 1:1:1 proportion and were kept in a glasshouse (air temperature 20-25°C and 16 h/8 h photoperiod). A factorial experiment with cultivars and inoculation as experimental factors was conducted. Six olive cultivars were used in this experiment. During the trial period, all olive plants were irrigated daily. Ten olive trees for each cultivar were inoculated whereas ten non inoculated plants were used as control.

Soil infestation test

The treatment took place in July 2010. Millet seed inoculum (about 20 $g.L^{-1}$) was added to the soil of plastic pots, as described by Robin *et al.* (1998). Ten plant for each cultivars were inoculated with *P. palmivora*. Sterile millet seeds were added to ten plants for each cultivars uses as controls. To stimulate zoospore release and disease development, pots were flooded for 48 h once every 2 weeks (Jung *et al.*, 1996). After each flooding treatment, excess water was drained off. Flooding was repeated three times.

Symptoms assessment

Symptoms development (wilting and mortality) was observed and recorded weekly during the entire experiment period. After three month form inoculation, each plant was removed from the pot and roots were washed finally dry weight was calculated. *P. palmivora* re-isolations were carried out after the end of the experiment from the taproot on selective PARPNH.

Data analysis

Results were treated performing an ANOVA. All significant main effects and interactions were compared using the least significant difference (Tukey Test, $P < 0.05$). Differences between cases of species mortality within the cultivars inoculated and un-inoculated were analyzed by means of the χ^2 test using the SPSS for Windows (SPSS, 2000).

RESULTS

At the end of the test, *P. palmivora* was positively re-isolated from the roots of all infested plants.

Symptoms on inoculated plants were observed within 50 days from the treatment. The showed symptoms were similar to those observed in the field: wilted, necrotic leaves, foliar necrosis, defoliation and some olive plant died. A lack of root growth was observed in treated plants (*Figure 1*). Dry weight of the root system of the different cultivars showed a reduction among 55 to 85% relative to control plants. In spite of that differences were not significant.

The $\chi 2$ test performed within each cultivars of the inoculated treatments revealed some significant differences. Mortality in 'Picual' and 'Arauco' plants showed significant differences between inoculated and control plants, whereas in 'Arauco', 'Manzanilla' and rootstock 'FS 17' did not show significan differences (*Table 1*).

Susceptibility tests have shown that 'Picual' was the most affected cultivar followed by 'Arauco' and 'Arbequina', although the latter no significant differences among the dead plants inoculated and un-inoculated. Mortality of 'Picual' was 100 %, whereas in 'Arbequina' and 'Arauco' was 75 and 66,7% respectively. However, 'Manzanilla' and 'Coratina' mortality was 60 and 50%, respectively.

It is interesting to note that not dead plants were observed in the rootstock FS-17, showing a very good disease resistance. This rootstock produced shorter trees with smaller trunk circumference and lower vigor therefore FS 17 allowed higher planting densities and potentially higher total yields (Fontanazza *et al.,* 1996; Sibbett *et al.,* 2005) (*Figure 2*).

DISCUSSION

The aim of this study was to check the susceptibility of some olive tree cultivars frequently used in Argentina against *P. palmivora*, species often present in national olive groves. It was concluded that some cultivars tested were susceptible to *P. palmivora* in varying degree. Also these results confirm as we have been observing in the field that 'Picual' behaves as a very susceptible cultivar in the Argentinean olive growing area. Others studies showed that 'Picual' is also one of the most susceptible cultivars to *V. dalhiae* Kleb. (López-Escudero *et al.*, 2004; Hegazi *et al.*, 2012). 'Arbequina' showed in our studies to be also susceptible to the disease. Iannota *et al*. (2006) proved that 'Arbequina' is little susceptible to *Camarosporium dalmaticum.*

The results of this study could have important implications in the management of *Phytophthora* root under field conditions. As we concluded in the present work, 'Picual' and 'Arbequina' are very susceptible to *P. palmivora.* Therefore those cultivars are not recommended in olive grove area where the pathogen was previously detected.

In this study, FS 17 was rated as little susceptible to resistant. However, previous studies reported that this rootstock had high field susceptiblity to *Alternaria alternata* in high density cultures or hedgerow planting (Moral *et al*., 2008).

Nowadays little is known about the susceptibility of different olive trees cultivars to the pathogens that could cause disease. Thereby this study makes up an important contribution to the knowledge of the olive tree resistance to an economic importance disease.

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Tables

Table 1: Chi-Square Test ($\chi 2$) calculated within each cultivar of the inoculated treatments.

Olive cultivars	Value for Chi Square	Df*	*P*-Value**
Arauco	7.500	1	.006
Arbequina	1.818	1	.178
Coratina	.000	1	1.000
Manzanilla	.267	1	.606
Picual	13.333	1	.000
Roostock	.a	.a	.a

.a: not calculated because no statistical dead plants was constant

*Df: degrees of freedom

Figures

Fig. 1. Picual olive plant where reduction in root system is observed by infection of *Phytophthora palmivora*. Left control plant and right inoculated plant.

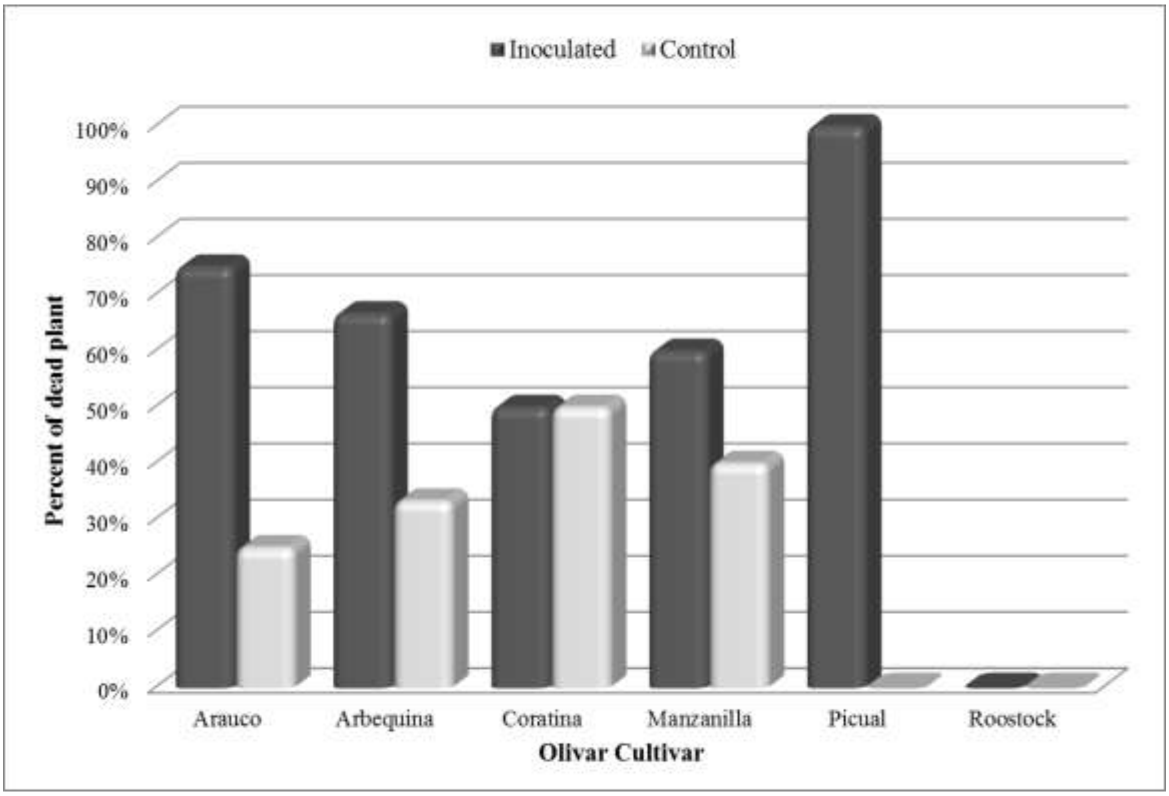

Fig. 2. Percentage of dead plants for each cultivar in controls and inoculated plants. Mean values not sharing same letter are significantly different for Tukey test ($P < 0.05$).