Phytophthora oleae, a new root pathogen of wild olives

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Phytophthora oleae, a new root pathogen of wild olives

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Abstract

Wild olive (*Olea europaea* subsp. *europaea* var. *sylvestris*) is an important component of Mediterranean forests and a key genetic source for olive improvement programs. Since 2009, a severe decline caused by *Phytophthora cryptogea* and *P. megasperma* was detected in a protected wild olive forest of high ecological value (Dehesa de Abajo, Seville, Spain). In this natural forest, samplings of roots and soil were done on 25 symptomatic wild-olives in 2014 and 2015. Apart from the already known *P. cryptogea* A1 and *P. megasperma*, a third *Phytopththora* species was consistently isolated from symptomatic wild-olive rootlets. These isolates conformed morphologically with the newly described species *P. oleae* and were confirmed by analysis of their ITS regions and Cox-1 sequences. Temperature-growth relationships showed a maximum growth at 19.9ºC on CA medium, being the lowest-temperature *Phytophthora* spp. infecting wild olive roots. Pathogenicity was confirmed on 1 year-old healthy wild olive seedlings and was similar respect to the previously known pathogenic Phytophthoras. As temperature requirements are quite different, the three *Phytophthora* spp. may be active against wild

olive roots in different seasons. However, the prevalence of *P. oleae* infecting wild olives in the last years could be due to its introduction as a new invasive pathogen. The likely invasive nature of *P. oleae*, together with increasing rain episodes concentrated in short periods frequent in southern Spain, would allow outbreak infections in wild olive forests, also putting at risk cultivated olive orchards.

Keywords: decline, invasive pathogen, Natural Reserve, *Olea europaea* var. sylvestris,

Introduction

Olive (*Olea europaea* subsp. *europaea*) is a fruit crop widely distributed in the Mediterranean region with economically important cultivars and also wild genotypes growing in natural forests. In Spain, the wild form (*O. europaea* subsp. *europaea* var. *sylvestris*) represents a distinct botanical variety of the subspecies *europaea* and is considered the best bioindicator of the Mediterranean Floristic Region (Rubio de Casas *et al*., 2002). Moreover, wild olives represent the main source of genetic traits for improvement of cultivated forms, commonly used in breeding programs for the high degree of resistance to diseases showed by some genotypes (Arias-Calderón *et al*., 2015).

Recently, a wild olive root rot caused by *Phytophthora megasperma* and *P. cryptogea* was reported in a Natural Reserve in southern Spain (Dehesa de Abajo, Seville) affecting near 5 ha of wild olive woodland of high ecological value (González *et al* 2017a). This forest grows around a natural pond with marked fluctuations in water tables due to the seasonal distribution of rainfall, alternating seasonal soil flooding and drought periods. As expected for *Phytophthora* soilborne pathogens, affected trees

exhibited different degree of symptoms depending on their distribution: moderate defoliation uphills, while severe defoliation and tree death were recorded downhills. *Phytophthora megasperma* was the only species isolated from declining wild olives in 2009. However, *P. cryptogea* was the main species associated with wild olive root disease in 2013 (González *et al*., 2017a). It was hypothesized that a higher-temperature *P. cryptogea* could replace the lower-temperature *P. megasperma* as a result of the global increase in temperatures in the Mediterranean region due to global climate change (Lindner *et al*., 2010). Given the intrinsic ecological and genetic value of wild olive populations, the main objective of this work was to know the evolution of *Phytophthora* spp. associated with the root disease, clarifying the role of temperature in the disease aetiology. Additionally, a new *Phytophthora* spp. associated with necrotic roots was isolated, identified, temperature-characterized, and checked for pathogenicity on wild olive.

Materials and methods

Samplings and isolations

In November 2014 and November 2015, two field surveys were carried out at the Natural Reserve "Dehesa de Abajo" (Seville, Spain, UTM 29: 37º12’33’’N; 6º10’16’’W). Soil is a mixture of gravel and sand with rainfall rates averaging 648 mm per year, mainly distributed in autumn and winter (Consejería de Agricultura, Pesca y Desarrollo Rural, 2017). Cardinal temperatures (minimum and maximum) recorded in the period 2001-2015 at the nearest meteorological station (La Puebla del Río II

Meteorological station; 37º04'48''N, 06º02'47''W, altitude 41 m.a.s.l.) are summarized in Table 1.

Using the methodology described in González *et al*. (2017a), samples of rootlets and rhizosphere soil were taken at approximately 1 m distance from the trunk at 10–30 cm depth of a total of 25 symptomatic (crown wilting or/and defoliation) wild olives. Samples from different trees were independently processed. For each sample, rotten feeder roots were cut in 3-4 mm segments, washed under running water for 2 h, and directly plated on NARPH medium (Hüberli *et al*., 2000). Soil samples were air dried and sieved (2 mm) and isolations from soil were performed using pieces of newly formed olive leaves as baits, according with González *et al*. (2017a). Six beakers per sample with a soil-water mixture (15:200 w:vol) and six floating baits each one, were incubated for 4 days at 22ºC under 12 h photoperiod. After the incubation period, the leaf pieces were washed and plated in Petri dishes containing NARPH medium. All the dishes (roots and soil baits) were incubated at 22ºC in the dark for 4 days. Colonies obtained from damaged roots and soil baits were grouped according to their morphology and transferred to 20% carrot agar (CA) medium. Five isolates per colony morphology were selected among the pure cultures obtained for molecular and morphological identification and growth-temperature characterization.

DNA extraction, amplification and sequencing

DNA of the isolates was obtained by harvesting the mycelium from 1-week-old pure cultures grown on PDA at 20°C in the dark. DNA was extracted using the PowerSoil DNeasy kit (Qiagen) following the manufacturer's protocol. The internal transcribed spacer (ITS1-5.8S-ITS2) region of the rDNA was amplified by PCR using the universal

primers ITS-6 (Cooke *et al*., 2000) and ITS-4 (White *et al*., 1990). The PCR reaction conditions used were an initial denaturing step of 94ºC for 2 min followed by 35 cycles of 94ºC for 30 s, annealing at 55ºC for 30 s and an extension at 72ºC for 45 s, with a final extension of 72ºC for 10 min. The mitochondrial cox1 gene was amplified using the primers OomCoxI-Levup and Fm85mod (Robideau *et al*., 2011) and were sequenced with the same primers. The PCR reaction conditions for cox 1 consisted of 3 min at 94°C followed by 35 cycles of 30 s at 94°C, 30 s at 50°C and 60 s at 72°C, with a final extension of 5 min at 72°C (Ruano-Rosa *et al*., 2018).

Morphological and growth-temperature characterization

For isolates molecularly identified but not previously associated with wild olive root rot, a morphological and growth-temperature characterization were carried out.

To characterize colony morphologies, the five selected isolates were grown at 20ºC in the dark on CA and PDA medium for 5 days to describe colony colour, pattern, edge shape and presence of aerial mycelium.

To describe sexual structures (oogonia, antheridia and oospores), isolates were individually transferred onto CA medium, incubated at 22ºC in the dark, and periodically observed under the inverted microscope (Olympus IMT-2, 40×). When sexual reproductive structures were detected in single cultures (after 10 days of incubation), small pieces of agar were placed on glass slides, stained with acid fuchsin in lactophenol (0.0005%) and observed under the microscope (Nikon Eclipse 80i, 100×). For each isolate, 30 mature oogonia, 30 antheridia and 30 oospores were selected and measured (NIS-Elements D 2.30, Nikon Instruments): oogonium and oospore diameter, antheridium length and thickness of the oospore wall.

To characterize asexual structures (sporangia), small CA plugs (5 mm diameter) from the edges of actively growing colonies were placed in the centre of 5-cm-diameter Petri dishes, and after that, sterilized saline solution (MSS, Chen & Zentmyer, 1970) was added to just cover the agar plugs. Petri dishes were incubated in darkness at 20ºC. Every 24 h, the saline solution was removed and replaced with fresh solution. After 4 days of incubation, mature sporangia present in the floating mycelium were removed and placed on glass microscope slides and stained with acid fuchsin in lactophenol (0.0005%) for observation under the microscope (Nikon Eclipse 80i, 100×). For each isolate, 30 mature sporangia were randomly chosen, and the following parameters were measured (NIS-Elements D 2.30, Nikon Instruments): sporangium length and breadth, and pore width. Other sporangial characteristics such as shape, presence or absence of papilla, were also recorded.

For growth rate tests, agar plugs of each isolate were plated in 9 cm Petri dishes with CA or PDA medium and incubated in the dark at 5, 10, 15, 20, 25, 30 or 35ºC. Three replicates (dishes) per isolate were prepared. Colony radius was measured daily until the colonies covered the agar surface and growth rate per day was calculated for each isolate, culture medium and incubation temperature. Maximum average data were adjusted to a regression curve using Statistix 10.0 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 ($p < 0.05$), coefficients of determination (R^2), coefficients of determination adjusted by degrees of freedom (R_a^2), and pattern of residuals. Maximum growth rates and optimum growth temperatures were estimated over the regression curves obtained.

Pathogenicity tests

153

154 Healthy wild olive seedlings (1-year-old) from a forest nursery of the Andalusian
155 Government (Vivero San Jerónimo, Seville) were inoculated with one isolate of the
156 *Phytophthora* spp. newly associated with wild olive root disease (Po group). To prepare
157 the inoculum, colonized agar plugs were placed in 9 cm Petri dishes containing 20 ml of
158 20% carrot broth and incubated at 22ºC in the dark for 20 days. The mycelium produced
159 was then filtered, washed, and shaken in an electric mixer (Osterizer pulse-matic 16)
160 with sterile water for 3 min at the highest speed to break mycelium masses and liberate
161 oospores. Oospore concentration was estimated by counting in a Neubauer chamber and
162 adjusted to 2.2×10^4 oospores$\times$ml^{-1}. For inoculation, 50 ml of this inoculum was
163 homogeneously added to the root ball of each seedling, before transferring them into
164 plastic pots containing 2 l of soil (sand:lime:peat 1:1:1 vol.). Ten seedlings (replicates)
165 were inoculated and 10 additional seedlings (uninoculated controls) were treated in the
166 same way, but only 50 ml of sterile water was applied to their root balls. All the pots
167 were incubated in an acclimatized greenhouse and flooded 2 days per week (Romero *et*
168 *al*., 2007). Foliar symptoms were assessed weekly for each seedling based on a 0–4
169 scale, according to the percentage of yellowing, wilted foliage or defoliation recorded (0
170 = 0%, 1 = 1-33%, 2 = 34-66%, 3 = more than 67% and 4 = dead foliage) (Sánchez *et*
171 *al*., 1998; González *et al*., 2017a). After 14 weeks, the relative area under the disease
172 progress curve (rAUDPC) was calculated as percentage regarding the potential
173 maximum value (Campbell & Madden, 1990). At this moment, root symptoms were
174 also assessed according to a similar 0-4 scale referred to root necrosis or rootlet absence
175 percentage as follows: 0 = 0%, 1 = 1–33%, 2 = 34–66%, 3 = more than 67%, 4 = dead
176 root (Sánchez *et al*., 1998; González *et al*., 2017a).

Data on foliar and root symptoms at the end of the experiment and rAUDPCs were tested for homocedasticity by the Levene's test and then, a one-way ANOVA was performed, and mean values compared by the Tukey's HSD test for $\alpha = 0.05$ (Statistix software 10.0).

Additionally, root segments from inoculated or control plants were carefully washed and plated on NARPH medium for re-isolation of the pathogen.

Results

Site temperatures

Table 1 shows the mean values of minimum and maximum temperatures recorded at La Puebla del Río II Meteorological station, grouped by season, for the period 2007-2015 and the wider historical temperature series available (2001-2015). It is remarkable the occurrence of two exceptional cold episodes suffered in winter 2007 (2.9ºC) and winter 2012 (3.1ºC) in comparison with the mean of minimum temperatures registered in the historical series (6.1ºC).

Species identification

Three different groups of isolates respect to their colony morphology on CA were found (Pm, Pc, and Po). The sequences of the ITS rDNA region (99% of homology with GenBank) conformed with *P. megasperma* (Pm) and *P. cryptogea* (Pc) as described in Gonzalez et al (2017a).

The third group of isolates (Po) was morphologically described since it was not previously known as associated with wild olive root rot. All these isolates showed colonies with scarce aerial mycelium and chrysanthemum growth pattern (Table 2). The sporangia of Po group were rarely produced in CA medium but very abundant in MSS saline solution. Gametangia were produced abundantly in CA single culture. Morphological characteristics of Po isolates are summarized in Table 2, and they were in good agreement with the description of *P. citricola* complex (Jung & Burgess, 2009), which includes *P. plurivora, P. multivora* and *P. citricola*. The sequence analyses of ITS rDNA regions conformed with *P. plurivora*, but only with a 95% homology with sequences in GenBank. Following the recent description of a new *Phytophthora* species, *P. oleae* (Ruano-Rosa *et al*., 2018), it was found that morphological data obtained for Po isolates were in a better agreement with features issued for *P. oleae* and, moreover, ITS rDNA regions and Cox-1 gene sequences conformed with *P. oleae* with a 100% homology.

Growth-temperature relationships

All five Po isolates had similar cardinal temperatures and growth rates at all temperatures. A 3rd degree polynomic model was selected: $y = aT^3 + bT^2 + cT + d$, as the best expression of growth rate *versus* incubation temperature, with y being the growth rate, T = incubation temperature, and a, b, c and d, the regression constants. According to the adjusted curve: $y = -0.0013\ T^3 + 0.0324\ T^2 + 0.253\ T - 1.85$ ($R^2 = 0.9932$), the estimated maximum growth rate for *P. oleae* isolates in CA medium was 5.8 mm day^{-1} at the optimum temperature, estimated in 19.9ºC (Figure 1a). The maximum growth rate in PDA medium was 2.5 mm day^{-1} at the optimum temperature,

226 estimated in 20.6ºC (Figure 1b) according with the adjusted curve $y = -0.0004\ T^3 +$
227 $0.0084\ T^2 + 0.164\ T - 0.9933$ ($R^2 = 0.9726$). Isolates did not grow neither at 5ºC nor at
228 30ºC in both culture media.
229
230 **Isolation frequencies**
231
232 Isolation frequencies (percentage of trees with positive isolation) of *Phytophthora* spp.
233 obtained in 2014 and 2015 from root and soil samples are in Figure 2. All the three
234 *Phytopththora* spp. (*P. cryptogea*, *P. megasperma* and *P. oleae*) were isolated from
235 roots and rhizosphere soil in both samplings. In 2014, *P. cryptogea* was the main
236 species associated with symptomatic roots (44% of positive isolation) and soil samples
237 (36% of positive isolation); while in 2015 it was replaced by *P. oleae* as the main
238 species in roots (36% of positive isolation) and rhizosphere (40% of positive isolation).
239 *Phytophthora megasperma* was isolated from roots and soil in both samplings, but
240 always at low frequencies (Figure 2). More than one *Phytophthora* spp. were recovered
241 from roots of the same tree at the same sampling, but only from four individuals.
242
243 **Pathogenicity**
244
245 The inoculated wild olive seedlings showed foliar symptoms like those observed in the
246 field: foliar yellowing and wilting, starting at the leaf edges and gradually spreading
247 towards the centre, drop of wilted leaves and twigs dieback. Necrosis and absence of
248 feeder roots were equally observed in the field and in inoculated seedlings.
249 Average values of foliar symptoms recorded weekly are in Figure 3a. Fourteen weeks
250 after inoculation, final foliar symptoms were significantly higher for wild olives

inoculated with *P. oleae* in comparison with uninoculated controls (F = 17.59, p = 0.0005). Respect to the disease progression, ANOVA analysis and comparison of means also indicated that average rAUDPC recorded for foliar symptoms was significantly higher in seedlings inoculated with *P. oleae* than in uninoculated control seedlings (F = 19.80; p = 0.0003) (Figure 3b).

Mean values of root symptoms at the end of the experiment are in Figure 3c. ANOVA showed significant differences between inoculated and uninoculated wild olives (F = 56.18; $p < 0.0001$), and the Tukey's test revealed that *P. oleae* caused a level of root symptoms significantly higher than those recorded for controls. Furthermore, *P. oleae* was consistently re-isolated from necrotic roots from inoculated seedlings (16.6% of positive isolation). No *Phytophthora* spp. were isolated from roots of uninoculated control seedlings.

Discussion

The present study shows *P. oleae* as a new pathogen for wild olive, causing root rot in a natural forest at southwestern Spain. This species is added to the other two *Phytophthora* species (*P. cryptogea* and *P. megasperma*) already known causing root rot in wild olive (González *et al*., 2017a). However, different *Phytophthora* species have been described causing root rot in olive cultivars in different parts of the world: *P. citricola* (Teviotdale, 2005), *P. drechsleri* (Teviotdale, 2005), *P. inundata* (Sánchez-Hernández *et al*., 2001), *P. megasperma* (Sánchez-Hernández *et al*., 2001), *P. nicotianae* (Vettraino *et al*., 2009) and *P. palmivora* (Vettraino *et al*., 2009).

Initially, Po isolates were tentatively identified as *P. plurivora* (González *et al*., 2017b), because of their similar morphology (Jung & Burgess, 2009), although sequence

homology appeared not strong enough (only 95%). In addition, Po wild olive isolates showed a low optimum temperature (20ºC) when compared with the 25ºC observed by Jung & Burguess (2009) for *P. plurivora*. After the recent description of *P. oleae* as cause of fruit rot in cultivated olives in southern Italy (Ruano-Rosa *et al*., 2018), a new molecular analysis (ITS and cox-1 regions) confirmed the identification of Po isolates as *P. oleae*. It is frequent to find records of a *Phytophthora* spp. causing fruit rots and root rots on woody plants; sometimes in different locations, as *P. palmivora* causing pomegranate fruit rot in India and Greece (Erwin and Ribeiro, 1996; Markakis *et al*., 2017) but crown and root rot in Turkey (Türkölmez *et al*., 2016); and sometimes both kind of symptoms occur simultaneously, as *P. citrophthora* causing fruit and root rot on *Citrus* spp. (Erwin and Ribeiro, 1996); or *P. parasitica* as pathogen on papaya fruits and roots in Hawaii (Hunter & Buddengagen 1969).

In previous samplings carried out in the same area between 2009 and 2013, González *et al*. (2017a) hypothesized that the root rot pathogen *P. cryptogea*, a high-temperature species, could be replacing a low-temperature one (*P. megasperma*) infecting wild-olives, considering a global increase in temperatures in the Mediterranean Basin (Lindner *et al*., 2010). However, the unexpected appearance of a new dominant species of low-temperature as *P. oleae*, led us to consider that it could be favoured by the extremely cold winter temperatures suffered in 2012 (minimum temperatures of 3.1ºC compared with the historical minimum mean of 6.1ºC), likely explaining the prevalence of this species in 2015. The prevalence of two low-temperature species infecting wild olive roots following exceptionally cold winter temperatures 2 years ago, as *P. megasperma* in 2009 (González *et al*., 2017a) and *P. oleae* in 2015, could be hypothesized as likely unusual considering the long, hot and dry summers prevailing in the area. However, some *Phytophthora* spp. with low optimum temperature and ability

to form thick-walled oospores and abundant sporangia, have been described as an adaptation to Mediterranean climates with long, hot and dry summer and wet winters (Brazee *et al*., 2016; Jung *et al*., 2017), including *P. oleae* (Ruano-Rosa *et al*., 2018). According with this hypothesis, *P. oleae* would be able to survive severe summer droughts in its dormant state (oospore) and rapidly resume growth and sporulation after autumn-winter rainfalls, acquiring advantage to compete for wild olive root infection when temperatures are cooler than usual. However, the prevalence of *P. oleae* infecting wild olives in the last years could also be a result of its arrival to Spanish natural forests as a new invasive *Phythophthora* pathogen. *Phytophthora oleae* was firstly described causing a soft rot of mature olive fruits in a restricted area in Italy (Ruano-Rosa *et al*., 2018) and, to our knowledge, this is the only report of this pathogen worldwide. Some of the most destructive and well-documented epidemics of trees and forests are caused by alien *Phytophthora* spp. Moreover, most of the ca. 150 currently known species of *Phytophthora* were unknown to science before they turned up in other continents as invasive aggressive pathogens of native plants or plantation crops (Jung *et al*., 2016). Due to a lack of co-evolution between the likely newly introduced *P. oleae* and wild olive, this endemic host can be highly susceptible to the pathogen and this fact could explain why it is replacing native *P. megasperma* and *P. cryptogea* in natural forests. Parameters commonly used as indirect indicators of the alien origin of a pathogen include consistent association with diseased common indigenous plant species and proven high aggressiveness to these plants in pathogenicity trials, as determined for *P. oleae* causing root rot of wild olive or fruit rot of cultivated olive (Ruano-Rosa *et al*., 2018). An stronger indicator is a low genetic variability and close phylogenetic relatedness to other non-native species (Jung *et al*., 2016). Identical cox1 and ITS1-5.8S-ITS2 region sequences were determined within the eight isolates characterized by

Ruano-Rosa *et al.* (2018), with only one isolate showing a single base insertion in ITS1. Additionally, the five isolates characterized in this work exhibit identical cox1 and ITS sequences. Despite the low genetic variability exhibited by *P. oleae*, which points to its alien origin, the low number of isolates analysed, together with their small area of provenance in Italy (two olive orchards in the same province) and Spain (one wild olive forest), make neccesary additional research to determine

~~For plant biosecurity and disease management, it is of utmost importance to know~~ whether *P. oleae* is indigenous in Europe or it must be considered as a new alien invasive pathogen. Moreover, to know it possible alien origin is of utmost importance to assess the risk of ~~may be~~ spreading from natural forest stands to olive plantation crops in Spain, although it will not necessarily successfully establish.

The likely invasive nature of *P. oleae*, together with increasing rain episodes concentrated in short periods frequent in southern Spain, would allow outbreak infections in wild olive forests, also putting at risk cultivated olive orchards.

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Figure 1 Growth rate patterns for *Phytophthora oleae* isolated from wild olives growing on CA (a) and PDA (b) media. Lines represent the adjusted third degree polynomic model on CA ($y = -0.0013\ T^3 + 0.0324\ T^2 + 0.253\ T - 1.85$; $R^2 = 0.9932$) and PDA ($y = -0.0004\ T^3 + 0.0084\ T^2 + 0.164\ T - 0.9933$; $R^2 = 0.9726$). Dots are the main values of the observed growth rates. Arrows indicate the estimated optimum growth temperature on CA (19.9ºC) and PDA (20.6ºC).

Figure 2 Percentage of trees with positive isolation of *Phytophthora oleae* (Po), *P. cryptogea* (Pc) or *P. megasperma* (Pm) from roots (a) and rhizosphere soil (b) of symptomatic wild olives sampled in 2014 and 2015.

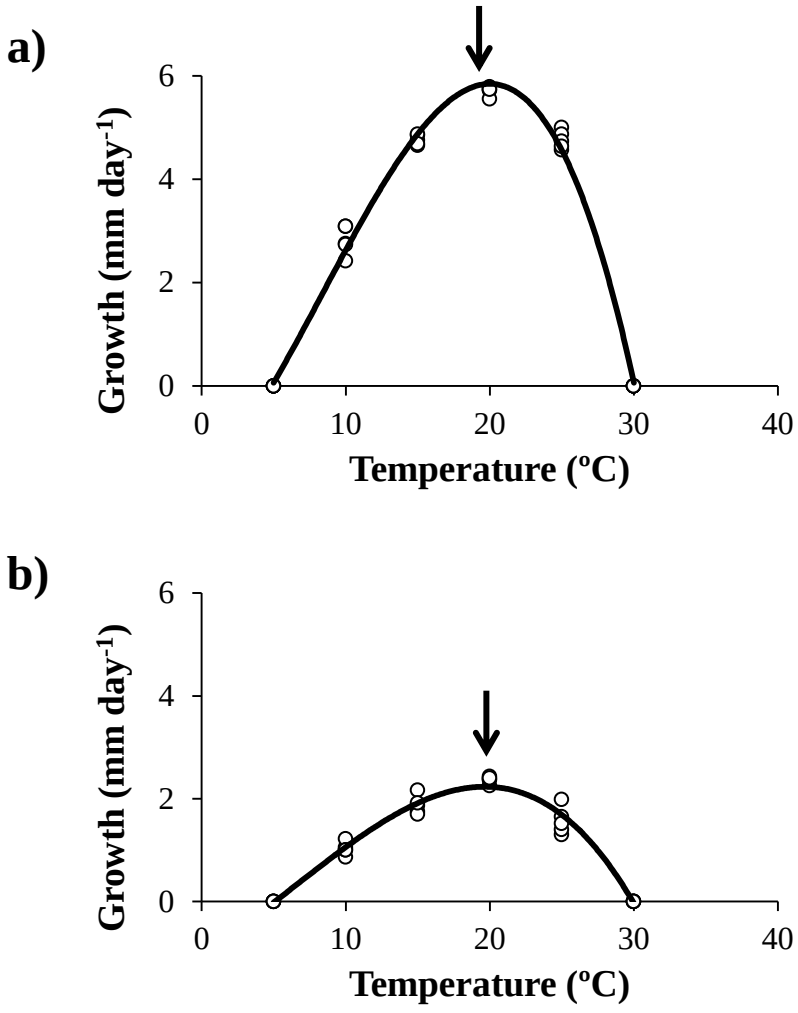
Figure 3 Average values and standard errors of foliar symptoms (a), relative area under the disease progress curve (rAUDPC) (b), and root symptoms (c), recorded on wild olives inoculated with *P. oleae* and submitted to periodical soil flooding for 14 weeks. For each graph, values with different letters differ significantly according with Tukey's HSD test ($p < 0.05$).

Table 1 Average seasonally minimal and maximal temperatures (ºC) between 2007 and 2015. Data obtained from 'La Puebla del Río II' Meteorological station (37º 04' 48'' N, 06º 02' 47'' W), Seville, Spain.

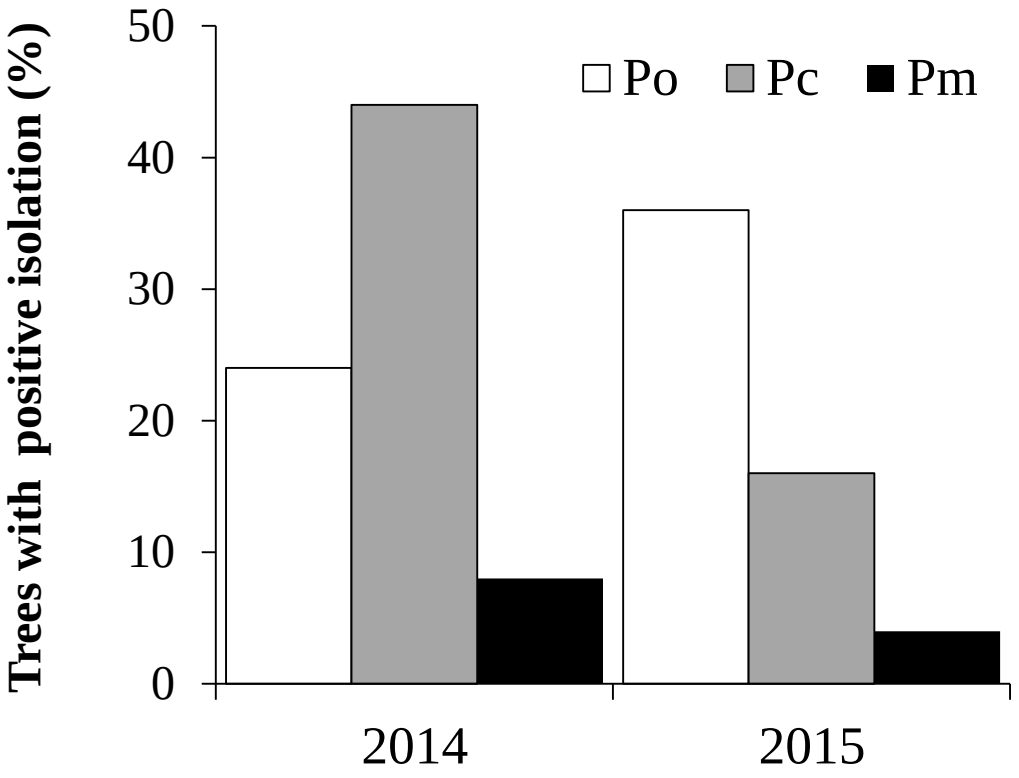
	Season	Year									Mean (2001-2015)
		2007	2008	2009	2010	2011	2012	2013	2014	2015	
Mean of minimals	Winter (Jan-Mar)	2.9	5.6	5.8	7.9	7.0	3.1	6.8	6.8	5.1	**6.1**
	Spring (Apr-Jun)	10.8	12.5	12.1	13.0	14.9	13.2	12.1	13.2	14.0	**13.2**
	Summer (Jul-Sep)	16.1	16.8	17.0	17.9	17.4	16.7	18.0	16.6	16.9	**17.3**
	Autumn (Oct-Dec)	6.8	7.2	10.2	9.1	9.4	10.4	8.6	10.2	11.0	**9.5**
Mean of maximals	Winter (Jan-Mar)	15.2	18.2	17.4	16.2	17.4	18.6	16.6	17.9	18.1	**17.4**
	Spring (Apr-Jun)	24.7	26.2	27.0	26.3	28.5	27.7	26.3	27.5	28.7	**27.0**
	Summer (Jul-Sep)	31.2	31.5	32.6	32.9	32.1	32.2	32.3	30.3	31.9	**31.7**
	Autumn (Oct-Dec)	19.3	18.6	21.8	19.4	21.5	20.2	20.9	20.9	21.4	**20.3**

Table 2 Morphological characteristics of Po isolates (*Phytophthora oleae*)

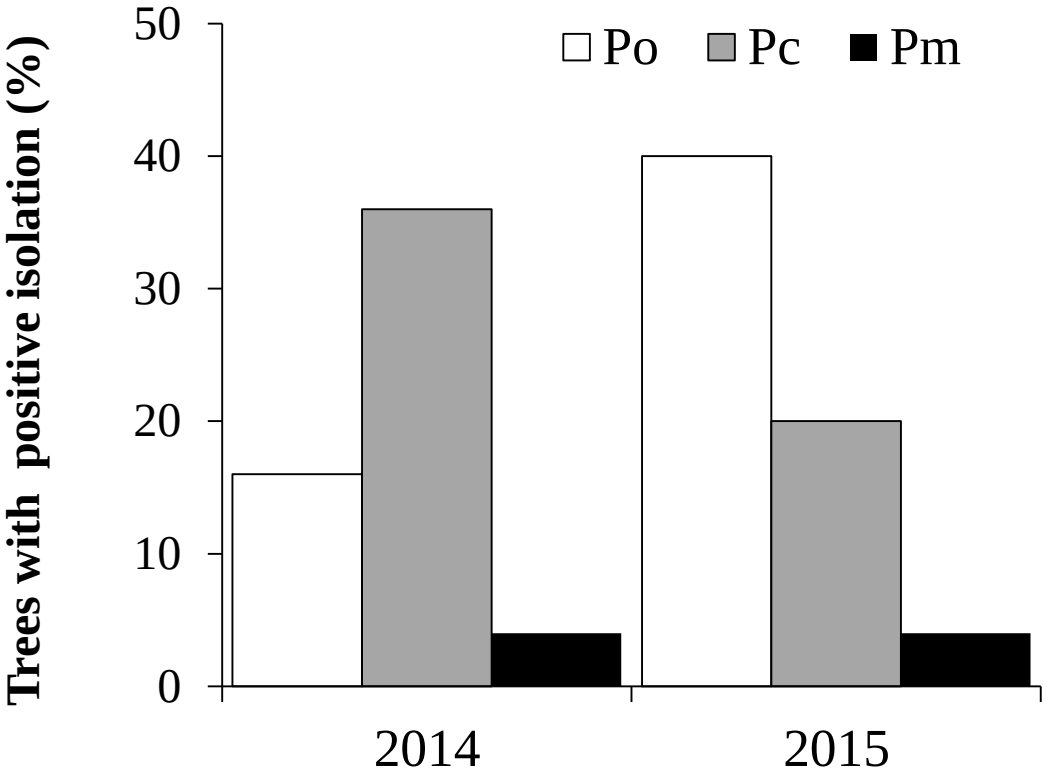
Colony morphology on CA (6 days at 20º C)	Chrysanthemum
Sexual system	Homothallic
Oogonia diameter (µm ± SE)	28.8 ± 0.3
Oospores	Aplerotic
Diameter (µm ± SE)	26.8 ± 0.2
Oospore wall thickness (µm ± SE)	1.5 ± 0.1
Antheridia	
Amphigynous	0%
Paragynous	100%
Length (µm ± SE)	6.3 ± 0.3
Oogonium diam:antheridium length ratio	4.6
Sporangia	
Shape	Obpyriform, ovoid
Length (µm ± SE)	39.1 ± 0.3
Breadth (µm ± SE)	28.2 ± 0.3
Length: breadth ratio	1.4
Papilla	Semi-papillate
Pore width (µm ± SE)	6.6 ± 0.1


a)
Growth (mm day-1)
0
2
4
6
0
10
20
30
40
Temperature (ºC)
b)
Growth (mm day-1)
0
2
4
6
0
10
20
30
40
Temperature (ºC)

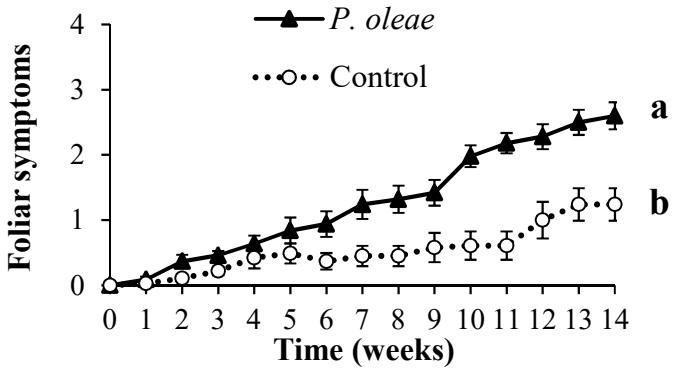
a)


Trees with positive isolation (%)
50
40
30
20
10
0
Po
Pc
Pm
2014
2015

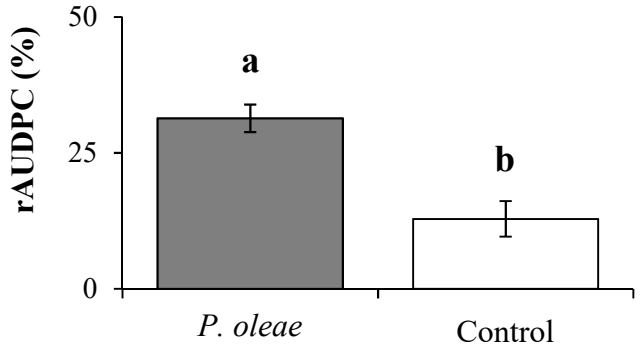
b)


Trees with positive isolation (%)
50
40
30
20
10
0
Po
Pc
Pm
2014
2015

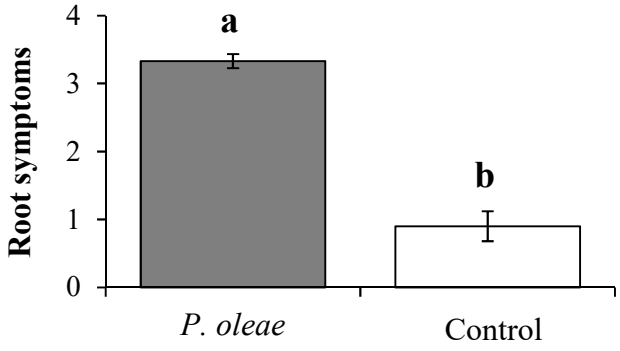
a)


P. oleae
Control
Foliar symptoms
Time (weeks)
a
b
0
1
2
3
4
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

b)


rAUDPC (%)
50
25
0
a
b
P. oleae
Control

c)


Root symptoms
4
3
2
1
0
a
b
P. oleae
Control