RESEARCH PAPERS

Experimental minimum threshold for Phytophthora cinnamomi root disease expression on Quercus suber

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Summary. Quercus suber seedlings were potted in soils infested with increasing concentrations of Phytophthora cinnamomi chlamydospores and submitted to weekly flooding for 3 months to favour root infections. Increasing quantities of chlamydospores led to an exponential increase in their ability to germinate. Root symptoms (necrosis and/or absence of feeder roots) were significantly more severe than those recorded in uninfested soil only for plants potted in soils infested with 61 cfu g⁻¹ or more. Although generated using potting mix, this minimum threshold represents a tool for checking the potential infectivity of infested soils or to assess the effectiveness of some control methods to reduce soil inoculum. However, a low level of root infection was recorded even at 3 cfu g⁻¹. Therefore, long-term disease risk may be present whenever the pathogen is detectable in oak forest soils.

Key words: chlamydospores, cork oak, infection, inoculum.

Introduction

Quercus is a genus especially threatened by Phytophthora cinnamomi in areas of Mediterranean climate, including Spain (Sánchez et al., 2002), California (Garbelotto et al., 2006) and Italy (Scanu et al., 2013). Highly variable P. cinnamomi soil inoculum densities have been reported for diseased oak ecosystems in southern Spain, ranging from 4–49 colony forming units (cfu) g⁻¹ dry soil (Romero et al., 2007) to 25-2500 cfu g⁻¹ (Gómez-Aparicio et al., 2012). Knowledge of the minimum threshold of P. cinnamomi inoculum provides an experimental reference point to check the potential infectivity of infested soils or to assess the effectiveness of control methods aimed at reducing soil inoculum (green or mineral amendments, biofumigation). The aim of the research reported here was to determine the threshold of inoculum necessary to cause root disease in cork oak plants under highly favourable experimental conditions for disease development.

Materials and methods

Experiment 1

Two P. cinnamomi isolates (PE90 from holm oak, and PA25 from cork oak) were used to obtain inocula at different concentrations. The mother inoculum was prepared following Sánchez et al. (2002) and consisted of a water suspension of chlamydospores from both isolates adjusted to 1.5 × 10⁴ chlamydospores mL⁻¹ (Romero et al., 2007). Additional inocula of 1500, 150 and 15 chlamydospores mL⁻¹ were prepared by successive water dilutions of the original suspension. Four containers, each with 30 L of soil (sand-peat 1:1 vol., 22.5 kg, pH = 6.36) were homogeneously infested with 1 L of each inoculum suspension and a fifth container (control) was prepared by adding 1 L of water (0 concentration). Three 15 mL soil samples per container (replicates), were plated on NARPH medium as described in Romero et al.
(2007). Individual Phytophthora cinnamomi colonies, each derived from at least one viable chlamydo-
spore, were identified and counted. After sampling, 
each soil was distributed into ten free-draining 3 L 
capacity plastic pots and 18 month-old Q. suber seed-
lings (replicates) were planted (one per pot) after re-
moving most soil without damaging them. The pots 
were each placed in a plastic tray without drainage 
(57 × 41 × 9 cm) and placed in an air-conditioned 
greenhouse (daily cycle of 25 ± 2°C for 12 h and 10 ± 
2°C for 12 h) in a randomized block design. For 2 d a 
week for the next 3 months the trays were partially 
filled with tap water, to periodically flood the soil 
(Serrano et al., 2012). After this time, root rot symp-
toms were assessed according to the percentage of 
root necrosis or root absence on a 0–4 scale (0 = 0% 
necrotic roots, 1 = 10–33%, 2 = 34–66%, 3 = more than 
67% necrotic roots, 4 = 100% dead root) (Serrano et 
al., 2012).

Experiment 2

To narrow the concentration range of inoculum 
necessary to cause significant root disease, a new 
mother inoculum suspension was prepared as de-
scribed above, adjusted to 3 × 10³ chlamydospores 
ml⁻¹ and diluted with water to obtain inocula con-
taining 2 × 10³, 1.5 × 10³, 10³, 500 or 50 chlamydo-
spores ml⁻¹. Soil mix (above) was infested and pro-
cessed as described above, and six 18 months-old Q. 
suber seedlings (replicates) were individually pot-
ted, incubated, harvested and assessed, as described 
above.

At the end of both experiments, root segments 
from plants potted in infested or control soils were 
plated on NARPH medium for re-isolation of the 
pathogen.

data analyses

Inoculum concentration data were transformed to 
[(cfu g⁻¹) + 0.5]¹/² for ANOVA analysis. A regression 
curve was performed with data from Experiment 1, 
to establish the relationship between the amount of 
chlamydospores added to the soils and the number of 
viable chlamydospores recovered. Data obtained 
from root symptom assessments were tested for ho-
moeedasticity by the Bartlett’s test, and when het-
erogeneity was detected, angular (Experiment 1) or 
logarithmic (Experiment 2) transformations were 

applied to the data. ANOVA was performed for 
root symptoms and mean values compared by the 
Tukey’s HSD test at P<0.05. Statistix 8.0 (Analytical 
Software) was used for data analyses.

Results and discussion

In Experiment 1, significant (DF = 5, F = 33.67, 
P<0.0001) differences in viable chlamydospores (cfu 
g⁻¹) were detected depending on the chlamydospore 
concentration added to the soils, following an expo-
nential relationship: y= 0.0416 e².0893x (R² = 0.9393) 
(Figure 1). Increasing quantities of chlamydospores 
in soil led to an exponential increase in their ability 
to germinate, while for other soilborne pathogens, 
such as Fusarium oxysporum f. sp. lini, chlamydospore 
viability suffers a slight decrease at high initial chla-
mydospore densities (Couteaudier and Alabouvette, 
1990). Only root symptoms recorded in plants grow-
in soils infested with 1.5 × 10⁴ or 1500 chlamydo-
spores mL⁻¹ (256.5 and 54.7 cfu g⁻¹) were significantly 
(DF = 4, F = 6.29, P<0.0001) more severe than those 
recorded for plants potted in soils infested with the 
two lowest chlamydospore concentrations and the 
control soil (Figure 2). Because of the constraints im-
posed by the pots and by the frequent flooding, all 
the experimental controls developed low levels of 
root necrosis; hence such controls need to be includ-
ed for a correct determination of disease symptoms.

Average numbers of viable chlamydospores ob-
tained from infested soils in Experiment 2 fitted well 
with the quantities expected according to the rela-
relationship presented in in Figure 1. This resulted (on 
average, and respectively) in 3, 21, 41, 61 and 82 cfu 
g⁻¹ from the initial 50, 500, 10³, 1.5×10³, and 2×10³ 
chlamydospores mL⁻¹ applied. Root symptoms of 
seedlings potted in soils infested with 61 or 82 cfu g⁻¹ 
were significantly greater (DF = 5, F = 9.48, P=0.0001) 
than those recorded for plants potted in control soil, 
while lowest concentrations of viable inoculum did 
not cause disease (Figure 2). This threshold of 61 cfu 
g⁻¹ was the minimum required to cause cork oak root 
disease in the highly favourable conditions artifi-
cially provided in this experiment. This value is similar 
to that obtained for P. capsici, which requires 41 oo-
spores g⁻¹ to produce 50% mortality in pepper plants 

Phytophthora cinnamomi was re-isolated from ne-
ecrotic roots of plants potted in soil infested with low 
inoculum concentrations (2.7 cfu g⁻¹ in Experiment 1,
Phytophthora cinnamomi inoculum threshold for oak root disease expression

or 3, 21 and 41 cfu g⁻¹ in Experiment 2), resulting in 14–39% of positive isolations. The pathogen was never recovered from control roots or from soil infested with 0.7 cfu g⁻¹ (Experiment 1). These results indicate that infections caused by these low amounts of inoculum lack the ability to progress into significant root mortality. It is possible, however, that in time, these lesions may lead to significant symptoms or new infections. Mitchell (1978) concluded that only 0.6 or 0.9 chlamydospores g⁻¹ of P. citrophthora or P. palmivora were needed to infect, respectively, Morenia odorata or Carica papaya, in growth chamber studies, although some of these results pertained to aerial infections. Additionally, these limited infections may allow for persistent survival of the pathogen in a site, as suggested by recrudescence of disease in absence of stringent inoculum eradication (Dunstan et al., 2010). Notwithstanding the role played by infections caused by low levels of inoculum, our results suggest that 61 cfu g⁻¹ represents a consistent threshold of inoculum capable of inducing significant root infection, at least in the experimental conditions applied in the present study.

Although the minimum threshold determined here can be useful as an experimental reference for checking the potential infectivity of infested soils, or to assess the effectiveness of some control methods to reduce soil inoculum, we are also aware that such a threshold may be different in natural forest soils. It is likely that in natural soils the threshold may be greater than the one determined here, thus our results may be valuable under this precautionary principle. We emphasize that these types of experiments are useful when monitoring disease spread in sites already known to be infested. When assessing risk, a site should be regarded “at risk” whenever the pathogen is detectable in the soil (Dunstan et al., 2010).

We conclude that presence/absence of P. cinnamomi needs to be accurately monitored at the large geographic scale to identify all cork oak sites at risk and all sites that may be a source of new infestations. Inoculum loads may be calculated to track disease expression and efficacy of disease management approaches. Results from inoculum load studies may indicate which sites may be more at risk and where

\[ y = 0.0416e^{2.0893x} \]

\[ R^2 = 0.9393 \]

Figure 1. Relationship between numbers of Phytophthora cinnamomi chlamydospores added to soil [log (chlamydospores ml⁻¹)] and viable chlamydospores detected (cfu g⁻¹). Dots are the obtained values and the line the adjusted exponential curve

![Figure 1](image1.png)

Figure 2. Mean severity of root disease symptoms recorded for cork oaks growing in soils infested with different numbers of Phytophthora cinnamomi chlamydospores. Lines are standard errors of ten replicates (Experiment 1, left chart) or six replicates (Experiment 2, right chart). Bars with different letters differ significantly according to Tukey’s HSD test (P<0.05).

![Figure 2](image2.png)
control measures may be more efficient. This will provide a way to prioritize choices when dealing with the widespread presence of *P. cinnamomi* in oak forest soils in southwestern Spain and southern Portugal (Romero *et al*., 2007), to indicate threats to the survival of oak forests in the region.

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