Comunicación invitada

STUDIES ON THE ASSOCIATION OF THE *Quercus suber* DECLINE DISEASE WITH *Phytophthora cinnamomi* IN PORTUGAL

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Summary

In Portugal, the decline disease has been described in evergreen oaks (*Quercus suber* L. and *Q.ilex* subsp. *rotundifolia* Lam.) since the end of the 19th century. The mortality of these species affects, particularly the central and southern regions of the country, being one of the most severe forest problems. *Phytophthora cinnamomi* Rands is the main pathogen responsible for the cork and holm oak mortality in Portugal. Several studies have been developed aiming at a better understanding of the effect of the *P. cinnamomi* action on the cork oak trees decline.

The present work describes preliminary results of some of these studies.

Resumen

ASOCIACIÓN DE *Phytophthora cinnamomi* CON LA SECA DE *Quercus. suber* EN PORTUGAL

En Portugal, la enfermedad de la "seca" se ha descrito en los Quercus de hoja perenne (*Quercus suber* L. and *Q.ilex* subsp. *rotundifolia* Lam.) desde el final del siglo XIX. La mortalidad de estas especies afecta, particularmente las regiones centrales y meridionales del país, siendo uno de los problemas forestales más graves. *Phytophthora cinnamomi* Rands es el principal patógeno responsable de la mortalidad de alcornoques y encinas en Portugal. Se han desarrollado varios estudios teniendo como objetivo una mejor comprensión del efecto de la acción de *P. cinnamomi* en el decaimiento de los Quercus.

El actual trabajo describe resultados preliminares de algunos de estos estudios.

Introduction

A heavy mortality and decline of evergreen oaks (*Quercus suber* L. and *Q. ilex* subsp. *rotundifolia* Lam.) has occurred across the southeast Iberian Peninsula.

In Portugal, the decline and death of cork oak (*Q. suber*), has been described and observed since the end of the 19th century (Almeida, 1898; Câmara-Pestana, 1898). The incidence of this problem markedly increased during the 1980s. Since then the mortality of this species became recognised as an important problem as a high number of trees is dying, namely in the central southern regions of the country (Alentejo and Algarve) (Fig. 1). This problem is a matter of high concern for the future of cork production. Moreover, this disease is a very serious environmental problem that threatens the survival of "Montado" in Portugal, an ancient agroforestry system. Actually, cork oak is a very important forest species occupying an area of *ca.* 730 000 ha (22.2% of the national forest area) concentrated mainly in Alentejo and Ribatejo (*ca.* 648 000 ha) and in Algarve (*ca.* 38 300 ha) (Moreira-Marcelino, 2001).

In earlier reports, this decline has been attributed to a complex involving biotic and abiotic stress factors, such as pathogenic fungi, *Biscogniauxia mediterranea* (de Not.) Kuntze (=*Hypoxylon mediterraneum* de Not. Ces. & de Not.), *Endothiella gyrosa* Sacc.; insect attacks, *Porthetria dispar* L., *Coraebus florentinus* (Herbst), *Euproctis chrysorrhoea* L., *Platypus cylindrus* F.; severe droughts, air pollution and cultural practices (Baeta Neves, 1947, 1948, 1949; Branquinho d'Oliveira, 1931). However, during 1990s studies by Portuguese and Spanish groups indicated that *Phytophthora cinnamomi* Rands could be the main cause of the evergreen oaks decline disease (Moreira *et al.*, 1993; Brasier *et al.*, 1993a; 1993b; Cobos *et al.*, 1993), and showed that cork and holm oak species are susceptible to this pathogen (Tuset *et al.*, 1996). In Portugal, during the last fourteen years, many different cork and holm oak areas situated in the southern regions were surveyed for infestation and infection by *P. cinnamomi.* The resulting analyses showed an increase of *P. cinnamomi* presence with time suggesting a build up of the pathogen population.

Figure 1. A- Cork oak tree with symptoms of decline disease, showing half of the crown dead; B- General view of a site with affected trees in the Caldeirão Mountain, Algarve

Some studies have suggested the joint influence of edaphic and physiographic factors, such as, soil type, site aspect and topography (Moreira & Martins, 2005) and alternating droughts and waterlogging periods (Brasier, 1993a; 1993b; Cobos *et al*., 1993; Gallego *et al*., 1999) with the *P. cinnamomi* activity. These factors may contribute to the severity of the disease and its rate of spread.

Phytophthora cinnamomi is a plant pathogen introduced in Europe and is known as responsible for the decline and death of a wide variety of native plant species, exotic fruit trees, forest species and ornamental plants, in different countries.

In Portugal, *P. cinnamomi* has been also isolated from maquis species (*Cistus* spp., *Calluna vulgaris, Ulex* spp., *Arbutus unedo, Genista triacanthos* Brot.) and from *Pinus pinaster Aiton,* associated to cork and holm oak diseased areas (Moreira & Martins, 2005). In a few sites with oak decline, plants from some shrub species, such as *Cistus* spp., *Calluna vulgaris, Genista triacanthos* showing disease symptoms were found. This was observed in areas where oak trees were severely affected by the disease or already succumbed to sudden death. Other plant species, like *Arbutus unedo,* appear to be infected but do not show any symptom.

The control of this pathogen is very difficult. It is a soil born plant pathogen, having a long survival in the soil as resistant structures and a large host range; when symptoms are evident the plant root system is already highly destroyed (Tsao, 1990). So, the knowledge of the infection process is very important in order to better understand its progression and develop control strategies.

Cytological studies

Our studies showed that *P. cinnamomi* invades quickly the root system in both species, cork (Fig. 2-A) and holm oak (Fig. 2-B). Penetration and intra and intercellular progression of the pathogen through the cortical parenchyma and vascular cylinder are similar in both species. However, *P. cinnamomi* invades holm oak root tissues more rapidly than cork oak ones (Moreira-Marcelino, 2001; Pires *et al*,. 2005).

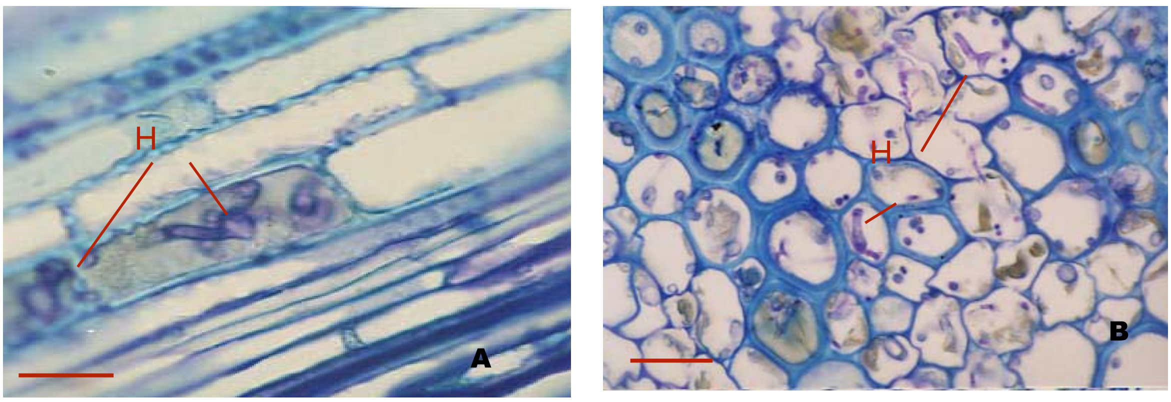

Figure 2. Invasion of the vascular cylinder by *P. cinnamomi* hyphae after inoculation, H-hypha. A- cork oak root; B- holm oak root. Bar= 10µm

In cork oak plants the accumulation of phenolic compounds was observed in association with pectins of intercellular spaces in phloem and parenchyma cells close to the hyphae (Fig.3).

The production of phenolic material is frequently referred to in several species as a response to the pathogen invasion. This may contribute to the reinforcement of the mechanical strength of the pectic material by decreasing its digestibility by the pathogen (Lheirminier, *et al.,* 2003). Pires *et al.* (2005) reported that these compounds may not prevent root invasion in this species, as hyphae invaded quickly the vascular cylinder. Studies are being carried out in order to clarify this point.

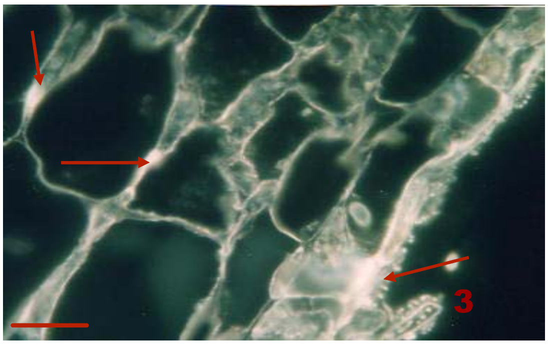

Figure 3. Accumulation of phenolic compounds (arrows) in the parenchyma cells close to the hyphae. Bar= 10µm.

The role of elicitors

Elicitins are low molecular weight proteins, often toxic, secreted by most species of *Phytophthora* and *Pythium*; they can cause hypersensitive reactions and induce systemic acquired resistance in some species, against a large number of pathogenic fungi and bacteria. The synthesis of a non-toxic elicitin that can elicit plant defenses without originating cell injury would be helpful to provide a non-toxic disease control.

The action on cork oak roots of a recombinant α-cinnamomin (Sousa, 2004), an elicitin secreted by *P. cinnamomi* during the infection process by *P. cinnamomi,* has been studied analysing the cellular alteration induced by the absorption of this protein by cork oak seedling roots. The cinnamomin treatment induced a cellular damage gradient. Severe damage of the roots was observed in the nearest site of the immersed zone decreasing towards the upper regions (Fig.4).

Phytophthora cinnamomi penetrates epidermal and cortical cells easily after cinnamomin treatment, but a high number of degraded hyphae were observed in the cortical parenchyma that presented a good preservation. The defense reaction alterations were not evident, although cinnamomin treatment induced important damage on the pathogen (Maia *et al.*, 2005).

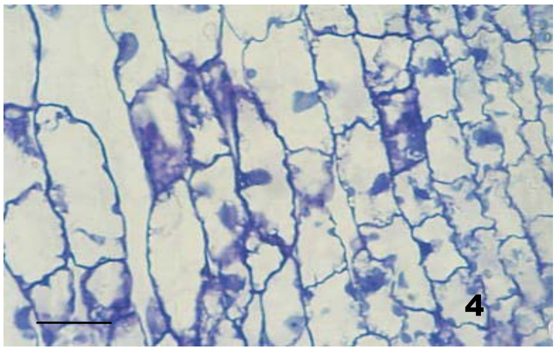

Figure 4. Light plasmolysis of cork oak cells after cinnamomin treatment.Bar= 10µm

Disease control

Several methods have been proposed to control *P. cinnamomi*. Chemical treatments were tested in Spain (Fernández-Escobar *et a*l, 1999) and are being used in some areas of Portugal to treat adult trees but their efficiency onwards is unknown. The use of more tolerant plants to *P. cinnamomi* could be an important way to overcome this problem, particularly in the reforestation of areas with a high pathogen density. This approach is being developed since 2004 in a joint cooperation project between teams from the University of Huelva, University of Algarve and Estação Agronómica Nacional-INIAP. This study is based on the selection of seedlings obtained from seeds that have been grown under controlled conditions, using a natural soil as substrate infested with three levels of inoculum of the pathogen. Seeds were collected from 180 trees from seven different regions of Portugal and Spain. Preliminary results showed that the seedlings had a high diverse response to the infection probably due to genetic variability (Raúl Tápias *et al*, 2005).

Cork oak seeds from the same families were sown at January 2004 in a natural infested soil with *P. cinnamomi*. The evaluation of the progression of plant's height showed an evident difference between regions. Plants from Alto Alentejo presented a better development than those from the other regions (Fig.5). However, no significant difference among families was observed in two years. This study must be carried on to follow the behaviour of the represented families of cork oak trees and clarify the relationship of the intraspecific genetic variability with the response to *P. cinnamomi* infection.

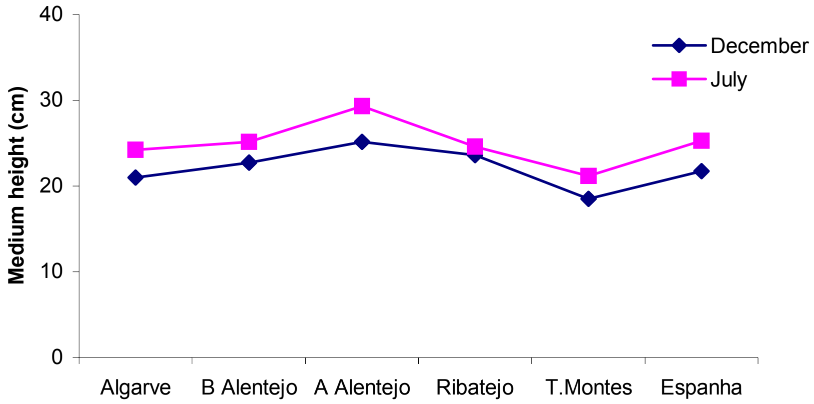

Figure 5. Medium height of the plants grown in a natural infested soil.

The role of abiotic factors

As several authors have stressed the effect of some environmental factors on the *P. cinnamomi* activity (Newhook and Podger, 1972; Sánchez *et al.*, 2002) studies were also undertaken to clarify the water availability influence on cork oak disease decline.

The interaction between water status and infection of *Q. suber* by *P. cinnamomi* has been followed in a greenhouse experiment in order to evaluate the performance of the two years old cork oak plants submitted or not to drought or flooding conditions. Under drought, infected plants presented lower relative water content (44%) than non-infected ones (62%), despite their similar soil water content (Fig. 6A). This result was probably due to the pathogen and was reflected on membrane integrity (Fig. 6B), as the pathogen infects and destroys the fine roots leading to a decrease in water and nutrient absorption (Robin *et al.*, 1998). Infected plants submitted to drought showed a higher membrane damage (64%) than non-infected ones (33%).

Concerning P_n, the pathogen induced a reduction in all treatments but the plants submitted to drought were the most affected. This tendency is similar to that observed in stomatal conductance.

Shoot and root mineral analysis showed that *P. cinnamomi* induced changes in mineral composition, increasing deficiency and / or toxicity of some elements, namely of Calcium and Copper. In fact, in infected plants, Ca presented a consistent decrease of *ca*. 20% both in roots and in shoots.

These results suggested that *P. cinnamomi* / drought interaction increased the negative effects of the pathogen (Quartin *et al*., 2005). This situation is also verified in natural conditions.

Acknowledgements

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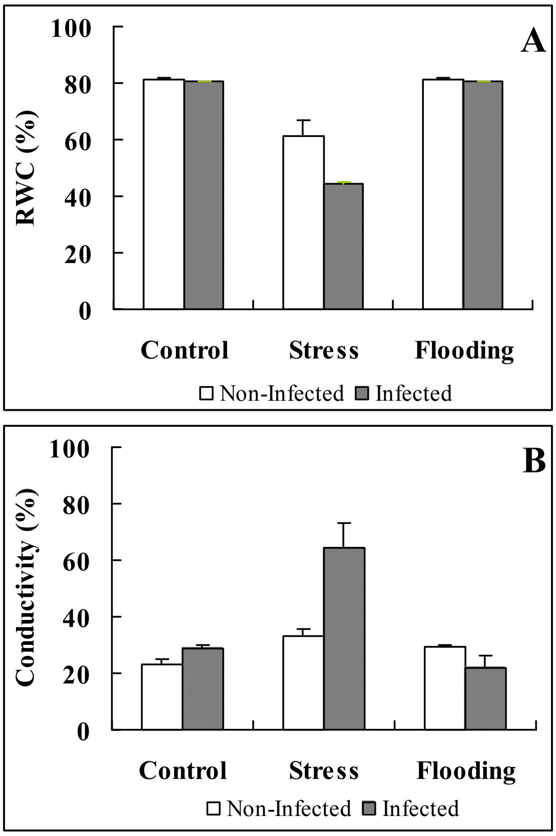

Figure 6. Effect of *P. cinnamomi* and water availability on RWC (A) and Conductivity (B) of oak plants. Values are mean+SE (n=3)

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