Integrated Protection in Oak Forests
IOBC/wprs Bulletin Vol. 76, 2012
pp. 93-96

Phytophthora root disease: a new threat for cork oaks at Doñana National Park (south-western Spain)

Paolo De Vita[1], María S. Serrano[1], Pierre Callier[1], Cristina Ramo[2]. Luis V. García[3], M. Esperanza Sánchez[1]*
[1]*Dpto. Agronomía. ETSIAM, Universidad de Córdoba, Apdo. 3048, 14080 Córdoba, Spain;*
[2]*Estación Biológica de Doñana (CSIC). Avda Américo Vespucio s/n, 41092 Sevilla, Spain;*
[3]*IRNAS (CSIC), Avda Reina Mercedes 10, 41012 Sevilla, Spain.*
**E-mail: ag1sahem@uco.es*

Abstract: The cork oak is an emblematic species of great ecological value in the ecosystem 'stabilized sands' at Doñana National Park (south-western Spain). The current oak population mainly consists of big scattered individuals representing only a 10% of the forest that occupied the area three centuries ago and which was devastated by massive cutting. After the protection of the area as Biological Reserve forty years ago, all silvicultural practices such as cutting, pruning and cork extraction ceased. However, the population of mature trees is suffering a quick decline that threatens their survival. The main menace to the survival of cork oaks comes from the high density of herbivores (deer, wild boar, cattle, horse) which eat almost all of the acorns and prevent young plants to grow and replace old trees. Additionally, a significant number of oaks are frequently occupied by a nesting colony of wading birds (storks, herons, spoonbills). These trees show signs of stress (defoliation), and eventually die much faster than trees not occupied. Finally, we detected a new problem: the sudden decline of trees, which may die within a few months independently to the presence of nesting birds. *Phytophthora cinnamomi* has been consistently isolated from roots and rhizosphere of these trees and an important increment in the number and size of these foci is expected. In this way, proposals to monitoring the affected trees and preventive application of phosphonates by trunk injection have been exposed to the Park authorities.

Key words: oak decline, *Phytophthora cinnamomi*, *Quercus suber*

Introduction

The cork oak is an emblematic species of great ecological value in the ecosystem 'stabilized sands' at Doñana National Park (south-western Spain). The current oak population mainly consists of big scattered individuals representing only a 10% of the forest that occupied the area three centuries ago and which was devastated by massive cutting. After the protection of the area as Biological Reserve 40 years ago, all silvicultural practices, such as cutting, pruning and cork extraction, ceased. However, the cork oak population is suffering a decline that threatens its survival. The main menace to the survival of cork oaks comes from the high density of herbivores (deer, wild boar, cattle, horse) which eat almost all of the acorns and prevent young plants to grow and replace old trees (Herrera, 1995). Additionally, a significant number of oaks which are frequently occupied by a nesting colony of wading birds (storks, herons, spoonbills) show signs of stress (defoliation), and eventually die much faster than trees not occupied (Ramo *et al.*, 2009). Cork oaks growing at Doñana National Park are evaluated every year for defoliation in a scale from 0 (defoliated and eventually dead tree) to 5 (well covered crown). Defoliation of the heavily-occupied trees tends to increase along the years (Ramo *et al.*, 2009). However, in 2009 the annual evaluation revealed the sudden decline of several trees, one of which died in 2010, in absence of nesting birds. The aim of the

present work was to elucidate the cause of this unusual decline and identify the pathogen associated with the disease threatening cork oaks at Doñana National Park.

Material and methods

Foci of disease were localized in the basis of the information generated in the 2009 evaluation trial for cork oak defoliation. A total of seven foci of trees showing decline symptoms in absence of bird nesting where located at the so called "Vera de Doñana" (Fig. 1). In autumn 2009, trees in the foci were assessed for symptoms and sampled. Samples of feeder roots and soil from the rhizosphere were taken from two different points of each tree, following the methodology described in Sánchez *et al.* (2002). Root excavations of chosen trees were carried out 1m from the base of the trunk, at a depth of 10-50cm. Samples of damaged feeder roots and soil from the rhizosphere (1kg approx.) were collected, placed in plastic bags, sealed and transported to the laboratory in a portable freezer.

Samples from different trees were independently processed. For each sample, feeder root segments were washed under running tap water for 2h, and directly plated on NARPH (Nistatin-Ampicillin-Rifampicin-Pentachloronitrobenzene-Hymexazol cornmeal agar) selective medium for *Phytophthora* species (Romero *et al.*, 2007). Soil samples were air dried and sieved (2mm pore diameter). A further 2g of clean soil was placed in plastic glasses with distilled water (1:6 vol.). Pieces of new formed cork oak leaves (4-5mm in surface area, avoiding the leaf margin and the central vein) were floated over the water, acting as baits for *Phytophthora* spp. Glasses were incubated 48h at 22°C under 12h light/12h dark. After the incubation period, leaf pieces were cleaned with sterile water, dried with sterile filter paper, and plated on NARPH medium (Sánchez *et al.*, 2002). All the plates were incubated at 22°C in the dark for 4 days. Colonies growing on selective medium were grouped according to morphology and selected isolates were transferred to CA (Carrot-Agar) medium (Dhingra & Sinclair, 1995).

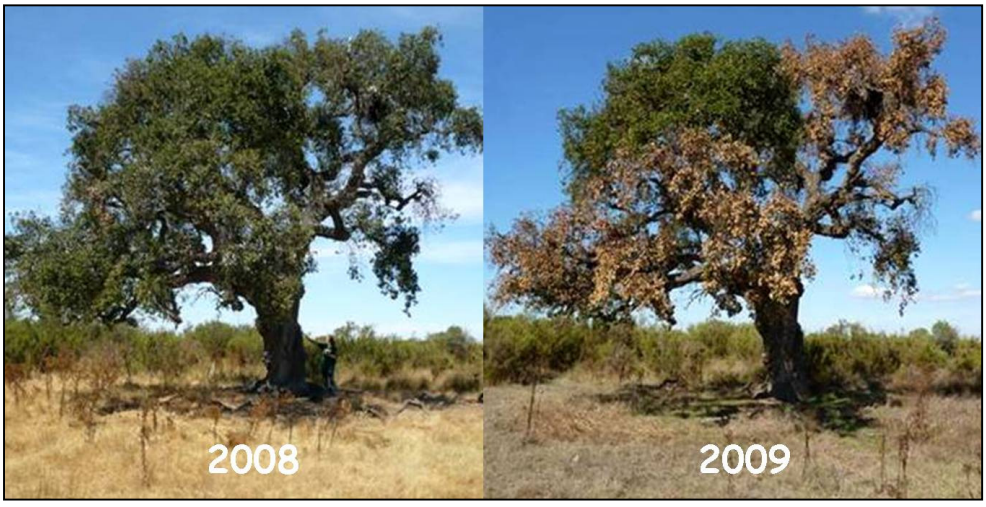

Figure 1. Healthy cork oak in 2008 and showing symptoms of decline (branch dieback) in 2009

All the isolates obtained were grown on CA medium at 20ºC in darkness. Between 6 and 30 days of growth, production of vegetative structures and resistant spores was assessed under an inverted microscope. Structures were observed after removal to a glass microscope slide and staining with acid fuchsin in lactophenol. Soil water extract was used to stimulate the

production of asexual reproductive structures (Ribeiro, 1978). Soil extract was prepared with natural soil from an olive tree orchard (Sánchez *et al.*, 2002). Sporangial production was assessed by direct observation under the inverted microscope. Sporangia were observed after removal to a glass microscope slide and staining as above described.

Results and discussion

A severe decline affecting Mediterranean *Quercus* species has been reported since the early 1990s in southern Spain and Portugal, leading to widespread mortality of the evergreen Mediterranean oaks (Brasier, 1996; Moreira *et al.*, 1999; Sánchez *et al.*, 2002; 2006). Root rot caused by *P. cinnamomi* has been described as the main disease associated with decline and the only one causing sudden death (Brasier, 1996; Sánchez *et al.*, 2002; 2006).

At Doñana National Park, aerial symptoms of cork oaks consisted on wilted leaves still attached to the branches (sudden death or apoplexy, Sánchez *et al.*, 2002). Branch dieback was frequent but never associated with the presence of any type of cankers. Epicormic shoots were rare. There was visual evidence of loss of feeder roots in the holes made to take damaged root and soil samples. It is remarkable that there was a total absence of symptoms and signs associated with other biotic factors of oak decline. These symptoms were previously described for holm oaks suffering sudden death caused by *P. cinnamomi* in Spanish rangelands (Sánchez *et al.*, 2002, 2006). At Doñana National Park the disease was detected 1 year ago and, at the moment, only sudden decline has been described. Only one colony type was isolated from necrotic feeder roots and rhizosphere. The oomycete exhibited rounded, branched colonies. Microscope observation of the structures present in the CA cultures revealed the presence of the typical coralloid hyphae with clustered chlamydospores and hyphal swellings. Sporangia were persistent, non-papillated, regularly formed terminally, mainly ovoid shape, but ellipsoid sporangia were also observed. Occasionally sporangiophores showed sympodial branching. These characteristics are in a good agreement with the description of *P. cinnamomi* (Erwin & Ribeiro, 1996; Sánchez *et al.*, 2002; 2006). *Phytophthora cinnamomi* was consistently isolated from all the sampled foci (Table 1).

Table 1. *Phytophthora cinnamomi* isolation frequency expressed as percentage of root segments yielding one colony when plated on NARPH medium

Foci code number	Tree code number	Tree diameter (cm)	Defoliation 2009	*Phytophthora cinnamomi* isolation frequency (%)
1	63	103	3	38
2	71	75	1	28
3	184	102	1	2
4	2008077	33	2	7
5	2008096	18	1*	19
6	2008100	35	3	19
7	2008298	21	1	52

* This oak died in 2010

The pathogenicity of *P. cinnamomi* on cork oak is well known (Sánchez *et al.*, 2002; 2006). The most frequent situation for disease development is seasonally moist soils followed by periods of drought (Shearer & Tippett, 1989; Sánchez *et al.*, 2002; 2006). Heavy rain episodes occurred during the winter 2009-2010. Along only 3 months (December 2009 to February 2010), rainfall rates more than duplicate the average rates for the same period registered in the Park for the last 30 years. These long periods of water soil saturation for cork

oaks appear as the main climatic factor that could favoured pathogen dispersal through the soil and new infections of healthy oaks in the Park. Then, as it seems to be a clear tendency towards the increase of these extreme climatic conditions (Brasier, 1996), it is expected an important increment in the number and size of *Phytophthora* disease foci. In this way, proposals to monitoring the already detected and eventually new foci, together with the preventive application of phosphonates by trunk injection, have been proposed to the Park authorities.

Acknowledgements

This work has been financed by the Spanish Ministry of Environment (Project 091/2009 DECALDO, Red de Parques Nacionales).

References

Brasier, C. M. 1996: *Phytophthora cinnamomi* and oak decline in southern Europe. Environmental constraints including climate change. Ann Sci For. 53: 347-358.

Dhingra, O. D. & Sinclair, J. B. 1995: Basic plant pathology methods. CRC Press, Boca Raton, 434 pp.

Erwin, D. C. & Ribeiro, O. K. 1996: *Phytophthora* diseases worldwide. St. Paul, MN, USA, APS Press.

Granados-Corona, M., Martín-Vicente, A., García-Novo, F. 1988: Long-term vegetation changes on the stabilized dunes of Doñana National Park (SW Spain). Vegetatio 75: 73-80.

Herrera, J. 1995: Acorn predation and seedling production in a low-density population of cork oak (*Quercus suber* L.). For. Ecol. Man. 76: 197-201.

Moreira, A. C., Ferraz, J. F. P. & Clegg, J. 1999: The involvement of *Phytophthora cinnamomi* in cork and holm oak decline in Portugal. In: Hansen, E. M. & Sutton, W. (eds.): Proceedings of the 1st IUFRO International Meeting on Phytophthoras in forest and wildland ecosystems, Grants Pass, Oregon, USA: 132-135.

Ramo, C., García, L. V., Díaz-Delgado, R., Marañón, T., Siljeström, P., Rodríguez, R., Garrido, H., Urdiales, C., Laffite, R., Ibañez, F. & Calderón, J. 2009: El declive del alcornocal en la Vera del Parque Nacional de Doñana: el papel de las colonias de aves nidificantes. In: Mutke, S. et al. (eds.): Actas V Congreso Forestal Español, Sociedad Española de Ciencias Forestales, Ávila: 1-9.

Ribeiro, O. K. 1978: A source book of the genus *Phytophthora.* J. Cramer, Vaduz, 417 pp.

Romero, M. A., Sánchez, J. E., Jiménez, J. J., Belbahri, L., Trapero, A., Lefort, F. & Sánchez, M. E. 2007: New *Pythium* taxa causing root rot on Mediterranean *Quercus* species in southwest Spain and Portugal. J. Phytopathol. 155: 289-295.

Sánchez, M. E., Caetano, P., Ferraz, J. & Trapero, A. 2002: *Phytophthora* disease of *Quercus ilex* in southwestern Spain. For. Path. 32: 5-18.

Sánchez, M. E., Caetano, P., Romero, M. A., Navarro, R. M. & Trapero, A. 2006: *Phytophthora* root rot as the main factor of oak decline in southern Spain. In: Brasier, C., Jung, T. & Oßwald, W. (eds.): Progress in Research on *Phytophthora* Diseases of Forest Trees, Forest Research, Farnham, UK: 149-154.

Shearer, B. L. & Tippett, J. T. 1989: Jarrah dieback: The dynamics and management of *Phytophthora cinnamomi* in the Jarrah (*Eucalyptus marginata*) forest of south-western Australia. CALM, Research Bulletin No 3. Department of Conservation and Land Management, Como, 76 pp.