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Original article

Impact of drought and *Hypoxylon mediterraneum* on oak decline in the Mediterranean region

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Summary — An association was evidenced in the last decade among changes in species composition, changes in rainfall distribution and incidence of stress-induced pathogens, such as *Hypoxylon mediterraneum,* in old oak coppices in central and southern Italy. *Quercus cerris* and *Q frainetto* were more affected by decline than *Q pubescens* following periods of summer drought. The role of *H mediterraneum* on drought-susceptible species was relevant by increasing their mortality. According to our results, changes in rainfall distribution and secondary biotic stress factors, such as *H mediterraneum,* seem to act as factors of balance by restablishing the original forest composition existing before the selective pressure of coppice managment.

oak / *Hypoxylon mediterraneum* / drought / decline / vegetation changes

Résumé — Effets de la sécheresse et d'une infection par *Hypoxylon mediterraneum* sur le dépérissement des chênes en zone méditerranéenne. *Des modifications de la composition floristique, de la pluviométrie, et de la sensibilité à un pathogène induit par les contraintes comme* Hypoxylon mediterraneum, *se sont produite de manière fortement interdépendante au cours de la dernière décennie, dans des taillis anciens de chênes en Italie centrale et méridionale.* Quercus cerris *et* Q frainetto *se sont révélés être plus sensibles au dépérissement résultant de périodes de sécheresse estivale que* Q pubescens. *L'impact de* H mediterraneum *sur les espèces sensibles à la sécheresse s'est traduit par une augmentation de leur mortalité. D'après nos résultats, la réduction de la pluviosité, et l'impact de contraintes biotiques secondaires comme* H mediterraneum *semblent agir comme facteurs d'accélération du retour à la composition floristique préexistante à la période de forte pression de sélection résultant du traitement en taillis intensif.*

***chêne / sécheresse / dépérissement / changement de flore /* Hypoxylon mediterraneum**

INTRODUCTION

Since the first reports on the effects of pollutants on tree health, forest decline is considered one of the most important issues for plant pathologists and physiologists. Forest decline not only affects species performances in a given environment (growth, photosynthesis, etc) but also has an impact at the ecosystem level through changes in forest species composition and structure.

There is a growing awareness that global changes, which are likely to occur in the near future, could have an important influence on the health status of trees, especially by modifying the interactions between trees and biotic stress factors such as insects and fungi. There is evidence that periods of drought have greatly increased susceptibility of trees to stress-induced pathogens, for example the fungus *Hypoxylon mediterraneum* (DeNot) Mill on *Quercus cerris* L in central and southern Italy (Vannini and Scarascia Mugnozza, 1991; Vannini and Valentini, 1994).

During the last decade, decline of oaks represented one of the most widespread decline syndromes in Europe and North America, affecting several species of the genus. Oak decline involves interactions between inciting abiotic or biotic stresses (drought, frost, defoliating insects) and subsequent attack by secondary organisms such as cork borers, root fungi, stem canker fungi (EPPO, 1990; Wargo, 1992). Highest incidence of decline is generally associated with predisposing factors such as poor site quality and advanced stand age (Becker and Lévy, 1982; Manion, 1991; Oak et al, 1991). In North America, symptoms include general and progressive dieback from the tip of the branches, production of chlorotic, dwarfed and sparse foliage, development of sprouts on main stems or branches, premature autumn leaf colour and leaf drop (Wargo et al, 1983). Symptoms in Europe include crown dieback, yellowing of leaves, bark cankers, abundant tannic exudations, reduced ring growth and tree mortality. On cross sections of the stem of declining trees, it is common to observe occlusion of xylem vessels and darkening of the surronding parenchima (Vannini, 1990b; Vannini and Valentini, 1994).

Oak species differ in their susceptibility to the decline syndrome: in France, *Quercus robur* L was more susceptible than *Quercus petraea* (M) Liebl (Landmann et al, 1993). In North America, species of the red oak group are more susceptible than those of the white oak group (Oak et al, 1988). In central and southern Italy, *Quercus cerris* L is more susceptible to decline than *Quercus pubescens* Will (EPPO, 1990).

The aim of the present work was to analyse what vegetation changes had occurred in the last decade in oak stands of central and southern Italy that had experienced decline periods, and the role of drought and *H mediterraneum* in such changes. The analysis was carried out in parallel with greenhouse experiments to assess the susceptibility of the oak species to water stress and *H mediterraneum*.

MATERIALS AND METHODS

Experiments were carried out on adult *Q cerris*, *Q pubescens* and *Q frainetto*: trees in the field and, for *Q cerris* and *Q pubescens*, 2-year-old seedlings kept in greenhouse.

Experimental plots of 1 000 m^2 were selected and observed starting from the year 1983 in central Italy and from 1989 in southern Italy. One of these plots was located in a 30-year-old natural coppice of *Q cerris* and *Q pubescens*, strongly affected by decline since 1983, in the Natural Reservation of Mt Rufeno (MR) (central Italy) at an elevation of about 690 m above sea level. The other three (G1, G2, G3) were located in a 40-year-old natural coppice of *Q cerris*, *Q pubescens* and *Q frainetto*, strongly affected by decline since 1989, in the district of Gravina, in Puglia, in southern Italy at an elevation ranging between 337 and 464 m above sea level.

In the experimental plots, each tree was tagged and, for each tree, the species identified. Observations on mortality of the trees were carried out by considering all the trees present in the 1 000 m^2 plots, and causes of death were determined where possible; the presence of black stromata along the stems of trees was evidence of *H mediterraneum* attacks (Vannini et al, 1991).

Predawn leaf water potential (PWP) and midday leaf water potential (MWP) of healthy trees were measured twice a month from May through September with a Scholander-Hammel pressure chamber (PMS instruments) using three shoots for each sample trees.

Pressure–volume curves were determined according to Hinckley et al (1983) for the same trees chosen for water potential measurements. Osmotic potential at full saturation ($\Psi\pi_{sat}$), osmotic potential at turgor loss point ($\Psi\pi_{TLP}$), and the relative water content at turgor lost point (RWC_{TLP}) were calculated from the PV curves using a software by Shulte and Hinckley (1985).

The effect of decreasing water supply on susceptibility of each oak species to secondary biotic stress factors was assessed using 2-year-old seedlings. Three groups of 30 seedlings of *Q cerris* and *Q pubescens* were treated with three different levels (600, 300 and 150 ml of water) of water supply every 48 hours (treatment A, B, C), respectively, and inoculated with *H mediterraneum*. Ten plants for each water treatment were inoculated with a 5 mm plug of mycelium of strain HL4 (ATCC = 90363), of *H mediterraneum*, taken with a cork borer from the advancing edge of a 3-day-old culture on Potato Dextrose Agar (PDA) (DIFCO). The colonised plugs were inserted into a bark wound made with a cork borer on the stem, covered with moistened, sterile cheesecloth and taped with parafilm.

Table I. Mortality of *Quercus cerris*, *Q pubescens* and *Q frainetto* in the experimental plots in central (MR) and southern (G1, G2, G3) Italy in the periods 1983–1990 and 1989–1993, respectively.

	Mortality (%)			
Species	*MR*	*G1*	*G2*	*G3*
Q cerris	56.8	52.3	39.4	38.8
Q pubescens	1.9	2.4	0.0	0.8
Q frainetto	–	49.2	38.4	53.3

Inoculations were assessed after 2 months according to Vannini and Valentini (1994). In all experiments, ten wounded but not inoculated plants and ten intact plants served as controls. PWP and MWP of seedlings for the three watering treatments were measured at the end of the experiment on three leaves from uninoculated controls. Statistical significance was determined by Student's *t*-test, using Systat software.

RESULTS

Decline syndrome began in central Italy in the years 1983–1984. In southern Italy, and particularly in the Gravina area to which the data are referred, heavy symptoms of oak decline began only in 1989.

Table I shows the mortality of the three species during the period of decline in all the experimental plots. For the central Italy experimental plot (MR), 56.8% of *Q cerris* trees died in the period 1983–1990 compared with 1.9% of *Q pubescens*. In this plot, *Q frainetto* was not present. One hundred percent of the dead *Q cerris* showed signs of *H mediterraneum* compared with 2.7% of *Q pubescens* dead trees.

In southern Italy, experimental plots *Q cerris* showed a mortality ranging from 38.8 to 52.3% in the period 1989–1993; *Q frainetto* ranged from 38.4 to 53.3% while *Q pubescens* showed a mortality rate ranging from 0 to 2.4%. *H mediterraneum* was widespread also in the Gravina area both on *Q cerris* and *Q pubescens*; but no data are available on the percentage of trees presenting signs of the pathogen.

Figure 1 shows the composition of the coppice in the plot in central Italy (MR) assessed in 1983 compared to the situation in 1990. *Q cerris* decreased from 73.7 to 53.8% of the total number of trees, while *Q pubescens* increased from 26.3 to 46.2%. In figure 2, the forest composition in southern Italy in 1989 is compared with that of

1993. *Q cerris* decreased on average from 24.7 to 20.8% of the total number of individuals, *Q frainetto* from 52.6 to 46.7%, while *Q pubescens* increased from 22.7 to 32.4%.

In figures 3 and 4, the average monthly precipitation of the two decades 1974–1983 and 1984–1993 are shown for the sites in central and southern Italy, respectively. Average annual precipitations for the two decades were 920 and 820 mm, respectively, in central Italy, and 590 and 478 mm, repectively, in southern Italy.

During the last decade, while the total annual precipitation did not change significantly compared with the previous one, a modification of annual distribution of precipitation and, in particular, a strong reduction of August rainfall have been recorded in both sites.

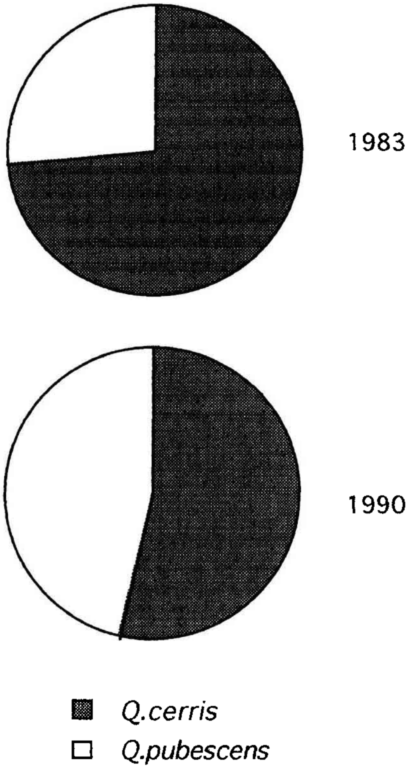


Fig 1. Oak species composition in the central Italy site (MR) before the decline syndrome in 1983 and in 1990 following a severe period of decline.

In table II, data concerning water relations of the studied species are presented. PWP measured in September 1990 in central Italy stand was –1.6 and –1.1 MPa for *Q cerris* and *Q pubescens*, respectively. In southern Italy, PWP measured in September 1992 was –2.5, –2.5 and –2.3 MPa for *Q cerris*, *Q frainetto* and *Q pubescens*, respectively. The osmotic potential at saturation was –0.8 and –1.5 MPa for *Q cerris* and *Q pubescens* in central Italy, and –0.9, –1 and –1.2 MPa for *Q cerris*, *Q frainetto* and *Q pubescens*, respectively, in southern Italy. In table II, water potential and RWC_{TLP} are also presented.

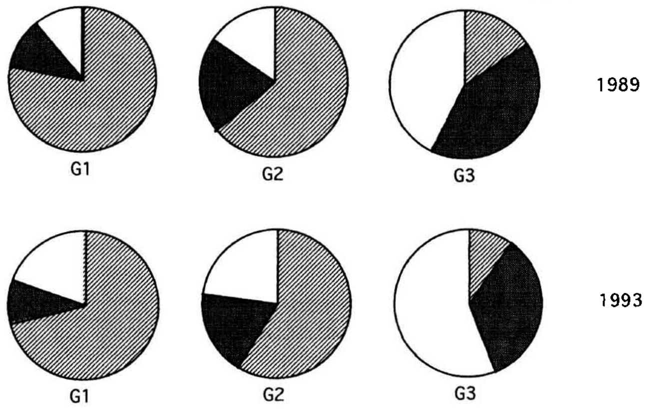
For the southern Italy plots, the 1992 trends of PWP and MWP are presented for the three study species (fig 5). *Q pubescens* mantains throughout the year the highest PWP and MWP.

Figure 6 shows the size of the necrosis produced by artificial inoculations of *H mediterraneum* on *Q cerris* and *Q pubescens* under different water supply treatments and the corresponding PWP value at the end of the experiment for each treatment. Data evidenced that there are no differences in susceptibility of the two species at the same value of PWP, but *Q cerris* shows substantialy lower values of PWP at the same water supply levels, to which correspond the maximum size of necrosis.

DISCUSSION

Historically, *Q cerris* and, in southern Italy, also *Q frainetto*, have been selected in coppice management in respect of *Q pubescens* due to their better growth performances and wood properties (Marinelli and Casini, 1989). This resulted in an unnatural forest composition with a greater presence of the formers compared with the lat-

Fig 2. Oak species composition in the southern Italy sites G1, G2 and G3 in 1989, and in 1993 following a severe period of decline.



ter species also in sites not suitable for them where *Q pubescens* represented the dominant species.

Our results show that in the last decade change in forest composition occurred both in central and southern Italy in abandoned oak coppices. In such conditions, *Q cerris* and *Q frainetto* tend to be largely replaced by *Q pubescens*.

This natural trend of replacement is, however, paralleled by a modification of annual distribution of rainfall of the last decade, which shows an extended period of drought in August. A statistical correlation between decreasing rainfall, particularly August precipitation, and incidence of decline was previously reported by Vannini (1990a) in an old *Q cerris* coppice in central Italy.

Our results from the survey in the plot in central Italy show that the mortality of *Q cerris* and related change in forest composition was strongly connected with *H mediter-*

Table II. Water relations of the three oak species measured in September 1990 and 1992 in central (MR) and southern (G) Italy sites, respectively.

Site	*Species*	$\Psi\pi_{sat}$ *(MPa)*	$\Psi\pi_{TLP}$ *(MPa)*	*RWC* $_{TLP}$ *(%)*	*PWP* *(MPa)*
G	*Q cerris*	-0.9^{a}	-2.0^{a}	66.8^{a}	-2.5^{a}
G	*Q pubescens*	-1.2^{b}	-2.2^{b}	66.4^{a}	-2.3^{b}
G	*Q frainetto*	-1.0^{a}	-1.8^{a}	67.7^{a}	-2.5^{a}
MR	*Q cerris*	-0.8^{a}	-1.8^{a}	58.3^{a}	-1.6^{a}
MR	*Q pubescens*	-1.5^{b}	-2.4^{b}	72.3^{b}	-1.1^{b}

^{ab} On each column and for each site, means followed by the same letter do not differ significantly at $P = 0.05$. $\Psi\pi_{sat}$: osmotic potential at full saturation; $\Psi\pi_{TLP}$: osmotic potential at turgor loss point; RWC_{TLP}: relative water content at turgor lost point; PWP: predawn leaf water potential.

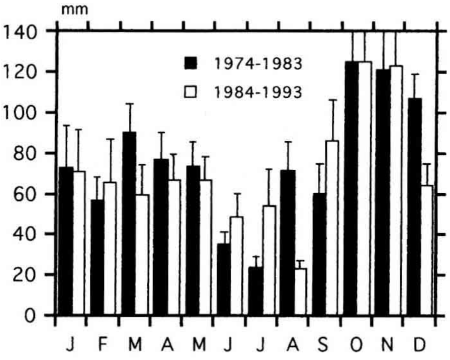


Fig 3. Average monthly distribution of precipitation for the periods 1974–1983 and 1984–1993 in central Italy. Bars represent the standard error.

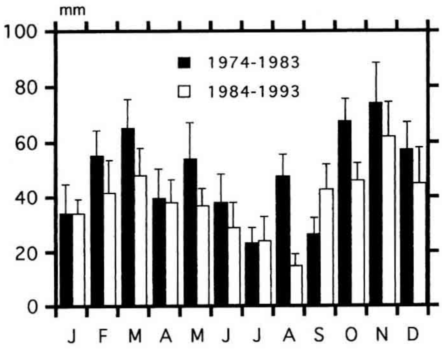


Fig 4. Average monthly distribution of precipitation for the periods 1974–1983 and 1984–1993 in southern Italy. Bars represent the standard error.

raneum attacks, while *Q pubescens* was not significantly affected by the fungus.

It has been widely reported that *H mediterraneum* is a pathogen on oak trees weakened by water stress. *H mediterraneum* on *Q cerris* was observed most frequently. Laboratory experiments and field tests have shown the importance of water stress and related physiological modification on the tree susceptibility to this fungus (Vannini and Valentini, 1994).

Data on seedlings inoculation with *H mediterraneum*, under different water supply levels, evidence that the higher susceptibility of *Q cerris* to the fungus, compared with *Q pubescens*, is associated with more negative PWP values than the latter species, at the same water supply level.

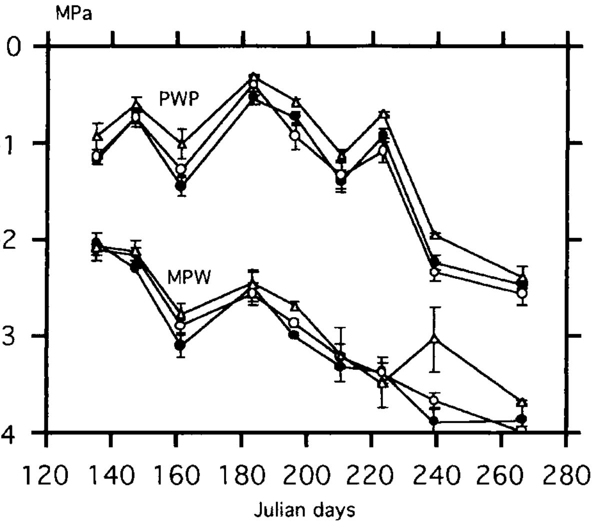


Fig 5. Patterns of predawn (PWP) and midday (MWP) leaf water potential of *Quercus cerris* (●), *Q frainetto* (O) and *Q pubescens* (Δ) from May through September 1992 in southern Italy. Bars represent the standard error.

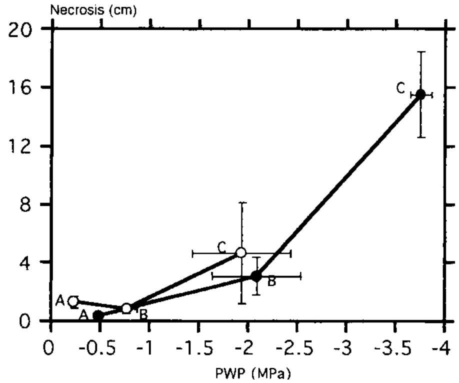


Fig 6. Size of the necrosis produced by *Hypoxylon mediterraneum* on inoculated *Q cerris* (●) and *Q pubescens* (**O**) seedlings in greenhouse, plotted versus predawn leaf water potential (PWP). Letters correspond to the three water supply treatments: 600 ml (**A**), 300 ml (**B**), 150 ml (**C**) every 48 hours. Bars represent the standard error.

Also in the field experiment, *Q cerris* always showed the most negative PWP values compared to *Q pubescens*, confirming that this higher mortality, mainly due to *H mediterraneum*, is related to the occurrence of water stress. In addition, measurements of $\Psi\pi_{sat}$ and $\Psi p\pi_{TLP}$ confirm the better performances of *Q pubescens* under drought conditions.

Q frainetto water relations are similar to those of *Q cerris* and also mortality in the last decade follows the same pattern.

The changes in oak forest composition occurring in the last decade in central and southern Italy are paralleled by changes in rainfall distribution. *H mediterraneum* seems to play a role in such modifications as the cause of death of drought-susceptible oak species.

Changes in rainfall distribution and secondary biotic stress factors, such as *H mediterraneum*, seem to act by reestablishing the original forest composition before the selective pressure of coppice management. Such a theory was also proposed by other authors (Becker and Lévy, 1982; Landmann et al, 1993), who consider the decline of *Q robur* in some sites in France as an ecological 'sanction' following the introduction by man of such species in unsuitable sites. In fact, *Q robur* was also historically favoured by man in forest management. In France, according to some forest inventory data, the retreat of *Q robur* is already advanced.

Our data also suggest important interactions between parallel climatic changes and vegetation changes through involvement of indigenous secondary biotic factors.

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