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Observations on *Armillaria* occurrence in declining oak woods of southern Italy

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Summary — The fungi of *Armillaria* genus have generally been recognized as being among the most important biotic causes of oak decline in the world. In order to assess which *Armillaria* species predominates on declining oaks in southern Italy, further surveys were carried out in numerous oak woods and additional *Armillaria* isolates were collected. Their identification was based on the characteristics of the diploid cultures obtained from infected roots and woody tissues, on haploid-haploid pairings with tester isolates and on the main features of the basidiomes. Most of the collected isolates were found to belong to *A gallica*, while *A mellea* and *A tabescens* were observed to occur less frequently. These observations conflict with previous surveys which considered *A mellea* as the most frequent *Armillaria* species in southern Italy. The fact that *A gallica* was found to be particularly widespread in the most seriously declining oak woods could confirm the opportunistic behaviour of *Armillaria* and suggest that it may depend on the remarkable weakness of the oak trees in very unfavourable site conditions.

***Armillaria* / root disease / oak decline / *Quercus* / southern Italy**

Résumé — Observations sur la présence d'armillaire dans les chênaies dépérissantes du sud de l'Italie. *Les champignons du genre* Armillaria *sont généralement considérés comme figurant parmi les plus importantes causes biotiques du dépérissement des chênes dans le monde. Dans le but d'établir l'importance relative des différentes espèces d'*Armillaria *dans les chênaies du sud de l'Italie, on a procédé à la récolte d'isolats de ces champignons dans de nombreuses chênaies (fig 1). L'identification des isolats a été basée : i) sur la morphologie des cultures diploïdes obtenues à partir des racines et des tissus ligneux infectés, ii) sur des confrontations entre haplontes faisant intervenir des testeurs d'espèces connues, iii) sur les caractéristiques morphologiques des carpophores. Les résultats ont montré l'appartenance à* A gallica *d'une forte majorité des isolats récoltés, la présence d'*A mellea *et* A tabescens *s'avérant moins fréquente (tableau I). Ces constatations sont en contradiction avec les observations antérieures selon lesquelles* A mellea *est l'espèce d'armillaire dominante dans le sud de l'Italie. La fréquence particulièrement élevée d'*A gallica *dans les chênaies où le dépérissement était le plus sérieux pourrait confirmer le comportement opportuniste de cette espèce et être mise en relation avec l'affaiblissement considérable des chênes sur certains sites où les conditions sont très défavorables.*

armillaire / *pathologie racinaire* / *dépérissement du chêne* / Quercus / *Italie du Sud*

INTRODUCTION

Root rot caused by the fungi of the *Armillaria* genus is one of the most important diseases of woody plants and affects hundreds of species of fruit, shade and forest trees, as well as other plants in temperate and tropical regions (Kile et al, 1994). They are also considered one of the most important biotic causes of oak decline, the complex syndrome that has been occurring for over a decade in many countries of Europe and North America, with very serious effects on oak vitality (Delatour, 1983; *OEPP/EPPO,* 1990; Ragazzi, 1993; Wargo, 1993).

Studies carried out since the late 1980s demonstrated the occurrence of different species of these Basidiomycetes (more than 30). In Italy five species of *Armillaria* were identified by means of mating tests (Korhonen, 1978) and observation of the morphological characteristics of diploid cultures (Intini and Gabucci, 1987). They were listed according to the current nomenclature (Marxmüller, 1992) as follows: *A cepistipes* Velenovsky, *A ostoyae* (Romagnesi) Herink, *A mellea* (Vahl: Fr) Kummer, *A gallica* Marxmüller and Romagnesi, and *A tabescens* (Scop: Fr) Emel. *A mellea* was found almost everywhere, regardless of altitude, climate conditions and plant species. *A ostoyae* was found to be specific to conifers in the Alps and Apennines. *A cepistipes, A gallica* and *A tabescens* occurred less frequently and, generally, only in some particular ecological sites (Anselmi and Lanata, 1989; Intini, 1989).

Research carried out on this subject in southern Italy partially confirmed that the most widespread species was *A mellea,* both in orchards and in woods (Tirrò, 1989; Tirrò and Rapisarda, 1989; Ippolito et al, 1991; Sicoli et al, 1992) and studies are in progress in order to ascertain the presence of *A cepistipes* and *A ostoyae* (Tirrò and Grillo, unpublished). Preliminary investigations were also carried out on *Armillaria* root rot in oaks affected by decline, in order to assess which *Armillaria* species predominates (Anselmi and Puccinelli, 1993; Grillo and Tirrò, 1993; Sicoli et al, 1993).

The aim of this work was to identify the species of *Armillaria* occurring on declining oaks and to check their distribution in southern Italy.

MATERIALS AND METHODS

Several declining oak woods were surveyed in Apulia, Basilicata and Calabria (fig 1). *Quercus cerris* L, *Q pubescens* Willd and *Q frainetto* Ten were found to be the prevalent oak species. The severity of decline was assessed as follows: each wood was divided into three discontinuous plots having a surface area of 225 m^2. In each plot, 30–40 oak trees were selected at random and classified according to the following empirical scale of decline: 0 = healthy plant; 1 = sparse or transparent crown; 2 = upper crown withered up to 50%; 3 = 50 to 100% withered crown; 4 = completely withered crown, epicormic shoots on the trunk up to 2 m from the collar; 5 = completely dead trunk, stump still alive (coppice shoots may be present); 6 = dead plant. The decline index was calculated by the mean of the values obtained per species and per wood.

Plant tissue samples for *Armillaria* isolations were collected from collar, roots and stumps of oak trees classified as 2 to 5 on the above scale. Roots were usually taken up to about 40 cm of depth and distance from the collar and had a diameter 1 cm. During above autumn, *Armillaria* was also isolated from basidiomes developing close to the declining oak plants.

The specimens from plant tissues were subcultured on a selective medium (Kuhlman and Hendrix, 1962) and all of the diploid isolates were grown in Petri dishes containing either 3% Difco malt extract agar (MEA) or Difco potato dextrose agar (PDA); they were kept in the dark at 23 ± 1 °C for 1 month. The monosporous isolates were used in mating tests with haploid testers (kindly provided by Dr Korhonen, Finnish Forest Research Institute, Helsinki, Finland) performed on 2% MEA according to Guillaumin et al (1991).

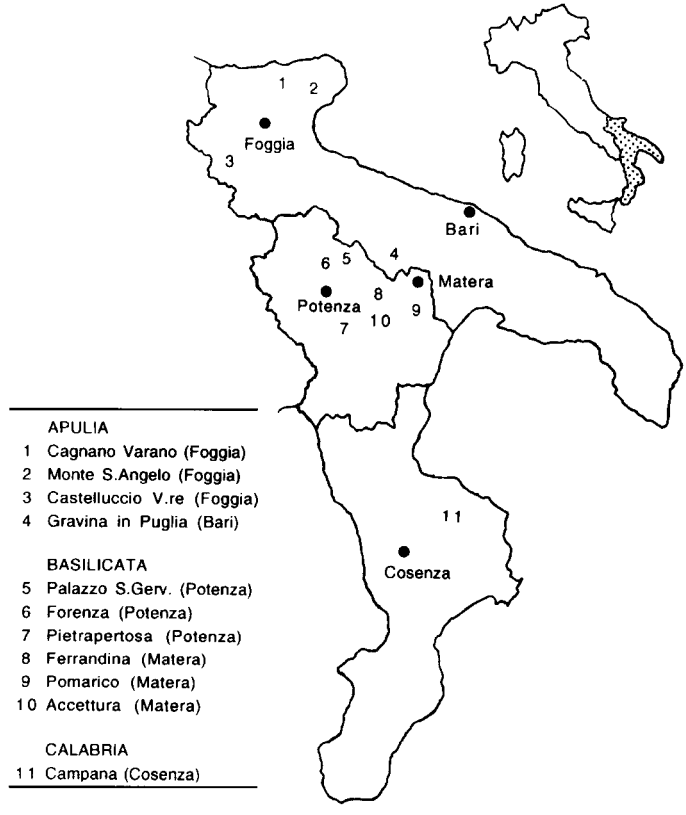
The identity of the diploid cultures was assessed on the basis of their morphological characteristics on PDA (method *a*), and that of the haploid ones on the results of mating tests (method *b*) (Sicoli et al, 1994). When possible, the features of the basidiomes were also taken into account (method *c*).

RESULTS

The examination of the diploid cultures on PDA showed that all the *Armillaria* isolates obtained belonged to the species *A mellea, A gallica* and *A tabescens*. The colonies appeared whitish in *A mellea* and reddish in *A gallica* and *A tabescens,* although the distinction between *A mellea* and *A tabescens* from the mycelial mats only was not definitive. The rhizomorphs were whitish, flattened and arborescent in *A mellea* and *A tabescens;* the only difference laid in their diameter which was larger in *A mellea*. The rhizomorphs of *A gallica,* instead, were thin, brown, cylindrical and monopodial, with few comb-shaped branches.

The results of mating tests carried out on almost 200 haploid isolates of *Armillaria* confirmed the identification of the above species. When the isolates were compatible in the haploid-haploid pairings, the colonies took on the morphological features of the diploid cultures according to Sicoli et al (1994).

These results were further confirmed on the basis of the morphological features of the basidiomes. *A tabescens* was characterized by ringless basidiomes. The other two species had very different rings: in *A*



APULIA
1 Cagnano Varano (Foggia)
2 Monte S.Angelo (Foggia)
3 Castelluccio V.re (Foggia)
4 Gravina in Puglia (Bari)

BASILICATA
5 Palazzo S.Gerv. (Potenza)
6 Forenza (Potenza)
7 Pietrapertosa (Potenza)
8 Ferrandina (Matera)
9 Pomarico (Matera)
10 Accettura (Matera)

CALABRIA
11 Campana (Cosenza)

Fig 1. Declining oak woods in southern Italy where plant samples and basidiomes were collected for *Armillaria* isolation.

gallica it was thin and fragile, while in *A mellea* it was thick and firm (Sicoli et al, 1994).

The species distribution of the *Armillaria* isolates obtained is summarized in table I. Almost all the isolates were shown to include *A gallica,* whereas *A mellea* and *A tabescens* were found less frequently and only in some of the investigated woods, including one at about 1 000 m elevation. With regard to host plants, these fungi were found on *Q cerris, Q pubescens* and *Q frainetto,* the latter resulting to be the most heavily declining species (Sicoli et al, 1993).

Table I. Distribution of *Armillaria* isolates and basidiomes collected in declining oak woods in southern Italy, according to their site of origin, host plant, decline index and fungal species.

Oak wood	*Altitude (m asl)*	*Host plant*	*Decline index**	*Species*			*Identification method***
				A mellea	A gallica	A tabescens	
Apulia							
Cagnano Varano (FOGGIA)	460	*Q cerris* L	2.97	6	7	0	a, b, c
"Bosco La Vallata"		*Q pubescens* Willd	2.23	1	0	0	a, b, c
Monte S Angelo (FOGGIA)	640	*Q cerris* L	1.35	1	9	0	a, b, c
"Bosco Quarto"		*Q pubescens* Willd	0.64	0	4	0	a, b, c
Castelluccio V re (FOGGIA)							
"Bosco Difesa"	500	*Q cerris* L	1.21	0	2	0	a, b, c
Gravina in Puglia (BARI)	450	*Q cerris* L	3.54	1	29	8	a, b, c
"Bosco Difesa Grande"		*Q pubescens* Willd	2.85	0	1	0	a, b, c
		Q frainetto Ten	3.83	0	2	3	a, b, c
Basilicata							
Palazzo S Gervasio (POTENZA)							
"Masseria Tufaroli"	450	*Q pubescens* Willd	2.46	1	4	0	a, b, c
Forenza (POTENZA)	750	*Q cerris* L	1.78	0	1	0	a
"Masseria S Antonio"		*Q pubescens* Willd	0.75	0	0	1	c
Pietrapertosa (POTENZA) "Monte Piano"	1 000	*Q cerris* L	0.34	0	0	4	c
Ferrandina (MATERA)	550	*Q cerris* L	3.57	0	2	0	a, b, c
"Monte Piano"		*Q frainetto* Ten	3.90	0	2	0	a, b, c
Pomarico (MATERA)	450	*Q cerris* L	2.82	0	3	0	a, b, c
"Bosco Manferrara"		*Q pubescens* Willd	2.07	0	3	0	a, b, c
Accettura (MATERA)							
"Gallipoli-Cognato"	450	*Q cerris* L	1.96	0	0	8	c
Calabria							
Campana (COSENZA)							
"Piano di Guerra"	980	*Q frainetto* Ten	2.63	0	5	0	a, b, c
"Cozzo Calamacca"	850	*Q frainetto* Ten	3.45	2	0	0	a, b, c

* Assessed on the basis of an empirical scale of 7 degrees, from 0 (= healthy plant) to 6 (= dead plant); ** see text for details.

Basidiomes of all three *Armillaria* species were found at the base of dying as well as dead trees. *A tabescens* was commonly observed at the collar of dead oaks in two lightly declining woods, but also close to declining trees in woods where the decline was severe. *A gallica* was very easily found on seriously declining and dead oaks, while *A mellea* more frequently colonized healthy or lightly declining trees. Sometimes, the basidiomes of *A tabescens* and *A gallica* were observed at the base of the same *Q cerris* tree, in September and in November, respectively, even though they colonized different parts of the stump. Moreover, *A mellea* was also detected on *Q trojana* Webb, *Q ilex* L and other shrubs occurring in some of the surveyed woods.

A cobweb-like and often powdery white mycelium of another fungus was frequently found growing on both young and old basidiomes of *A tabescens*. It had previously been observed on *A mellea* in other oak woods in central and northern Italy (Luisi and Sicoli, unpublished) and was identified as *Cladobotryum dendroides* (Bull: Mérat) W Gams & Hoozemans, on the basis of the features of its conidia and conidiophores (de Hoog, 1978).

Finally, other decay fungi, such as *Phellinus torulosus* (Pers) Bourd and Galz, *Ganoderma lucidum* (Curt: Fr) Karst and *Collibya* sp, were isolated, although less frequently, from epigeous tissues of declining oaks.

DISCUSSION

The most recent data concerning the presence of *Armillaria* species on oak in southern Italy demonstrate the widespread occurrence of *A mellea* and *A tabescens* (Guillaumin et al, 1993). However, whereas *A tabescens* is generally considered to be a typical colonizer of the maquis, in this study this species occurred also on deciduous oaks in mesophile woods of the Mediterranean region. This behaviour may be explained if it is considered that, despite the altitude and the presence of mesophile plant species, sites such as Pietrapertosa still belong to the *"Lauretum"* zone, according to Pavari's phytoclimatic areas (Pavari, 1916; Cantore et al, 1987). Moreover, the results of the surveys carried out in the declining oak woods show that *A gallica* is more widespread than generally acknowledged. Even though Anselmi and Puccinelli (1993) and Grillo and Tirrò (1993) detected mainly *A mellea* in these ecosystems, *A gallica* seems to be a well-established constituent of the declining oak rhizosphere, as had already been argued by Guillaumin et al (1985) and Wargo (1993). It still remains to be demonstrated what role this species plays in southern Italy: whether it is really just an opportunistic parasite, able to attack only weakened trees after *A mellea* primary infections, or whether it may be a contributing factor, active like other detected microorganisms, regardless of the occurrence of *A mellea.*

Further pathogenicity tests and more thorough investigations concerning the root system of oaks at different stages of decline could clarify these aspects of the phenomenon in southern Italy. Furthermore, ther is a need to investigate more closely the occurrence and role of *Collybia fusipes* (Bull: Fr) Quél, elsewhere considered an important cause of oak decline (Guillaumin et al, 1985), but still not clearly identified in this survey.

Cladobotryum dendroides, already detected on *A borealis* Marxmüller et Korhonen and on *A ostoyae* in central Europe (Holdenrieder and Marxmüller, unpublished), is not known to play an effective role as a hyperparasite of *Armillaria.* Nevertheless, it might be useful to continue investigating this aspect.

In conclusion, *A gallica* was observed to be more widespread than *A mellea* in the

declining oak woods of southern Italy, but its pathogenic role requires further investigation. Moreover, drought conditions and incorrect silvicultural practices seem to be the main predisposing factors to *Armillaria* attacks and the most serious obstacles to oak wood recovery.

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