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RESEARCH PAPERS

Pathogenicity of *Armillaria* isolates inoculated on five *Quercus* species at different watering regimes

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Summary. One of three fungal isolates of *Armillaria mellea* (Vahl: Fr.) P. Kummer, *A. gallica* Marxm. *et* Romagn. and *A. tabescens* (Scop.: Fr.) Emel. was inoculated on 1,440 three-year-old potted seedlings of five *Quercus* species (*Q. cerris* L., *Q. ilex* L., *Q. pubescens* Willd., *Q. robur* L. and *Q. trojana* Webb.) grown at different watering regimes in a greenhouse. Inoculum was represented by a piece of an oak branch colonised with the fungus (or sterile, as a control), which was attached to the unwounded main root of each oak seedling. During the growing season, differences in water availability among seedlings were measured monthly using minimum water potential assessments on non-inoculated seedlings receiving an equal amount of water. Although all three *Armillaria* isolates induced infection, the *A. mellea* isolate was most pathogenic in all cases, while the *A. gallica* isolate showed a statistically equal degree of pathogenicity only on the least watered seedlings. Of the *Quercus* species, *Q. ilex* showed the greatest number of infected seedlings, *Q. robur* the smallest. Reducing the water supply to potted oak seedlings could be a useful indicator for detecting differences in pathogenicity between *Armillaria* species.

Key words: root rot, water stress, minimum water potential.

Introduction

Basidiomycetes of the genus *Armillaria* commonly known as "honey fungi" comprise about 40 species and are distributed worldwide. Many of these species are plant pathogens, causing root rot and wood decay, and producing tree mortality ranging from 3% to over 50% in plantations (Shaw and Kile, 1991).

Armillaria species also occur in oak stands where they generally act as saprotrophs, but some of them sometimes become co-factors causing plant dieback (depending on tree vitality) and even oak decline (Delatour, 1983; Guillaumin *et al.*, 1985; Rishbeth, 1985; Wargo and Shaw III, 1985). In southern Italy the most common Armillarias in oak stands are *Armillaria mellea, A. gallica* and *A. tabescens* (Luisi *et al*., 1996), which are thought to differ in their pathogenicity (Guillaumin *et al.*, 1993).

The importance of drought as a factor predisposing to or causing oak decline has long been a subject of study (Wargo, 1996; Thomas *et al*., 2002). Furthermore, some root-rot agents such as the *Armillaria* species, that were regarded as contributing to the decline, have been reported to be in re-

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ality opportunistic parasites, that attack oaks previously weakened by drought (Houston, 1992; Wargo, 1993; Thomas *et al.*, 2002). It therefore seemed of interest to carry out a study in which *Armillaria* spp. were inoculated on oak seedlings growing under a range of different but constant water regimes. The results of this study are here presented.

Materials and methods

Isolates and hosts

Isolates from three species, *A. mellea, A. gallica* and *A. tabescens*, were selected from some 200 *Armillaria* isolates mostly from declining oak stands at different sites in southern Italy. *A. mellea* (M181) came from a *Quercus* sp. at Gravina in Puglia (Bari), *A. gallica* (G66) from *Quercus frainetto* Ten. a Campana (Cosenza), and *A. tabescens* (T172) from *Q. cerris* growing at Vico del Gargano (Foggia). All isolates had tested positive on *Quercus* seedlings before (Luisi, unpublished data).

Five oak species (*Q. cerris*, *Q. ilex*, *Q. pubescens*, *Q. robur* and *Q. trojana*) were tested. Oak seedlings were 3 years old, grown in 25-cm, 4.5-l hard plastic pots containing standardised soil composed of 70% arable plant-debris–lacking loam that impeded the harbouring of *Armillaria* inoculum, and 30% peat. After potting, the seedlings were grown under natural lighting and exposed to air in a greenhouse, uniformly pruned and regularly watered for 3 months before inoculation.

Watering, monitoring of water content and predisposition to inoculation

To induce a gradient of water stress the seedlings were divided into three groups that, during the vegetative season, before and after inoculation, received 1 l of water per seedling every:

2 days: maximum watering (Max);
4 days: intermediate watering (Mid);
7 days: minimum watering (Min).

The water potential of the seedlings was recorded monthly during the vegetative season on the twigs of a separate group of 90 seedlings (6 per species per watering regime) by a minimum water potential (MWP) assessment carried out at midday just before watering using a pressure chamber (Skye SKPM 1420, Skye Instruments Ltd., Powys, UK). The MWPs were as follows:

Max MWP = -1.0 to -1.3 MPa;
Mid MWP = -1.6 to -2.0 MPa;
Min MWP = -2.9 to -3.4 MPa.

For the inoculations, 24 replications were prepared for each host–pathogen combination. Therefore, 96 seedlings per oak species per water regime were inoculated either with one of the three *Armillaria* isolates or with sterile inoculum as a control. Altogether 1,530 (90+1,440) seedlings were distributed on 15 banks in the greenhouse. One hundred and two seedlings per species per water regime were allocated to each bank.

Inoculum preparation and seedling inoculation

Inoculum was prepared from branches of *Q. ilex* cut into pieces 6.0 cm long and 2.0–2.5 cm in diameter. The pieces were washed under tap water, dried and autoclaved at 121°C for 40 min in 1-l glass jars containing 300 ml of 2% malt extract solution in distilled water. Each jar, containing about 25 wood pieces, was inoculated with three plugs of one of the *Armillaria* isolates removed from a fungal colony grown on 2% malt extract agar in 10-cm Petri dishes for 3 weeks at 23±1°C in the dark. The inoculum was incubated for about 3 months under the same conditions as the cultures in the Petri dishes.

Inoculation was carried out in September 2002 by vertically attaching the inoculum (colonised or sterile) to the taproot of the seedling, before watering and after removing 6 cm of soil, and without causing any wounds at the roots around the inoculation area.

Assessment of results and data analysis

One year after inoculation with *Armillaria* all oak seedlings were uprooted and the inoculum examined for viability and rhizomorph occurrence. Concurrently, seedling infections were recorded using an empirical scale based on five classes of symptom severity (Fig. 1):

0, no mycelial fans extending under the periderm (failed attack, the seedling remained healthy);
1, mycelial fans and/or rhizomorphs developed in the contact area between the inoculum and the root;
2, mycelial fans extending beyond the inoculation point but without girdling the root;
3, mycelial fans colonising half the root system, the plant survives;

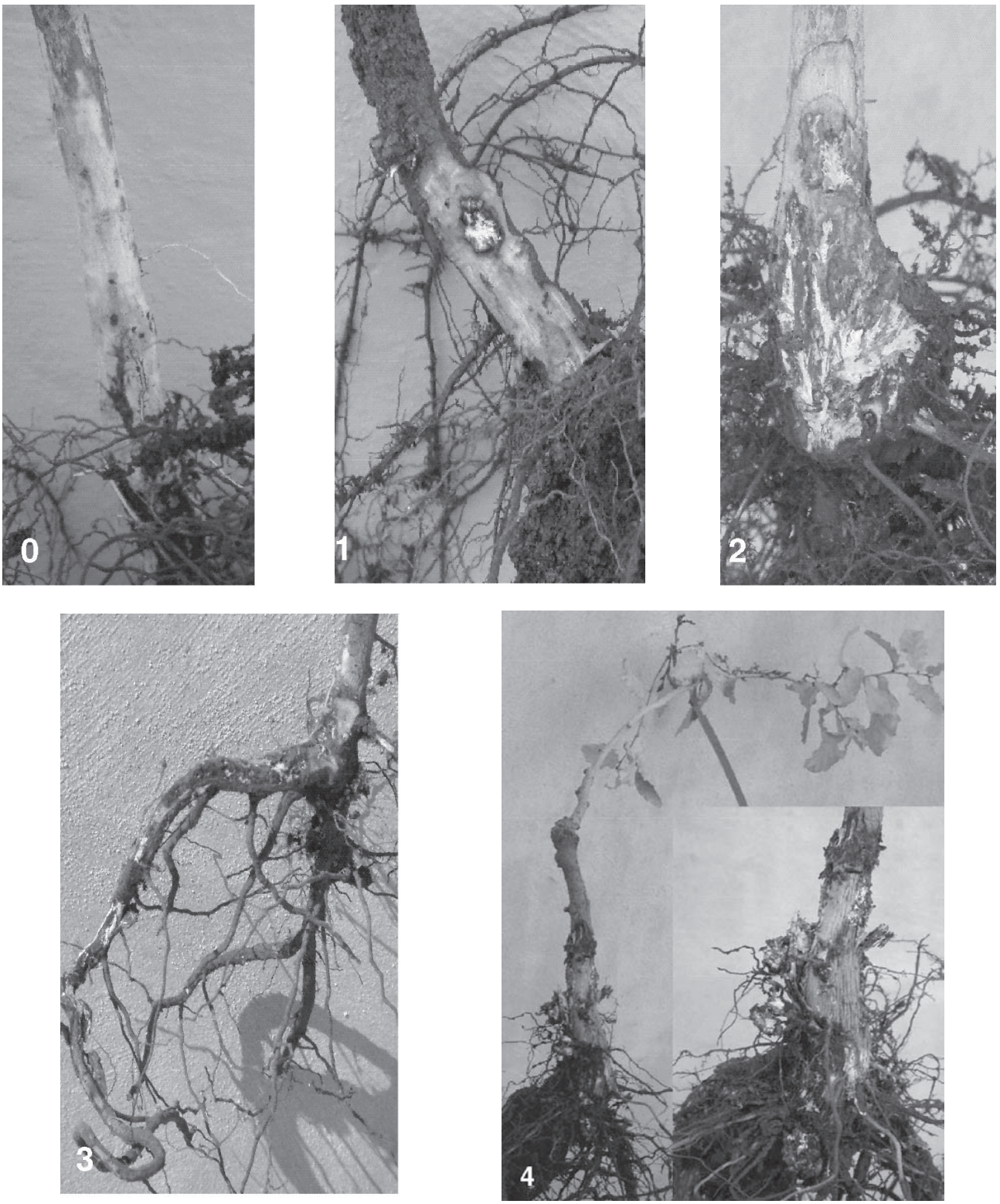

Fig. 1. Severity classes (0 – 4) for symptoms on oak seedlings assessed 1 year after inoculation with *Armillaria*.

4, mycelial fans invading more than half of the root system, the plant dies.

Data were statistically processed by means of variance analysis and Duncan's test (S.A.S. Institute Inc., 1999, Carey, NC, USA).

Results

One year after inoculation, all inoculum plugs showed viability in the form of the typical mycelial fans in the wood observed after removing the bark. About 55% of seedlings inoculated with the fungi were infected, although fewer than 5% had severe root rot (classes 3 and 4) and only a few seedlings were infected with *A. tabescens* (Table 1). Specifically, symptoms of infection occurred in 63.1% of the least watered seedlings and in 44.2% of the most watered seedlings. No infections were observed in the controls.

Symptom severity varied significantly with the water regime and with the isolate inoculated (Table 2). The least watered seedlings had the severest symptoms (1.24), and the most watered seedlings had the least severe (0.62). The *A. mellea* isolate caused the most severe infection (mean symptom severity 1.30), while the *A. tabescens* isolate was the least pathogenic (mean symptom severity 0.27). *Q. ilex* seedlings were most susceptible to *Armillaria* (1.36) and *Q. trojana*, *Q. robur* and *Q. cerris* were least susceptible (0.79, 0.70 and 0.67 respectively).

Differences in pathogenicity among isolates persisted within watering regimes, except in the minimum water regime, where *A. gallica* (1.69) and *A. mellea* (1.64) were statistically equally pathogenic (Table 3). Differences in symptom severity between oak species were weak but statistically significant except for *Q. ilex*, which was the most susceptible regardless of water regime and fungal isolate (data not shown).

Finally, the majority of *A. gallica* inoculum plugs (89%) produced rhizomorphs, which caused infection in 73% of cases, though mostly at an early stage (Fig. 2). With *A. mellea*, rhizomorphs occurred in

Table 1. Number of seedlings affected with *Armillaria* root rot in each symptom severity class 1 year after inoculation.

Symptom severity class	Isolate			Total	
	A. mellea	*A. gallica*	*A. tabescens*	No.	%
0	77	96	309	482	44.6
1	159	185	30	374	34.6
2	77	40	11	128	11.9
3	30	16	6	52	4.8
4	17	23	4	44	4.1
Total infected	283	264	51	598	55.4
Inoculated	360	360	360	1,080	100.0

Table 2. Mean infection severity values one year after inoculation of three *Armillaria* isolates on seedlings of five oak species grown under three water regimes.

Factors	Symptom severity
Water regimes	
Min	1.24 A[a]
Mid	0.82 B
Max	0.62 C
Armillaria isolates	
A. mellea	1.30 A
A. gallica	1.11 B
A. tabescens	0.27 C
Oak species	
Q. ilex	1.36 A
Q. pubescens	0.94 B
Q. trojana	0.79 B C
Q. robur	0.70 C
Q. cerris	0.67 C

[a] Values marked with the same letters are not statistically different between factors ($P \leq 0.05$).

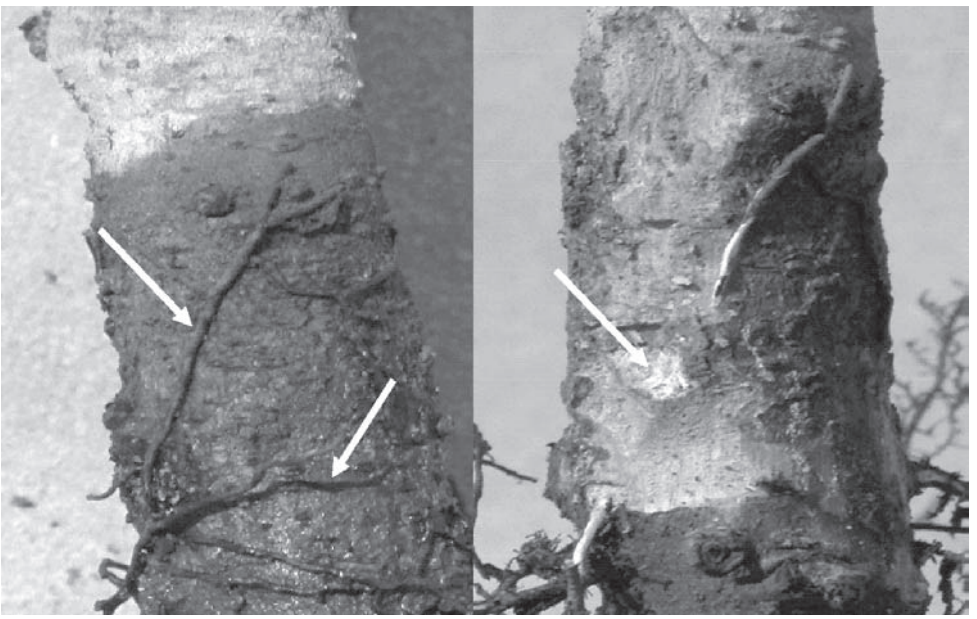

Fig. 2. Rhizomorphs of *A. gallica* attached to the taproot of a *Q. ilex* seedling just below the collar (left, see arrows) where an early infection was induced (right, see arrow).

Table 3. Pathogenicity of three *Armillaria* isolates inoculated into oak seedlings watered at three water regimes and assessed 1 year after inoculation.

Isolates	Water regimes		
	Min	Mid	Max
A. mellea	1.64[a] **A** *A*	1.21 **A** *B*	1.04 **A** *B*
A. gallica	1.69 **A** *A*	0.97 **B** *B*	0.66 **B** *C*
A. tabescens	0.37 **B** *A*	0.27 **C** *A*	0.15 **C** *A*

[a] Values marked with the same bold letter in columns and same italic letter in rows are not statistically different ($P \leq 0.05$).

only 37% of seedlings and these attacked the roots in only 35% of cases, and for the most part (classes 3 and 4) even caused extensive root rot. The *A. tabescens* isolate did not produce any rhizomorphs.

Discussion

Numerous inoculation trials on a number of plant species and using varying types of inocula and inoculation techniques have shown differences in disease severity caused by different *Armillaria* species (Guillaumin, 1977; Davidson and Rishbeth, 1988; Tsopelas and Tjamos, 1999; Sicoli *et al.*, 2002). Nevertheless, the effect of infection on trees weakened by water stress or insect defoliation has only rarely been studied (Parks *et al.*, 1994; Omdal *et al.*, 1995; Piercey-Normore and Bérubé, 2000; Lung-Escarmant *et al.*, 2003).

The three *Armillaria* isolates inoculated in these tests had already been shown to be pathogenic on oak seedlings reflecting a pathogenic gradient *A. mellea* > *A. gallica* > *A. tabescens* for these species (Guillaumin *et al.*, 1993; Tsopelas and Tjamos, 1999), although the pathogenicity of an *Armillaria* species is known to require assessments based on several isolates (Omdal *et al.*, 1995). However, the present study also demonstrated that the amount of watering significantly affected the severity of Armillaria root rot in Mediterranean *Quercus* seedlings. In support of this finding, we showed that differences in isolate pathogenicity agreed with the above gradient when the isolates were tested on seedlings watered every 2 and every 4 days, but the gradient was different: namely *A. mellea* ≃ *A. gallica* > *A. tabescens,* in the seedlings watered every seven days. In this, the least wa-

tered group, the *A. mellea* and the *A. gallica* isolates showed no statistical difference in pathogenicity but were equally infective, most likely because the seedlings were suffering from water stress. Lastly, the three watering regimes significantly influenced the pathogenicity of the *A. gallica* isolate, while the *A. tabescens* isolate was statistically the least pathogenic with all watering regimes on account of the small number of infected seedlings.

As for rhizomorph production and rhizomorph-induced infection, here the *A. gallica* isolate was more infective than the *A. mellea* isolate, but infection with the latter mostly occurred via direct contact between the inoculum and the taproot of the host, thus revealing that on the whole *A. mellea* was more pathogenic. This activity is consistent with the finding that *A. mellea* has a more restricted rhizomorph-forming ability than *A. gallica* (Redfern and Filip, 1991), and that the less pathogenic *Armillaria* species, except *A. tabescens*, form more abundant and more vigorous rhizomorphs than do the more pathogenic species (Prospero *et al.*, 2004).

Differences in susceptibility among oak species were slight but statistically significant, with *Q. ilex* showing the severest symptoms. This was probably because the seedlings were uprooted so soon (after only 12 months) instead of after 24–36 months, which is the recommended incubation period for experiments of this type (Morrison and Pellow, 2002; Prospero *et al.*, 2004). A 12-month incubation period nevertheless proved sufficient to discriminate between the three water regimes measurable by their water potential; such regimes changed the pathogenicity gradient of the three isolates of *Armillaria*. The pathogenicity of *A. gallica* could be enhanced by reducing the amount of water delivered to the oak species.

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