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Occurrence and pathogenicity of *Armillaria tabescens* on almond in Greece[1]

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Armillaria tabescens was detected in almond orchards in three different localities of central Greece (Fthiotis county). Mycelial fans of the fungus were observed in the roots and the base of the trunk of dead or dying trees. Symptoms of gummosis were also evident on the bark of infected trees. One of the orchards had been established on land cleared of a *Quercus coccifera* forest. *A. tabescens* was identified from basidiocarps developed *in vitro*, as well as in haploid–haploid mating tests with testers from France. This is the first report of *A. tabescens* on almond in Europe. Although the fungus has been characterized as a weak parasite or even non-pathogenic to other hosts, it caused considerable damage in almond orchards. In inoculation trials, it was shown that one-year-old almond trees were susceptible to three *Armillaria* species tested: *A. mellea*, *A. gallica* and *A. tabescens*. The most virulent species in these tests was *A. mellea* while the least virulent was *A. tabescens*. The pathogenicity tests verified the ability of *A. tabescens* to be pathogenic to almond.

Introduction

Armillaria root rot has been reported as a serious disease of stone fruits in many areas of the world. Species of *Prunus* are considered as very susceptible hosts to *Armillaria*, especially almond (Guillaumin *et al.*, 1989a; Gregory *et al.*, 1991). However, most of the reports on infection of almond come from older literature and refer to *Armillaria melea sensu lato*. There is no information on which species of *Armillaria* attack almonds.

Armillaria tabescens (Scopoli) Emel (synonym: *Clitocybe tabescens*) has always been distinguished from other *Armillaria* species, because its basidiocarps do not bear an annulus on the stipe. It is referred as an exannulate *Armillaria* species (Guillaumin *et al.*, 1993). However, basidiocarps of the fungus are not very common in nature, since they appear for short periods of time. Some confusion may therefore exist in the records of *A. tabescens*, because the symptoms of the disease are indistinguishable from those of other *Armillaria* species. Also, the morphology of cultures from vegetative isolates of the fungus is very similar to that of the other species.

A. tabescens has been reported to occur in Europe, North America, North Africa and certain areas of Asia. However, considerable doubt exists that the fungus reported in all these areas belongs to the same species (Guillaumin *et al.*, 1989b; Gregory *et al.*, 1991). *A. tabescens* is thermophilic. In Europe, it is distributed mainly in southern areas, although some records of the fungus exist from south-east England (Guillaumin *et al.*, 1993). In North America, it is quite common in southern USA (Gregory *et al.*, 1991).

In Europe, *A. tabescens* is mostly reported as a saprophyte on broad-leaved forests, common in the 'Mediterranean maquis' (Guillaumin *et al.*, 1993). However, *A. tabescens* has been found to cause mortality of cork oak (*Quercus suber*) in Portugal (Azevedo, 1976) and it was also aggressive on

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eucalyptus species in southern France (Lung-Escarmant *et al.*, 1985) and Tunisia (Delatour, 1969). In the USA, *A. tabescens* has been reported as a serious pathogen of cultivated trees (Gregory *et al.*, 1991).

This work reports the occurrence of *A. tabescens* in almond orchards in Greece. The pathogenicity of the fungus towards almond trees was also examined in inoculation trials. Two other *Armillaria* species were included in these tests: *Armillaria mellea* (Vahl: Fries) Kummer and *Armillaria gallica* Marxmueller & Romagnesi, which are very common in Greece.

Materials and methods

A number of almond orchards in Fthiotis county of Greece were examined for the presence of *Armillaria* root disease. Dead and dying trees, or trees with symptoms of decline, were excavated to a depth of about 30 cm and the root collar and a number of roots were examined for the presence of mycelial mats under the bark. Root samples were taken to the laboratory for isolation of the fungus. Isolations were done on potato dextrose agar (PDA) selective medium, amended with fungicides and antibiotics (Tsopelas & Korhonen, 1996). *Armillaria* isolates were grown on 3% malt extract agar (MEA) and carrot agar (CA) for observation of the morphology of cultures and possible identification of the *Armillaria* species (Intini & Gabucci, 1987).

In order to obtain basidiocarps *in vitro*, two types of substrates were used in 500 mL Erlenmeyer flasks. In the first, small segments of oak (*Quercus pubescens*) branches were used with the addition of 150 mL deionized water. In the second substrate, we used a mixture of whole grain rice (25 g), beech sawdust (15 g) and peptone in deionized water (1 g in 200 mL H_2O) (Tirro, 1991). The flasks were autoclaved for about 1 h at 115°C. After inoculation with the fungus the flasks were incubated at 23°C for about 6 weeks in the dark. Then the temperature was lowered to 15°C and the flasks were exposed to light (Guillaumin *et al.*, 1989b) with a photoperiod of 11 h.

Monospore cultures were isolated from basidiocarps produced *in vitro*. A modified Pasteur pipette was used to pick germinated spores from the surface of 1% MEA under the microscope and transfer them to fresh medium (Korhonen & Hintika, 1980).

The *Armillaria* species isolated from almond was identified initially from cultures of the fungus on MEA and CA and was verified on the basis of the morphology of basidiocarps produced *in vitro* and also from compatibility tests. The monosporous haploid isolates were paired with haploid testers of different *Armillaria* species on 2% MEA (Guillaumin *et al.*, 1989b). The haploid testers that were initially used were from France (J.J. Guillaumin), but we also used haploid isolates of *A. tabescens* from southern Italy (G. Sicoli) and diploid isolates of this fungus from Slovenia (A. Munda), to compare Greek isolates with populations of *A. tabescens* from different areas of Europe.

Inocula were prepared by using oak-branch segments 8 cm long and about 1.5 cm in diameter. These were placed in bundles of 15–20 in 750 mL glass jars and 150 mL of deionized water was added; they were then autoclaved for about 1 h at 115°C. After autoclaving, the water was poured off and 150 mL of 3% MEA melted medium was added to each jar (Shaw, 1977). The jars were inoculated with vegetative (diploid) isolates of three *Armillaria* species: *A. mellea, A. gallica* and *A. tabescens*. Two isolates from each species were used. The jars were incubated in the dark at 23°C for 3 months.

One-year-old bare-rooted almond trees were planted in 12-L plastic pots in 1992-03. The potting medium was a mixture of mineral soil and sphagnum peat (2: 1 v/v). The trees were inoculated 2 months later (1992-05). For inoculation, a section of plastic pipe, 2 cm in diameter and 12 cm in length was placed adjacent to each tree during planting. The inocula were placed in contact with the main root after removal of the plastic pipe. At the same time we used autoclaved oak branch segments in control plants (Shaw, 1977; Rishbeth, 1984). For each of the two isolates of each *Armillaria* species eight trees were inoculated.

The trees were placed outdoors and were watered twice a week during the dry season. Inoculated trees were examined regularly for the presence of disease symptoms. Dead and dying trees were examined for the presence of mycelial mats under the bark of the roots. In some cases the fungus was

reisolated from infected roots and its identity was examined. Analysis of variance (ANOVA) was applied on the infection percentages (infected dead and also infected live + dead). The averages between the two isolations of each *Armillaria* species were compared among the three species using the Duncan test ($P = 0.05$).

Results

Armillaria root disease was detected in almond plantations at three localities of Fthiotis county: Afrati, Jannitsou and Palamas, widely apart from each other. In the locality of Afrati, the almond orchard was established on cleared forest land, where the original vegetation was evergreen broad-leaved shrubs (maquis), the main species being *Quercus coccifera*. This type of vegetation covered the area around the orchard (Fig. 1). The infected trees were usually scattered inside the orchards, but we also observed diseased trees in restricted areas of the field.

Gradual death of the trees was evident in most cases, with symptoms of twig and branch dieback. In some cases, sudden death of the trees was observed. Gummosis was common on the bark of infected trees at the base of the trunk. In the roots and the root collar, mycelial mats were found beneath the bark (Fig. 2), which are the most conspicuous symptoms of the disease. Rhizomorphs were not detected in our observations in the roots of infected almond trees.

All the isolates of *Armillaria* from the almond orchards examined were identified as *A. tabescens*. The morphology of the cultures on MEA and CA is very characteristic and the fungus was identified from them. However, *A. tabescens* was identified more accurately from basidiocarps developed *in vitro* and in mating tests.

Basidiocarps of *A. tabescens* developed on both types of substrates 6–8 weeks after the exposure of the cultures in light conditions. A large number of basidiocarp primordia (40–70) developed in the initial stages, but only 3–5 basidiocarps grew to full size in the small space of the flasks. Sometimes the basidiocarps grew outside the flasks through the opening. The basidiocarps were characteristic of *A. tabescens*, without an annulus on the stipe and with a small pileus 15–30 mm in diameter.

Monosporous (haploid) isolates from the basidiocarps developed *in vitro* were compatible with testers of *A. tabescens* from France, but incompatible with testers of other *Armillaria* species. These monosporous isolates were also compatible with haploid isolates of *A. tabescens* from southern Italy and diploid isolates from Slovenia.

In the inoculation trials, all isolates of the three *Armillaria* species were pathogenic to almond trees, although their virulence varied greatly. Infected trees were identified from the foliar symptoms. Certain trees, especially those infected with *A. mellea*, showed sudden death, while other trees showed a gradual decline with symptoms of twig dieback. All the dead trees had the characteristic white mycelial mats under the bark of the root an/or the root collar. Trees that had died recently showed mycelial mats in certain parts of the root system, while other parts of the root were still living. However, in plants that were examined a few weeks after their death, almost every part of the root system was colonized by the fungus. Rhizomorphs were observed growing from the inocula of *A. mellea* and *A. gallica*, but not from those of *A. tabescens*. Some of the trees infected by the first two pathogens also had rhizomorphs attached on the roots. We did not observe any dead trees or symptoms of dieback in the control plants. All three *Armillaria* species were reisolated from some of the infected trees and their identity was confirmed.

A. mellea proved to be the most virulent species in the inoculation trials (Table 1). Three months after the inoculation, three out of 16 trees were killed by the fungus (1 by isolate I and 2 by isolate II). The rest of the trees, except one, died within one year after the inoculation. The tree that survived the first year was killed by *A. mellea* a year later.

A. gallica was less virulent than *A. mellea*. One of the inoculated trees was found dead 7 months after inoculation. In a 2-year period, five more trees were killed by *A. gallica*. By the end of the experiment (3 years after inoculation), five more trees were found infected by the fungus but they were still alive with symptoms of branch dieback (Table 1).

Fig. 1. Almond orchard with trees killed by *Armillaria tabescens*. The orchard was established on cleared forest land of maquis vegetation.

Fig. 2. Mycelial mats of *Armillaria tabescens* on the base of the trunk of an almond tree.

Table 1. Infection and mortality of almond trees, 3 years after inoculation with *Armillaria* species

Armillaria species and isolate (%)	Trees infected live (%)	Trees infected dead (%)	Total infected (live + dead) (%)	Trees not infected (%)
A. mellea I	0	100 (8)	100 (8)	0
II	0	100 (8)	100 (8)	0
A. gallica I	37.5 (3)[1]	37.5 (3)	75 (6)	25 (2)
II	25 (2)	37.5 (3)	62.5 (5)	37.5 (3)
A. tabescens I	12.5 (1)	25 (2)	37.5 (3)	62.5 (5)
II	12.5 (1)	12.5 (1)	25 (2)	75 (6)
Control	0	0	0	100 (8)
Means				
A. mellea		100a	100a	
A. gallica		37.5b	68.75b	
A. tabescens		18.75c	31.25c	

[1] Numbers in brackets show the number of plants. Eight plants were inoculated with each isolate.
[2] Means followed by different letters differ significantly as determined by Duncan test ($P = 0.05$).

A. tabescens was the least virulent of the three *Armillaria* species. Two years after inoculation, three trees out of 16 had died as a result of infection by the fungus (2 by isolate I and 1 by isolate II). Two more trees were found infected by *A. tabescens* (one from each isolate) with symptoms of dieback (Table 1), in the same 2-year period. We did not observe any other trees infected by *A. tabescens* in the end of the 3-year period.

The rate of infection and mortality of almond trees due to the three *Armillaria* species at the end of the 3-year period is summarized in Table 1. The differences in virulence among the three *Armillaria* species, expressed by the percentage of infected dead and/or total infected (live + dead) trees were statistically significant ($P = 0.05$).

Discussion

A. tabescens is considered a significant pathogen of orchard trees and ornamental plants in southern USA (Gregory *et al.*, 1991). However, reports on the occurrence of the fungus in Europe on cultivated plants are rare. Laville & Vogel (1984) reported *A. tabescens* on citrus trees in Corsica (FR) and Rishbeth (1985) cited some information on the attack of pear trees (*Pyrus communis*) in Portugal. This work reports for the first time in Europe the occurrence and pathogenicity of *A. tabescens* on almond.

As mentioned in the introduction, a certain doubt exists whether *A. tabescens* found in Europe is the same species as the one reported in North America and other places in the world. Guillaumin *et al.* (1989b, 1993) suggested that compatibility tests between isolates from Europe and USA showed them to belong to different species. However, Darmono *et al.* (1993) reported that haploid isolates from Europe were compatible with haploid isolates from USA, and that isolates from different areas of North America all belonged to the same species. Our results of compatibility tests show that isolates of *A. tabescens* from Greece, France, Italy and Slovenia also belong to the same species.

Rishbeth (1984, 1985) reported the results of inoculation trials with different *Armillaria* species on pine. In these experiments, *A. tabescens* was non-pathogenic to young pine trees, while *A. mellea* was the most virulent and *A. gallica* showed limited infection. Similarly, our results show that *A. mellea* is the most virulent species and *A. tabescens* the least virulent, with *A. gallica* intermediate.

However, our results clearly showed that *A. tabescens* is pathogenic to almond, which seems to be very susceptible to *Armillaria* species in general. In the inoculation trials of Guillaumin *et al.* (1989a), with rootstocks of different *Prunus* species, almond was very susceptible to *A. mellea*, but no other species of *Armillaria* were tested. The pathogenicity of *A. tabescens* in North America was proved by Plakidas (1941), in inoculation tests with pear. Our results confirmed for the first time in Europe the pathogenicity of *A. tabescens* in inoculation trials.

In the almond orchards of Greece in which *A. tabescens* was detected, the fungus appears to behave as a primary parasite and in some cases causes significant damage. However, it is possible that the infected trees may have suffered from drought in certain years, since they were not irrigated and periods of reduced rainfall are very common in Greece. Infections by *A. tabescens* and other *Armillaria* species are prevalent in sites where the trees have been weakened by moisture stress (Gregory *et al.*, 1991).

Présence et pouvoir pathogène d'*Armillaria tabescens* sur amandier en Grèce

Armillaria tabescens a été détecté dans des vergers d'amandiers dans trois localités du centre de la Grèce (comté d'Ethiotis). Le mycélium du champignon a été observé dans les racines et à la base des troncs d'arbres morts ou mourants. La présence de gomme était également visible sur l'écorce des arbres infectés. Un des vergers avait été établi sur une coupe de maquis de chêne kermes *Quercus coccifera*. *A. tabescens* a été identifié d'après des basidiocarpes cultivés *in vitro* et par des tests de croisement haploïde-haploïde réalisés avec du matériel obtenu en France. Il s'agit du premier signalement d'*A. tabescens* sur amandier en Europe. Ce champignon a été caractérisé comme étant un parasite faible ou même non pathogène pour d'autres hôtes, mais il semble causer des dégâts considérables dans les vergers d'amandiers. Au cours de tests d'inoculation, il a été montré que les amandiers d'un an sont sensibles à trois espèces d'*Armillaria* testées: *Armillaria mellea*, *A. gallica* et *A. tabescens*. L'espèce la plus virulente était *A. mellea*, et la moins virulente *A. tabescens*. Les tests de pouvoir pathogène ont vérifié qu'*A. tabescens* peut être pathogène sur amandier.

Появление и патогеничность Armillaria tabescens на миндале в Греции

Armillaria tabescens был обнаружен в миндальных фруктовых садах трех различных мест центральной Греции (район Фтиотис). Мицелиальные веера этого гриба наблюдались в корневой системе, а также в основании ствола мертвых или отмирающих деревьев. На коре зараженных деревьев были видны симптомы гуммоза. Один из фруктовых садов был расположен на территории, очищенной от леса *Quercus coccifera*. *A. tabescens* был выявлен на базидиокарпах, развившихся *in vitro*, а также путем гаплоид-гаплоидового спаривания на французских тестерах. Это была первая регистрация *A. tabescens* на миндале в Европе. Несмотря на то, что этот гриб был охарактеризован как слабый и даже как непатогенный вредитель по отношению к другим хозяевам, он вызывал существенный ущерб на миндальных плантациях. На инокуляционных испытаниях было показано, что одногодки миндальных деревьев проявляли чувствительность по отношению к отдельным видам *Armillaria: A. mellea, A. gallica* и *A. tabescens*. Наиболее вирулентными видами в этих тестах оказался *A. mellea*, в то время как наименее вирулентной был *A. tabescens*. С помощью тестов проверялась патогеничность *A. tabescens* по отношению к миндалю.

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