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INCIDENCE OF ROOT DISEASES IN THE FIR FOREST OF MOUNT PARNIS NATIONAL PARK, GREECE

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SUMMARY

Incidence of root rot pathogens was investigated over an area of 2500 ha of fir forest in the National Park of Mount Parnis. Dead trees and stumps were examined in 20 (0.1 ha) permanent sample plots. *Heterobasidion annosum* (F intersterility group) was widely distributed in the fir forest, occurring in 70% of the plots. *Armillaria mellea* was detected in 25% of the plots, while *Armillaria gallica* was present in 10% of the plots. In 85% of the plots, at least one of the pathogens was found. However, incidence of these fungi was relatively low in the dead trees examined. Upon examination of 329 stumps and trees, which were dead until 1997, *H. annosum* was isolated from 11.55%, *A. mellea* from 1.21% and *A. gallica* from 0.91% of them. From 68 trees that died over a 3-year period (1998-2000) in the plots, 7.35% were found infected by *H. annosum*, 4.41% by *A. mellea* and 1.47% by *A. gallica*. During this 3-year period, 70.59% of the dead trees were observed in the summer and fall of 2000, a year of very limited precipitation. Dead and dying fir trees were found infested by the bark beetles *Phaenops knoteki* and *Pityokteines spinidens*. Another important factor of fir mortality in Mount Parnis National Park is mistletoe (*Viscum album*). Of the trees died over the 4-year period, 85.29% were infected by the mistletoe. The most common saprotrophic fungi detected on fir stumps and dead trees were *Fomitopsis pinicola* and *Trichaptum abietinum* These two fungi seem to play an important role as antagonists of *H. annosum* and *Armillaria* spp.

INTRODUCTION

Mount Parnis is located north to the city of Athens, at a distance of about 30 km (38°10' N). An area of 3800 ha at the high altitudes of the mountain has been designated as National Park since 1961. Most of the area of the Park is covered by fir (*Abies cephalonica* Loud.) at elevations 700-1400 m. Aleppo pine (*Pinus halepensis* Mill.) is the dominant species in lower elevations, forming also mixed stands with fir at elevations 700-1000 m. Soils in the fir-forest are mostly shallow and rocky derived from hard limestone and flysch parent material. The mean annual precipitation at elevations 1020 m has been reported to 750 mm, reaching 1000 mm in certain years, but during dry years this is limited to less than 500 mm (Tsopelas et al. 2001).

Records of fir mortality in Mount Parnis date back to the 1930's. Extensive tree mortality has been reported in certain years in the 1950's and 1960's as well as the 1980's. In all these cases fir mortality has been associated with periodic droughts (Kailidis and Georgevits 1968, Amorgianiotis and Angelopoulos 1996).

Root rot fungi are important pathogens in the fir-forests of Greece. *Heterobasidion annosum* (Fr.) Bref (the F intersterility group), *Armillaria mellea* (Vahl.:Fr.) Kumm. and *Armillaria ostoyae* Velen. have been reported to cause considerable damage in many fir-forests of the country (Tsopelas and Korhonen 1996, Tsopelas 1999). The objectives of this work was to study the incidence of root rot pathogenic fungi in the fir-forest of Mount Parnis National Park and to determine the role of these pathogens in fir-mortality.

MATERIALS AND METHODS

Data were collected from 20 circular sample plots, each 0.1 ha, which were established in 1997 to monitor forest decline. These were systematically located along transects of a 1250 x 1250 m grid, over an area of about 2500 ha of dense fir-forest.

In 1997, all dead trees and stumps in the plots were examined for the presence of root rot fungi. They were included trees that had been dead for about 20 years or less. This was judged by the presence of intact bark in more than half of their circumference at the root collar (Filip and Goheen 1982). Also, they were examined all the trees that died in the following 3 years (1998, 1999, 2000). At least two roots, on opposite sides of trees and stumps, were excavated and examined for sings of root rots. A section 10-15 cm from the roots was cut, placed in a plastic bag and transferred to the laboratory. Following root excavation each tree was felled and trunk disks were cut at ground level and were also transferred to the laboratory.

The presence of *H. annosum* in trees and stumps was detected from the characteristic white, stringy decay in the roots and the trunk of felled trees. Hollow stumps were also examined for the presence of basidiocarps of *H. annosum*. The roots and the root collar were inspected for the presence of the characteristic mycelial mats of *Armillaria* species and the occurrence of rhizomorphs on the roots (Filip and Goheen 1982, Tsopelas 1999). During the autumn the plots were inspected for the presence of basidiocarps of *Armillaria* spp.; they were collected basidiocarps from the sample plots as well as from other areas of the fir-forest. Also, basidiocarps of other wood rotting basidiomycetes were noted and collected for identification from the sample plots.

Isolations from root samples and trunk disks were carried out without surface disinfection. The samples were washed well in tap water and dried. The isolation medium was potato dextrose agar (PDA) amended with fungicides and antibiotics (Tsopelas and Korhonen 1996). Also, isolations from basidiocarps of *H. annosum* and *Armillaria* were performed on the same type of medium. Only heterokaryotic cultures were obtained in this study. Pure cultures were maintained on 2% w/v malt extract agar (MEA). This substrate, along with carrot agar, were also used for identification of *Armillaria* species, on the basis of morphological characteristics of the vegetative mycelium (Guillaumin et al. 1989, Tsopelas 1999). However, in most cases, identification of *Armillaria* species was confirmed in pairings on 2% MEA of the isolates (putatively diploid) with haploid testers (Korhonen 1978a, Guillaumin et al. 1991). These were belonging to the three *Armillaria* species that occur in southern Greece: *A. mellea*, *A. gallica* and *A. tabescens* (Tsopelas 1999). The intersterility group of the isolates of *H. annosum* were determined in pairings with homokaryotic tester strains F, P and S, on 1% MEA (Korhonen 1978b, Tsopelas and Korhonen 1996). In certain plots, when *H. annosum* was isolated from neighboring trees and stumps, pairings were carried out between these isolates to determine the vegetative incompatibility groups (VIG's) and study the spread of the fungus between trees (Korhonen 1978b).

RESULTS AND DISCUSSION

Three species of root pathogenic fungi were identified in the fir-forest of Mount Parnis: *Heterobasidion annosum* (the F intersterility group), *Armillaria mellea* and *Armillaria gallica* Marx. & Rom. In 17 of the 20 sample plots (85%) at least one of these fungi was present. *H. annosum* was found with the highest frequency; it was recorded in 14 (70%) of the plots. *A. mellea* was present in five (25%) of the plots and *A. gallica* was detected in two (10%) plots. In two of the plots, *H. annosum* and *A. mellea* occurred together; in one plot *A. mellea* and *A. gallica* were found together and in another plot *H. annosum* and *A. gallica* occurred together (both were found on the same tree which was dead for many years). Although these fungi were well distributed over the entire area of the fir-forest, the incidence of infection was relatively low. Upon examination of 329 stumps and trees which were dead until 1997 (approximately the last 20 years), *H. annosum* was isolated from 11.55%, *A. mellea* from 1.21% and *A. gallica* from 0.91% of them. From 68 trees that died in the following 3 years (1998-2000), 7.35% were found infected by *H. annosum*, 4.41% by A. mellea and 1.47% by *A. gallica* (Table 1). However, very often the roots of fir trees were growing in fissures between rocks of limestone and only a small

part of the root system was excavated. Therefore, it is possible that some of the roots of the dead trees were infected by these fungi and were not detected in our investigation.

In two of the plots, *H. annosum* was detected only in a single tree. In the rest (12) of the plots, the number of trees and stumps, which were infected by *H. annosum*, ranged from two to nine. In nine of the plots they were found genotypes (VIG's) of *H. annosum* that had spread secondarily to more than one tree or stump, two to four genotypes were determined in each plot. The longest distance that a single genotype was present in all sites was 7 m. However, very often adjacent trees or even the same tree were infected by different genotypes of the fungus. *A. mellea* was mostly found singly in the plots, except in one case that the fungus was isolated from three adjacent trees. This fungus though has been observed in many areas of Mount Parnis to cause mortality of fir-trees in groups of two to seven trees. *A. gallica* was found in one of the plots that have infected two small fir trees suppressed in the understorey, while in the other plots its presence was saprotrophic.

Table 1. Incidence of root pathogens in sample plots

Fungal species	Percent of infection (%)	
	Stumps and trees dead until 1997[1]	Trees that died from 1998 to 2000[2]
Heterobasidion annosum	11.55 (38)[3]	7.35 (5)[3]
Armillaria mellea	1.21 (4)	4.41 (3)
Armillaria gallica	0.91 (3)	1.47 (1)

[1] Total number of trees and stumps 329, [2] Total number of trees 68, [3] Number of trees and stumps infected

H. annosum was isolated from trees that had been dead for several years as well as from recently dead trees, inside and outside the plots. In old stumps and trees that had been dead for many years, *H. annosum* was detected in decayed wood below the ground line, while other types of decay were present above the ground line. Similar observations have been reported by Filip et al. (1992) in fir-tree stumps in forest of western United States. According to these authors, the presence of viable *H. annosum* below ground with other types of rot above ground is an indication that the roots were infected live and further colonization of the root system occurred after the death of the tree. Infections of *H. annosum* in root system of living fir-trees may be common in Mount Parnis; in a few cases, the fungus was isolated from wood cores taken from the base of the trunk of live trees. This is further supported by the presence of recently windthrown trees with extensive root infection, in which the crown was still green without any obvious symptoms of disease.

A. mellea was detected in recently dead fir-trees by the presence of mycelial mats in the cambial zone of roots and the root collar, often extending in the above ground portion of the trunk up to 2.5 m in height. However, in trees which had been dead for several years, mycelial mats could not be found in the above ground portion of the trunk or even the root collar; only in roots below ground could be detected. Rhizomorphs of *A. mellea* were not very frequent in Mount Parnis. More often they were detected rhizomorphs of *A. gallica* (mostly outside the plots) in old stumps.

Basidiocarps of *H. annosum* were mostly found inside the hollow stumps, where moisture conditions were favourable for their development. Very rarely basidiocarps of this fungus were observed around the root collar of trees at the ground level, usually covered by the litter. Fruitings of *Armillaria mellea* were observed in many areas of the fir-forest of Mount Parnis, during the years of observation. They appeared usually in October and were associated with dead trees and stumps; in a few cases, they were also associated with live trees, in which, only a part of the root system and root collar was infected. Basidiocarps of *A. gallica* were relatively rare during the four years of observation. However, they had been recorded in the fir forest a year earlier (1996) in abundance. They were mainly fruiting on stumps but also on bare ground, connected though to wood material with rhizomorphs.

Fir mortality was 0.96% in 1998 and 0.28% in 1999, but it was significantly increased in the year 2000 to 3.65%. This is interpreted to 7.0 trees/ha in 1998, 2.0 trees/ha in 1999 and 26.5 trees/ha in 2000. In the year 2000, in which high tree mortality was recorded, precipitation was significantly reduced. Although we do not have information from Mount Parnis data from other areas of the Attika region show that precipitation during the hydrologic year 1999-2000 was reduced by more than 50%. High fir mortality has been observed in many areas of Greece during periods of extensive droughts (Kailidis and Georgevits 1968, Tsopelas et al. 2001) Generally, tree mortality was more severe in southern and eastern facing slopes, in sites of low productivity. In certain areas of Mount Parnis, fir grows under adverse site conditions, in rocky shallow soils. Fir mortality has been severe in these areas over the years, especially near the margins of the fir range, at elevations 800-1000 m. As a result, Aleppo pine is taking over in certain sites where fir was growing a few decades ago (Tsopelas et al. 2001). Root pathogenic fungi were widely distributed in the fir forest and they certainly play a role in tree mortality of many trees. However, the majority the fir-trees that die every year in Mount Parnis do not seem to be affected by these pathogens.

Dead and dying fir-trees were found infested by bark beetles, whether they were infected by root rot fungi or not. The most common bark beetle was *Phaenops knoteki* Reitt. (Buprestidae), this was observed in almost all of the dead trees. Also, a considerable number of the dead trees were infested by *Pityokteines spinidens* Reitt. (Scolytidae). Bark beetles play an important role in fir mortality. They infest and kill trees suffering from water deficiency and/or affected by other abiotic and biotic factors (Ferell and Hall 1975). Among the biotic factors are root pathogenic fungi. Another important biotic factor that affects tree vigor and mortality in Mount Parnis is the mistletoe (*Viscum album* L.). Of the trees that died over the 3-year period, 85.29% were infected by mistletoe; a significant proportion of these trees were heavily infested by this parasite.

The most common wood decay fungi in the fir forest of Mount Parnis were *Trichaptum abietinum* (Fr.) Ryv. and *Fomitopsis pinicola* (Fr.) Karst. *T. abietinum* was identified by its basiocarps in all of the sample plots, occurring in stumps and dead logs, occasionally it was also found on the dead portion of the trunk of living firs. *F. pinicola* was detected in 13 of the plots (65%), but it may be underestimated. Basidiocarps of *F. pinicola* were not very frequent; the fungus was isolated from stumps and dead trunks, causing characteristic brown rot. These two decay fungi seem to play an important role as antagonists of root pathogenic fungi. They were observed quite often on trees and stumps also infected by *H. annosum* and *A. mellea*. Other decay fungi identified on fir stumps and dead trunks were: *Pleurotus ostreatus* (Jacq.:Fr) Kumm., *Ischnoderma benzoinum* (Wahl:Fr.) Karst., *Ganoderma carnosum* Pat., and *Pholiota aurivella* (Batsch:Fr.) Kumm.

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