

***Sphaeropsis sapinea* Dyko & Sutton Associated with Shoot Blight on *Pinus brutia* Ten. in Southwestern Turkey**

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Abstract – This study was conducted in order to determine the agents of shoot blight and dieback of Calabrian pines at Aşağı Gökdere, Isparta province, in 2005 and 2006. Ninety trees were selected systematically in the stand. One dead branch from each tree was cut and the shoots were investigated under the stereomicroscope for the presence of fungal structures. *Sphaeropsis sapinea* Dyko & Sutton and *Truncatella hartigii* (Tubef) Steyaert were common fungi, with the observation frequencies of 21.1% and 46.7%, respectively. Pathogenicity of two fungi was investigated by winter and spring inoculations on Calabrian and Crimean pine. After eight months incubation period, the lesion sizes were measured. *S. sapinea* was found to be quite aggressive and large lesions formed on both hosts while lesions caused by *T. hartigii* did not differ significantly from the control.

Calabrian pine / Crimean pine / dieback / pathogenicity

Kivonat – A *Sphaeropsis sapinea* Dyko & Sutton és a *Pinus brutia* Ten. hajtáspusztulása Délnyugat-Törökországban. E tanulmány célja a kalábriai fenyők hajtáspusztulásáért és száradásáért felelős kórokozók meghatározása Aşağı Gökdere térségében, Isparta vidékén 2005-ben és 2006-ban. Az állományban rendszeresen kilencven fát választottunk ki. Minden fáról levágtunk egy-egy elhalt ágat és sztereómikroszkóp alatt megvizsgáltuk a gombaszervezetek jelenlétét a hajtásokon. A *Sphaeropsis sapinea* Dyko & Sutton és a *Truncatella hartigii* (Tubef) Steyaert gombák esetében 21,1%-os, illetve 46,7%-os gyakoriságot állapítottunk meg. A két gomba patogenitását téli és tavaszi mesterséges fertőzéssel vizsgáltuk a kalábriai és a krimi fenyőn. Nyolc hónap inkubációs idő elteltével megmértük a nekrozisok nagyságát. A *S. sapinea* meglehetősen agresszívnek bizonyult, mindkét gazdán nagyméretű nekrozisokat okozott. A *T. hartigii* okozta nekrozisok a kontrolltól szignifikánsan nem különböztek.

kalábriai fenyő / krimi fenyő / pusztulás / patogenitás

1 INTRODUCTION

Mediterranean region of Turkey exhibit a rich diversity of forests ranging from coniferous to deciduous trees belonging to this mild climatic zone. There are single-species forests of softwood and hardwood trees, and also mixed forest formations. The most frequently occurring coniferous forest in the Mediterranean belt consists of *Pinus brutia* Ten., *Pinus*

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nigra Arn. (Lamb.) Holmboe, *Abies cilicia* Carr. and *Cedrus libani* A. Rich. The highway from Isparta to Antalya where the Calabrian pine plantation was managed is attractive and famous for domestic and international guests with its special mountainous landscape, *Liquidambar orientalis* L. protection area and also for many other natural beauties.

Shoot dieback of coniferous trees caused by fungi have economic importance when it causes tree to loose its vitality (Butin 1995). *Sirococcus conigenus* (DC.) P. Cannon & Minter (syn. *S. strobilinus* G. Preuss) (Hartig 1893, Klein 1987, Minerbi 1987, Wulf – Maschnig 1992, Neumüller 1994, Anglberger – Halmshlager 2003), *Sphaeropsis sapinea* Dyko & Sutton (syn. *Diplodia pinea*) (Brookhouser – Peterson 1971, Peterson 1977), and *Gremmeniella abietina* (Lagerberg) Morelet. (Yokota et al. 1974, Setliff et al. 1975, Gibbs 1984, Laflamme 1993) are among most common agents of the disease all over the world.

Shoot dieback of *P. brutia* has devastating effects from the beginning of 2004 in western part of Taurus Mountains located in Mediterranean site of Turkey. Calabrian pine plantation (app. 16 352 ha) in Aşağı Gökdere, Isparta province exhibits unhealthy appearance, specifically characterised with branches carrying dead shoots.

The objective of this study was to find out the causal agent of the serious damage observed on Calabrian pine plantation at Aşağı Gökdere.

2 MATERIAL AND METHOD

A field study was conducted in July 2005 at Aşağı Gökdere. A Calabrian pine stand, which was as homogenous as possible regarding to tree size and shoot blight symptoms, was chosen for the investigation. Ninety trees were selected systematically within the stand. One dead branch from each tree was cut and the shoots were investigated under the stereomicroscope for the presence of fungal structures. The fungi were identified based on conidial and pycnidial morphology (Sutton 1980). Fungi were also isolated from the shoots having dieback symptoms and kept as PDA slants for further studies.

Virulence of the fungal isolates was investigated in 2005-2006, by inoculating branches of *P. brutia* and *P. nigra*. Pathogenicity tests were carried out in two different inoculation trials using one isolate of each fungus with ten replicates.

Healthy looking shoots of 15 years old *P. brutia* trees were inoculated in December 2005 and April 2006 (as winter and spring inoculations) in the same area where the field study was performed. In addition, shoots of 15-year-old *P. nigra* were inoculated in April 2006 near the main campus of Süleyman Demirel University.

Inoculations were performed on a healthy branch (about Ø 15 mm) of the trees. In each tree outer bark was removed with a cork borer (Ø 5 mm), and the hole was filled with a plug of actively growing mycelium of each fungus cultured a week on PDA at 25°C. The hole was covered with parafilm to retard desiccation. After an eight month incubation period, branches were cut and the length of the cankers was measured. Fungi were re-isolated from the cankers.

Data were statistically analysed by using SPSS software programme and means were compared by Duncan's multiple range test ($p = 0.05$).

3 RESULTS AND DISCUSSION

As a result of the field work, *Sphaeropsis sapinea* Dyko & Sutton (syn. *Diplodia pinea*) and *Truncatella hartigii* (Tubef) Steyaert were commonly found on the shoots of 90 collected branches, with the observation frequencies of 21.1% and 46.7%, respectively. Similarly, it was reported that *T. hartigii* was one of the most common fungi occurring on Austrian pines

infected with *S. sapinea* (Diminia 1994, Jurc 2005, Milijasevic- Karadzic 2007). *S. sapinea* and *T. hartigii* were also found to be the most common fungi isolated from pine cones and seeds (Vujanovic et al. 2000).

S. sapinea is a latent fungal endophyte of coniferous trees occurring throughout the world (Eldridge 1961, Punithalingam - Waterston 1970, Nicholls et al. 1977, Sutton 1980, Swart - Wingfield 1991 a, b, Hausner et al. 1999, Flowers 2001). Although the existence of the fungus was reported in Marmara Region (Unligil - Aytekin 1993), its damage, pathogenicity and distribution have not been studied for Turkey. The fungus causes devastating effects in plantation sites, especially where susceptible *Pinus* spp. are extensively planted. As it is an opportunistic pathogen, predisposition due to a variety of biotic and abiotic stress factors can also result in this naturally being fungus causing substantial deaths in pine plantations (Bega et al. 1978, Swart et al. 1987, Nicholls - Ostry 1990, Palmer 1991, Swart - Wingfield 1991, Stanosz - Cummings-Carlson 1996). Poor sites, draught, hail, snow and insects are among stress factors (Chou 1987). Our plantation site is surrounded with mountains located in a large valley, which has high summer temperatures causing draught stress for the trees. Pine Stem Borer, Pine Sawfly and Pine Engraver Beetle are the common insects in this location. Apart from the site properties, origin of the seeds or seedlings is known among important factors for the adaptation and healthy growth of the trees in the plantation sites. Reliable data confirming the origin of the planting material does not always exist in Turkish forestry practice. Appropriate silvicultural treatments such as pruning and thinning also affect future health and survival of the plantation.

In the pathogenicity test, winter inoculations on *P. brutia* and spring inoculations on *P. brutia* and *P. nigra* with both fungi resulted in lesions with different sizes. Lesion sizes caused by *T. hartigii* did not differ significantly from that on the control shoots, but *S. sapinea* was found to be quite aggressive and cause larger lesions (Table 1 and Figure 1). Results of Milijasevic and Karadzic (2007) who mentioned that *T. hartigii* occurred as a weak parasite or saprophyte on shoots colonised by *S. sapinea*, supported our findings.

Table 1. Lesion lengths on *P. brutia* and *P. nigra* branches caused by *S. sapinea* and *T. hartigii* inoculations

Treatments	Lesion length (mm)		
	Winter inoculation	<i>P. brutia</i> Spring inoculation	<i>P. nigra</i> Spring inoculation
<i>S. sapinea</i>	47,6 a*	96,9 a	44,5 a
<i>T. hartigii</i>	9,2 b	17,1 b	7,4 b
Control	5,4 b	5,8 b	7,0 b
Means	B	A A	B

* Means in the same column followed by the same lowercase letter and means in the same row shown by the same uppercase letter were not significantly different from each other according to Duncan's Multiple range test (P=0.05)

Spring inoculations with *S. sapinea* caused bigger lesions than winter inoculations on *P. brutia*. It can be due to higher temperatures, causing rapid fungal growth and predisposing the trees. Between two host species, *P. brutia* seems more susceptible against both fungi than *P. nigra*. But climatical conditions of two sites may be the main factor causing the significant difference between lesion lengths on the trees. *P. nigra* plantation area has lower temperature and humidity than Aşağı Gökdere. Extensive pycnidia formation was observed under the barks of *S. sapinea* inoculated shoots.



Figure 1. Lesion inoculated with *S. sapinea* (right hand) and control treatment (left)

In conclusion, *S. sapinea* was found to be the main agent of the shoot blight disease of Calabrian pines in the Mediterranean region of Turkey. This is the first report of the fungus for this part of our country. Further research is needed on the biology, host range and economical importance of the pathogen.

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