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Research article

First records of *Sirococcus conigenus* causing shoot blight on *Pinus peuce* in Bulgaria

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Abstract: Macedonian pine (*Pinus peuce*) is a southern Balkan Peninsula endemic, growing in high mountains of Bulgaria, between 1400 and 2100 m a.s.l. Stands of *P. peuce* form the upper tree line forest areas. During a survey in 2020–2022 on Macedonian pine natural stands and plantations in Bulgaria, symptoms of shoot blight were observed in the Pirin Mts, the Rila Mts and Mt Vitosha. The fungal pathogen *Sirococcus conigenus* was identified as the causal agent of the disease that appeared for the first time on *Pinus peuce* in Bulgaria and Balkan Peninsula. Incidence of blighted shoots on individual trees varied, but was as high as 70–80% in the Rila Mts and Mt Vitosha.

Keywords: Balkan Peninsula, Bulgaria, Macedonian pine, shoot blight, *Sirococcus conigenus*

Introduction

Macedonian pine (*Pinus peuce* Griseb.) is endemic to the southern Balkan Peninsula, growing in high mountains of Albania, Bosnia and Herzegovina, Bulgaria, Greece, Kosovo, Montenegro, North Macedonia and Serbia. In Bulgaria the species is distributed in the Rila Mts, the Pirin Mts, the Western Rhodopes, the Central Balkan Range, Mt Slavyanka and Mt Vitosha, between 1400 and 2100 m a.s.l. Stands of *Pinus peuce* form the tree line forest areas in the mountains, where play an important ecological role for the stability of the forest ecosystems. Its restricted range, coupled with the effects of limited exploitation and its potential susceptibility to climate change mean that it is currently assessed as 'near threatened' (Alexandrov & Andonovski, 2011; Thomas, 2019).

In the temperate zone, the main fungal pathogens causing damage to forest tree crowns are *Gremmeniella abietina* (Lagerb.) M. Morelet, *Diplodia sapinea* (Fr.) Fuckel, *Sirococcus conigenus* (Pers.) P.F. Cannon & Minter (Capretti et al., 2013), *Dothistroma septosporum* (Doroguine) Morelet (Drenkhan et al., 2016) and *Lecanosticta acicola* (Thümen) Syd. (Tubby et al., 2023). These pathogens are among the best known agents of serious epidemics on pine species (*Pinus* spp.) (Tubby et al., 2023).

Sirococcus Preuss is a genus of asexually reproducing fungi that includes important pathogens causing shoot blight and tip dieback of conifers. *Sirococcus conigenus* is an anamorphic fungus responsible for shoot tip blight on seedlings, saplings and mature trees of several species of conifers in the temperate and boreal forests of the northern hemisphere (CABI, 2023). Subsequently, the disease was reported on a wide range of conifer hosts from genera *Picea, Pinus, Larix, Tsuga, Pseudotsuga* in Europe and North America (Peace, 1962; Sutherland et al., 1987; Butin, 1995; Smith et al., 2003; Sinclair & Lyon, 2005; Dobreva et al., 2017), and on *Picea*

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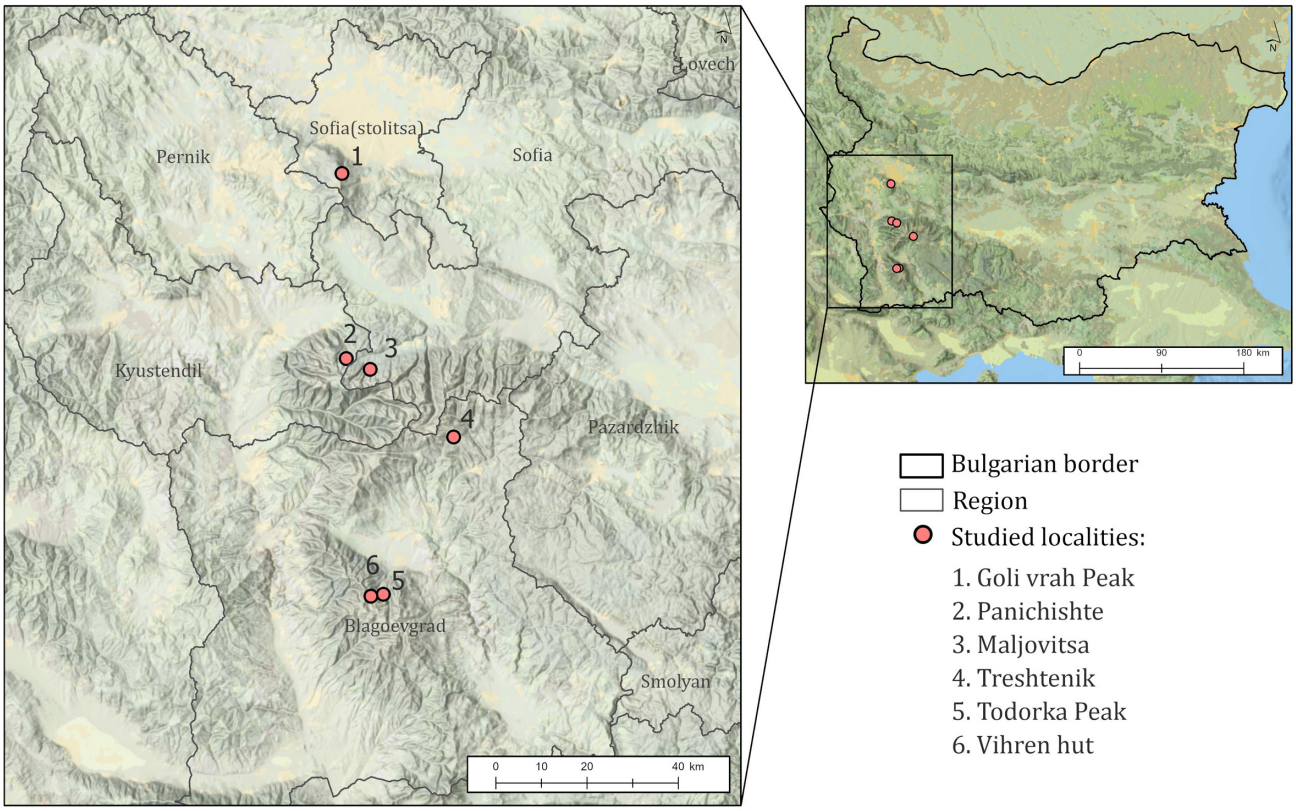

Fig. 1. Map of the studied sample plots.

spinulosa (Griff.) Henry in Bhutan, South Asia (Kirisits et al., 2007).

Sirococcus shoot blight was reported mostly from temperate and alpine forests, but also from the Mediterranean basin on Aleppo pine (*Pinus halepensis* Mill.) in Spain (Muñoz-Lopez, 1997), Italy (Danti & Capretti, 1998), and in Morocco, northern Africa (Morelet, 1972). In Bulgaria, damage on Norway spruce, *Picea abies* (L.) Karst. seedlings infected by *S. conigenus,* were reported for first time in 2016 in a forest nursery in Yundola Village (Southwest Bulgaria) (Dobreva et al., 2017).

The present note reports *Sirococcus conigenus* as a new fungal pathogen on *Pinus peuce,* causing shoot blight, which appeared for the first time in Bulgaria on this host species.

Materials and methods

In 2020, six permanent sample plots (three in natural stands in Rila, two in natural stands in Pirin and one plantation in Mt Vitosha), were selected to study the structural-functional characteristics and health status of Macedonian pine (*P. peuce*) (Fig. 1; Table 1).

In the period 2021–2022, symptoms of shoot blight were observed on Macedonian pine trees in all sample plots. The extent of the crown that was affected was estimated once a year during the period July – August for forty trees in each sample plots and recorded as follows: none (1–10%), slight (11–25%), moderate (26–60%), severe (61–99%), dead (100%) (Eichhorn et al., 2006, 2020). Records of damage caused by abiotic and biotic factors were also conducted.

Samples (symptomatic shoots and cones) were collected and transferred to Forest Research Institute, Sofia. Symptomatic needles and cone scales were surface washed with tap water for 5 min, surface sterilised for 1 min in 96% ethanol, subsequently rinsed once in sterile distilled water. Sterilised parts were placed in Petri dishes on moistened filter paper and incubated for 4–5 days at 22±2°C under artificial light. Isolations of fungal pathogen were made by placing pycnidia onto malt extract agar Difco (MEA: 20 g malt extract; 16 g agar-agar; 1000 ml tap water),

Table 1. Main characteristics of studied Macedonian pine stands.

N	Sample plots	Mountains	Origin	Latitude	Longitude	Altitude, m	Age, years
1	Goli Vrah	Vitosha	plantation	42.591972	23.292611	1814	90
2	Panichishte	Rila	natural stand	42.228167	23.325194	1948	150
3	Maljovitsa	Rila	natural stand	42.208972	23.390028	1760	110
4	Treshtenik	Rila	natural stand	42.082167	23.618028	1915	100
5	Todorka Peak	Pirin	natural stand	41.767306	23.449167	1993	150
6	Vihren Hut	Pirin	natural stand	41.761917	23.416944	1969	150

Table 2. Assessment of the degree of affected tree crowns in studied Macedonian pine stands in 2021 and 2022.

N	Sample plots	Mountains	Damaged part of crowns, %			Defoliation class (by Eichhorn et al., 2006)
			Average values		Range	
			2021	2022		
1	Goli Vrah	Vitosha	37.7	41.3	20–70	slight to severe
2	Panichishte	Rila	43.5	47.5	30–70	moderate to severe
3	Maljovitsa	Rila	53.8	48.0	30–80	moderate to severe
4	Treshtenik	Rila	23.8	26.3	10–40	none to moderate
5	Todorka Peak	Pirin	38.8	33.7	20–60	slight to moderate
6	Vihren Hut	Pirin	17.5	14.5	10–30	none to moderate

supplemented after autoclaving with 100 mg streptomycin sulphate to suppress bacterial growth. The shape and size of pycnidia were measured at magnification 40× using an ocular micrometer of Zeiss and conidia at 125× using Carl Zeiss NU2 light microscopes equipped with a digital camera DinoEye AM-423X. The identification of size and shape of conidia was made according to Butin (1995) and (Rossmann et al., 2008).

Results and discussion

During a survey on the structural and functional characteristics, and perspectives for diverse use of endemic relict coniferous forest communities in Bulgaria, symptoms and signs of the disease resembled those described for Sirococcus shoot blight were noticed on Macedonian pine (*Pinus peuce*). In the period 2021–2022, the health status of six stands of *Pinus peuce* was assessed in the Pirin Mts, the Rila Mts and Mt Vitosha. Records of damage caused by abiotic and biotic factors were conducted. Abiotic damage by strong wind, wet snow and ice-break were registered in studied stands. In the springs of 2021 and 2022, symptoms of wilting and dieback of the current year shoots were noticed in all studied stands.

In 2021, the tree crowns in different sample plots were affected by Sirococcus shoot blight on average between 17.5% and 53.8%, and in 2022 – between 14.5% and 48.0% (Table 2). Incidence of blighted shoots on individual trees varied widely, but was as high as 70–80% in Panichishte and Maljovitsa (Rila Mts), and in Goli Vrah (Mt Vitosha). In Treshtenik (Rila Mts) and Vihren Hut (Pirin Mts) none to moderate damaged crowns were observed.

Infections on shoots caused characteristics droop downward of fully-expanded needles (Fig. 2A).

Fig. 2. *Sirococcus conigenus* on *Pinus peuce:* A – typical symptoms of shoot blight disease; B – pycnidia on cone scales; C – pycnidia on needles; D – conidia.

Pycnidia (0.3–1.0 mm) long abundantly at the base of dead needles and cone scales (Fig. 2B, C), dark brown at maturity. Under humid conditions conidia oozed from pycnidia to form spore horns. Conidia with fusiform shape, one-septa, often slightly curved, 11.0–14.4 (–12.7) × 2.5–2.9 (–2.7) µm (Fig. 2D).

Based on morphological characteristics of the symptoms and reproductive structures, the pathogen *Sirococcus conigenus* (Ascomycota, Diaporthales, Gnomoniaceae) was observed as the causal agent of the disease. The current identification of this pathogen on Macedonian pine trees is the first report for Bulgaria and the Balkan Peninsula. In 2016, damage of *S. conigenus* were identified on Norway spruce (*Picea abies*) seedlings in a nursery in Yundola Village, Southwest Bulgaria (Dobreva et al., 2017).

In the present study, the pathogen was established in stands in high altitude between 1760 m and 1993 m a.s.l. The fungus was encountered on cone scales and needles of infected trees in all studied sample plots. The large numbers of spores produced on cones can contribute to infection and branch mortality of older trees or infection of seedlings and young trees growing near the older infected trees (Rossman et al., 2008). The fungus particularly affects stands in cold, humid and low light conditions in early spring (Butin, 1995). Infection in nurseries is often related to the physiological stress of seedlings in early spring when significant evapotranspiration occurs and they cannot be compensated by the absorption of water by roots.

In recent years, damage caused by the fungal pathogens were established on *Pinus peuce* trees in

the Rila Mts and Mt Vitosha (Georgieva & Marković, 2018; Mirchev et al., 2021). Among the biotic factors, the main damage was caused by fungal pathogens *Heterobasidion annosum* (Fr.) Bref., *Diplodia sapinea, Cenangium ferruginosum* Fr., *Cytospora pinastri* Fr. and *Lophodermium conigenum* (Brunaud) Hilitzer. and bark beetles *Ips sexdentatus* (Börner), *I. amitinus* (Eichhoff).

In conclusion, the fungal pathogen *Sirococus conigenus* was reported for the first time on *Pinus peuce* in Bulgaria and the Balkan Peninsula as whole. Sirococcus shoot blight appeared to cause severe damage on Macedonian pine stands in high mountains in Bulgaria. The fungus has the potential to cause intense infections on other conifer species not only in nurseries and young plantations, but also in mature stands and ornamental trees in urban areas (Halmschlager et al., 2000).

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