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RESEARCH ARTICLE

Spread of the invasive pathogen *Lecanosticta acicola* on species of *Pinus* in Bulgaria

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Abstract

The brown spot needle blight, caused by the fungal pathogen *Lecanosticta acicola*, has been the most serious and damaging disease on needles of *Pinus* spp. in recent years. In Bulgaria, the pathogen was reported for the first time in 2017 in a generative plantation of *Pinus sylvestris* in the region of the State Forestry Ardino, the Eastern Rhodopes. The newly- established invasive pathogen is considered highly adaptable to new hosts and environmental conditions. The life cycle and symptoms of the disease strongly suggest that the new emerging pathogen has the potential to cause severe damages and is a serious threat to naturally distributed species of *Pinus* in the country. In the period 2018-2019, a spread of *L. acicola* from the initial outbreak was established throughout stands of *P. sylvestris* and *P. nigra* on the territory of Kardzhali District.

Keywords

Brown spot needle blight disease, *Pinus* spp., Bulgaria

Introduction

The brown spot needle blight disease (BSNB) caused by the fungal pathogen *Lecanosticta acicola* (Thümen) Sydow (teleomorph *Mycosphaerella dearnessii* Barr.) was established for the first time in Bulgaria in August 2017 in a 50-year-old generative plantation of *Pinus sylvestris* L. in the region of Svetulka Village, State Forestry (SF) Ardino, Kardzhali District (Stamenova et al., 2018). The causative agent *L. acicola*

was officially confirmed by the Plant Health Laboratory for the French Agency for Food, Environmental and Occupational Health & Safety (ANSES). The pathogen is currently listed as a quarantine by the European Plant Protection Organisation (EPPO) (Pehl et al., 2015), and is considered an invasive species, highly adaptable to new susceptible hosts at favourable environmental conditions.

In order to prevent further spread of the pathogen, phytosanitary measures in the infected generative plantation were taken in 2018, aiming to eradicate the disease. All infected pine trees were cut down and removed from the site where the pathogen was localised. Despite the taken control measures, in November 2019, severe damages by BSNB was observed on newly- infected pine stands on the territory of SF Ardino.

The aim of this study was to present data for the spread of *L. acicola* causing brown needle blight disease across forest stands of *P. nigra* and *P. sylvestris* in the Kardzhali District, South Bulgaria.

Material and methods

During the period 2017-2019, samples of needles with symptoms of BSNB were collected from the generative plantation and nine locations in the region of SF Ardino at a distance of 25 km from the first outbreak of the disease (Fig. 1). Symptomatic

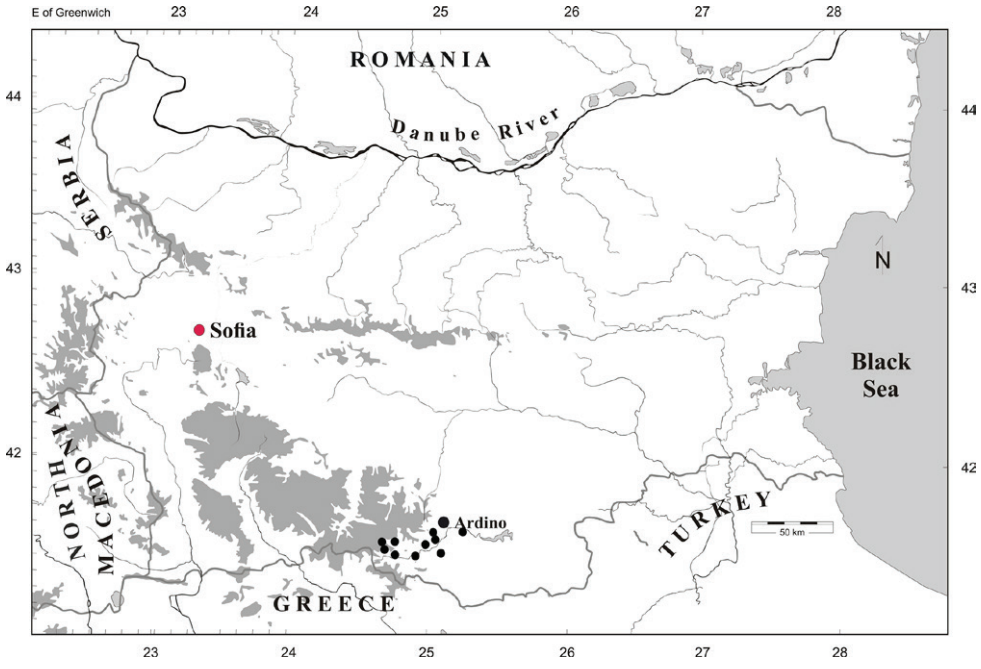

Figure 1. Locations of sites for sample collections

Table 1. Characteristics of the studied locations

N	Location	Longitude	Latitude	Altitude	Host species	Type of analysis
1	Svetulka	41.559766	25.088129	639	*Pinus sylvestris* *Pinus nigra*	PCR (Ioos et al., 2010) Morphological (Barnes et al., 2004)
2	Padina	41.555148	25.084476	603	*Pinus sylvestris*	PCR (Ioos et al., 2010)
3	Byal izvor	41.542411	25.084476	661	*Pinus nigra*	PCR (Ioos et al., 2010)
4	Srunsko	41.548981	25.106127	803	*Pinus nigra*	PCR (Ioos et al., 2010)
5	Podvis	41.567231	24.825938	773	*Pinus sylvestris*	Morphological (Barnes et al., 2004)
6	Rovina	41.564514	24.855476	752	*Pinus sylvestris*	Morphological (Barnes et al., 2004)
7	Svetulka	41.559692	25.089948	623	*Pinus sylvestris*	Morphological (Barnes et al., 2004)
8	Varbinski most	41.545231	24.976153	615	*Pinus nigra*	Morphological (Barnes et al., 2004)
9	Chubrika	41.584444	25.206111	593	*Pinus nigra*	Morphological (Barnes et al., 2004)
10	Hromitsa	41.619035	25.222807	558	*Pinus nigra*	Morphological (Barnes et al., 2004)

needles were taken from both *P. nigra* and *P. sylvestris* trees at sites at altitudes between 558 and 803 m a.s.l. (Table 1).

In 2017, symptomatic needles of BSNB were collected during the annual monitoring for quarantine pests on forest species, conducted by experts of the phytosanitary control. They were sent to the Plant Health Laboratory of the French Agency for Food, Environmental and Occupational Health & Safety (ANSES), where the causal agent *L. acicola* was confirmed. In April 2018, samples of infected needles were collected from four sites at a distance of up to 20 km from the generative plantation. Then, they were sent to the Central Laboratory of Plant Quarantine in Sofia, where a molecular analysis was done using species-specific PCR primers according to Ioos et al. (2010).

A part of the samples was transferred to the Laboratory of Phytopathology of the Forest Research Institute, Sofia, for examination of pathogen infections. Needles were sterilised in 96% ethanol for 10 s, then rinsed in sterile water. After surface sterilisation, needles were cut into segments, 4-6 mm long, under sterile conditions and then placed in Petri dishes. Conidiomata formed on the needles were aseptically excised, rolled onto 2% malt extract agar medium (15 g agar BD Difco™ per litre of distilled water with 100 mg/L streptomycin), in order to release conidia from the conidiomata as described by Barnes et al. (2004). The isolated conidiomata were incubated for one to two days at 23 °C. The plates were examined using a dissection microscope Zeiss NU2. The single conidial isolates were grown for 1-3 weeks in a natural day light cycle, at 22-23 °C.

In November 2019, a survey was carried out at seven new locations at a distance of 25 km along the Arda River in the west direction of the initial site in Svetulka Village and 25 km – in the north-east direction), to explore the rate of disease spreading from the initial outbreak. Samples of symptomatic needles were collected and tested for the presence of *L. acicola* according to Barnes et al. (2004).

Results

Symptoms of extensive brown spot necrosis on needles was recorded for first time in 2017 on *P. sylvestris* trees in the generative plantation close to the Svetulka Village (SF Ardino, the Eastern Rhodopes). Based upon the molecular analysis, the fungal pathogen *L. acicola* was identified as a causer agent of brown spot needle blight.

During the next spring (April 2018), the study on the spread of *L. acicola* in the generative plantation showed severe damages (between 40% and 80%) on infected trees. It was established that the disease developed upward and infected the whole crowns. Symptomatic needles were confined to the two- and three-year old internodes. The needles had necrotic spots with brown tissue and yellow periphery. On the spots, subepidermal pycnidia formed resin-black bodies with irregular shape. Pycnidia breaking through the epidermis through longitudinal slits were observed. Conidia were two- to four-celled, curved, pale olive-brown and 16-34 × 2-4 μm. The fungus was isolated in pure culture on malt agar, firstly a white aerial mycelium appeared which then turned greenish-olive to dark olive, forming stromatic and erumpent colonies. The molecular detection of *L. acicola* on trees of *P. sylvestris* were confirmed in the Svetulka and Padina Villages (Table 2).

Two pathogens with close symptomatic characteristics were molecularly identified on the same trees of *P. nigra* in the region of Byal Izvor: *L. acicola* causing BSNB and *Dothistroma septosporum* (Dorog.) Morelet causing *Dothistroma* needle blight (DNB) (Table 2). From the highest location (803 m a.s.l.) near the Srunsko Village (approximately 20 km from the initial outbreak), only *D. septosporum* was identified on trees of *P. nigra*. There the severity of DNB ranged from 20-30%.

In 2019, a spread of severe occurrence of BSNB infection, caused by *L. acicola* on trees of both *P. sylvestris* and *P. nigra* was established at a distance of 25 km (Pod-

Table. 2. Identified pathogens on species of *Pinus* on the territory of SF Ardino

N	Location	Host species	Identified pathogens	Degree of defoliation
1	Svetulka (2018)	*Pinus sylvestris* *Pinus nigra*	*Lecanosticta acicola* *Dothistroma septosporum*	40-80% 20-30%
2	Padina (2018)	*Pinus sylvestris*	*Lecanosticta acicola*	30-50%
3	Byal izvor (2018)	*Pinus nigra*	*Lecanosticta acicola* *Dothistroma septosporum*	30-60% 30-60%
4	Srunsko (2018)	*Pinus nigra*	*Dothistroma septosporum*	20-30%
5	Podvis (2019)	*Pinus sylvestris*	*Lecanosticta acicola*	50-100%
6	Rovina (2019)	*Pinus sylvestris*	*Lecanosticta acicola*	50-100%
7	Svetulka (2019)	*Pinus sylvestris*	*Lecanosticta acicola*	50-60%
8	Varbinski most (2019)	*Pinus nigra*	*Lecanosticta acicola*	30-50%
9	Chubrika (2019)	*Pinus nigra*	*Lecanosticta acicola*	25-30%
10	Hromitsa (2019)	*Pinus nigra*	*Diplodia sapinea*	20-25%

vis Village), in west direction from the Svetulka Village. A progressive browning of the needles from the lowest branches upwards was observed more severe on trees of *P. sylvestris* where defoliation of two- and three-year-old needles was detected. The degree of defoliation varied between 50-100% (Table 2). The infections resulted in the death of small branches, in addition to the needles, and the most severely affected trees of *P. sylvestris* were killed by the pathogen. On trees of *P. nigra*, the oldest needles close to the stem were infected by the disease, spreading upwards along the branches.

In the north-eastern direction from the initial outbreak of *L. acicola*, the disease was also detected but the rate of its pathogenicity was lower (defoliation up to 30%). At a distance of 10 km, the symptoms of BSNB was not observed, only damages on shoots caused by the pathogen *Diplodia sapinea* (Fr.) Fuckel were recorded.

Discussion

The brown spot needle blight disease caused by the fungal pathogen *L. acicola* has been the most serious and damaging needle diseases of *Pinus* spp. in recent years. It is the only species in the genus known to be a significant pathogen that is spreading rapidly in Europe and in the north-eastern parts of North America (van der Nest et al., 2019). In Bulgaria, the pathogen was reported for the first time in 2017 in a generative plantation of *P. sylvestris* in the region of SF Ardino, the Eastern Rhodopes (Stamenova et al., 2018). In 1938, a short report of brown-spot fungus *L. acicola* (as *Septoria acicola*) was published by Kovacevski (1938), but based on the description of the conidia, it was most likely that it was a species of genus *Dothistroma* (Mullett et al., 2018).

This study presents the new results on the spread of *L. acicola* at a distance of 25 km from the initial outbreak of the disease in the region of SF Ardino, Kardzhali District. The main component that facilitates spread of conidia is moisture, but other factors may also aid in their dispersal (van der Nest et al., 2019).

During the last years, introduced pathogens have turned into a growing threat for the natural pine species, disturbing the biodiversity and ecological dynamics in forest and urban ecosystems (Georgiev et al., 2017; Georgieva, Hlebarska, 2018). *Dothistroma septosporum* was confirmed for the first time as the causal agent of *Dothistroma* needle blight disease on trees of *Pinus sylvestris* and *P. nigra* planted in the region of Panichkovo Village, Kardzhali District (Mullet et al., 2018). In the same location, *D. septosporum* was recorded also on trees of *Pinus contorta* Douglas (Georgieva, unpubl.). In 2018, the new pathogen *L. acicola* was identified to cause severe damages on generative plantation of *P. sylvestris*, situated in the same district in the Eastern Rhodopes (Stamenova et al., 2018). The teleomorh of two newly-established invasive pathogens are included in the EPPO quarantine lists (EPPO, 2005, 2008) and considered highly adaptable to new hosts.

Lecanosticta acicola is widespread and has been causing serious losses in China, Eastern United States, Central and South America. In Europe, *L. acicola* has been observed for the first time in 1940s (Janoušek et al., 2016). The pathogen was detected as a major cause of needle blight on several native European pine species, especially *P. sylvestris*, *P. nigra* and *P. mugo* (La Porta, 2000). Currently, *L. acicola* has been reported in 31 countries and on 53 pine species and pine hybrids (van der Nest et al., 2019).

The distribution and population size of BSNB disease vectors is heavily affected by local climate. Because the spread of the pathogen facilitated by movement of free-living hosts or vectors, the risk of spread of *L. acicola*, when accounting for heterogeneous landscape features, could be estimated. The high pathogenicity of the diseases strongly suggests that these newly emerging in Bulgaria pathogens have the potential to cause severe damages and are a serious threat to species of *Pinus* throughout the country.

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