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RESEARCH ARTICLE

First report for Massaria disease on plane trees in Bulgaria

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Abstract

Massaria disease caused by the fungal pathogen *Splanchnonema platani* (anamorph *Macrodiplodiopsis dermazieresii*) was found for the first time in the winter of 2023 on fallen branches of plane trees (*Platanus* × *acerifolia*) in park 'Rupite', southwestern Bulgaria. In 2023-2024, the symptoms of the disease were found on the fallen branches of ornamental trees in green city areas of Yambol, Gabrovo, Stara Zagora and Sofia. The life cycle of the Massaria disease was studied in the period June, 2023 – June, 2024 on plane trees planted in the Arboretum of Forest Research Institute in Sofia. Conidia of the anamorph state *Macrodiplodiopsis dermazieresii* were observed during the entire year, while the ascospores of the telemorph state *Splanchnonema platani* was reported in February to April 2023-2024. This study was the first report of Massaria disease on *Platanus* × *acerifolia* in Bulgaria.

Key words

Massaria disease, *Platanus* × *acerifolia,* Bulgaria

Introduction

Massaria is a fungal disease that infects branches of plane trees. The infected branches, weakened by the disease, break off from the stem or from the main branches of the tree (Kehr, Krauthausen, 2004). The ascomycete *Splanchnonema platani* (Ces.) Barr (Dothideomycetes, Pleosporales) (syn. *Massaria platani* Ces.) was the causal agent of the disease. The fungus is known as a common weak parasite of plane trees growing in the Mediterranean countries and in the North America (Nalli, 1981; Ciccarone,

1988; Grosclaude, Romiti, 1991; Schmitt et al., 2014). The disease is a bark inhabiting organism of dead branches (Sutton, 1980; Sinclair, Lyon, 2005). Lesions are developed and expanded on the upper part of the branches, which is difficult to be noticed from the ground. As the affected plane trees are widely used as an urban species in parks and public gardens in Bulgaria, the activity of the pathogen has resulted in a risk to human and animal safety.

The Massaria disease was first observed in the Mediterranean countries, but in the mid 1990s *S. platani* presumably spread to southern Germany (Kehr, Krauthausen, 2004). By 2005 the disease had spread to entire central Europe (Dujesiefken, Kehr, 2008). In 2023 the anamorph state of the disease *Macrodiplodiopsis dermazieresii* (Mont.) Petr. was observed for the first time on fallen branches of *Platanus* × *acerifolia* trees planted in park 'Rupite', Southwest Bulgaria.

The aim of this research was to report the presence of Massaria disease in Bulgaria and to study its life cycle.

Materials and methods

In the period 2023-2024, *Platanus* × *acerifolia* trees, aged between 20-70 years, were surveyed for health status assessment in six localities in Bulgaria (Table 1).

Table 1. Characteristics of studied plots of *Platanus* × *acerifolia*

Sample plot	Locality	Date of sample collection	Age, years	Number of trees	Longitude, °E	Latitude, °N	Altitude, m
1	Rupite (Petrich)	20.01.2023	20-30	5	41.459024	23.263304	114
2	Gabrovo	25.04.2023	60-70	3	42.871370	25.316522	395
3	Sofia (Arboretum of FRI)	05.05.2023	60-70	6	42.631641	23.354475	634
4	Sofia (Zoo garden)	16.07.2023	20-30	8	42.660002	23.333338	600
5	Yambol	30.07.2023	60-70	7	42.483700	26.503900	135
6	Stara Zagora	01.04.2024	60-70	10	42.431588	25.622794	263

Fallen branches with symptoms of Massaria disease were taken from the studied localities. They were observed in the Laboratory of phytopathology at the Forest Research Institute in Sofia. The branches were washed under running water to eliminate saprophytic and epiphytic microflora for 30 minutes (Stancheva, 2004) and placed in a humid chamber on sterile filter paper for 7 days at an air temperature of 22±2°C to observe the appearance of fruit bodies and to record the sporulation.

The identification of the causative agent of Massaria disease was carried out according to the morphological characteristics of the reproductive structures of the fungal pathogen (fruiting bodies and spores) using macroscopic and microscopic observations. Fruiting bodies were observed under a Zeiss Stemi 305 binocular, and

conidia and ascospores under an Optica B810 microscope. Images of the reproductive structures were taken using Dino Light and Opticam PR08 Digital Camera. Variations in the dimensions of length and width (min – max) were observed on 100 pcs. conidia and ascospores. Morphological characteristics of the reproductive structures were compared with those reported in the literature sources of Kehr, Krauthausen (1982) and Shear, Davidson (1936).

The life cycle of the disease was observed in a one-year period (June, 2023 - June, 2024) on two plane trees, planted in the Arboretum of Forest Research Institute in Sofia. Spore traps (microscope slides coated with Vaseline®) were attempted to determine the presence of wind dispersed ascospores and conidia.

Results

Fallen branches of *Platanus* × *acerifolia* induced the symptoms of Massaria disease were observed for the first time in January, 2023 in park 'Rupite' (Petrich, southwestern Bulgaria). An assessment of the health status of 40 symptomatic plane trees in six sample plots was assessed from April to September (Table 2). Symptoms of the disease were observed mainly in the middle and lower part of the crown, and less often in the upper part. The diameter of the studied fallen branches were 3-6 cm. Defoliation of trees varied between 20-50% (average 30%). The highest percentage of damage was reported in the Zoo garden in Sofia, where defoliation of crowns was up to 50%. The percentage of symptomatically dead branches varied from 10% to 40%.

Table 2. Table with characteristics of studied plots of *Platanus* × *acerifolia*

Sample plot	Locality	Percentage of defoliation (min-max)	Percentage of dead branches in the crown (min-max)
1	Rupite (Petrich)	20-30	10-30
2	Stara Zagora	20-30	10-30
3	Gabrovo	10-20	10-20
4	Sofia (Arboretum of FRI)	30-40	10-30
5	Sofia (Zoo garden)	40-50	10-40
6	Yambol	30-40	10-30

Massaria disease symptoms included orange-coloured strips and lesions on the upper surface of the branch. Lesions were associated with wood decay, characterised by soft rot. Under the epidermis, black fruiting bodies were observed, in groups or individually (Fig. 1A). The disease symptoms on the cross section of branches showed brown discoloration with stage of decay in the xylem (Fig. 1B). After placement in a humid chamber, fruiting bodies (pycnidia with diameter 0.5-1.0 mm) of the anamorph state of the disease were found (Fig. 1C). Brown, 4 – celled conidia, 35.5-46.8(40.3) × 13.2-18.5(15.4) µm in size were observed under microscopic analysis (Fig. 1E). On the basis of conidia morphology (shape, dimension and number of

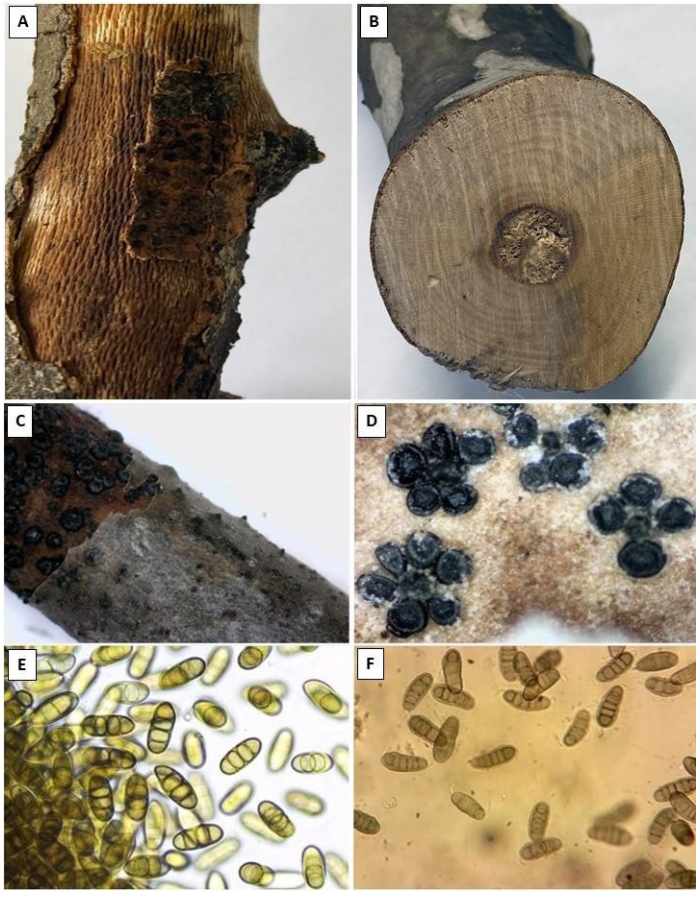

Fig. 1. Symptoms of Massaria disease: A – Branches of *Platanus* × *acerifolia* with fruiting bodies, B – cross sections with decay pattern; C – Pycnidia of *Macrodiplodiopsis dermazieresii***;** B –Peritecia of *Splanchnonema platani*; E – Conidia of *M. dermazieresii*; D – Ascospores of *S. platani.*

septa), the fungus *Macrodiplodiopsis dermazieresii* was identified. The fruiting bodies (apothecia) of its teleomorph stage (*Splanchnonema platani*) were found under the bark (Fig. 1D). Peritecia (0.5-0.8 mm in diameter) were dark coloured and contain asci measured 109-184(146.8) × 33.2-35.0(34.2) μm, contained eight 6-celled brown ascospores, 45.2-68.5(56.2) × 14.5-17.0(15.8) μm (Fig. 1F).

The life cycle of the Massaria disease was studied in the period June, 2023 – June, 2024 on the spore traps installed in the Arboretum of Forest Research Institute in Sofia. For a one-year period both reproductive states of the disease were detected on the spore traps (Fig. 2). Conidia dissemination was noticed during the entire year with a peak in April-May, while the ascospores of the teleomorph state was reported only in the period February-April.

Discussion

In Bulgaria, plane trees (*Platanus* × *acerifolia*) are widely used for landscaping in urban green areas as ornamental trees. Fungal pathogens *Apiognomonia platani* (Sacc. & Speg.) Hohn, *Erysiphe platani* (Howe), *Phytophthora* sp., *Armillaria mellea* (Vahl) Kumm, *Cytospora platani* Fuckel, and insects pests *Corythuca ciliata* Say and *Phyllonorycter platani* (Staudinger), are the major causal agents of damage on the plane trees in Sofia parks and urban streets (Georgieva et al., 2023).

In 2023, Massaria disease was detected for the first time in southwestern Bulgaria. In the following year, the disease was found in other localities mainly in Southern and Central Bulgaria. The causal agent *Splanchnonema platani* (Dothideomycetes, Pleosporales) is widespread in North America (Share, Davidson, 1936), Germany (Kehr, 2007, 2011; Kehr, Krauthausen, 2004), Italy (Wijayawardene et al., 2014), Austria (Kessler, Cech, 2008), The Netherlands (Kehr, Groot, 2008), Britain (Tubby, Perez-Sierra, 2015), etc. With our surveys, it was found that the fruiting bodies of the anamorph state (pycnidia) appeared in January, and then – in February – the peritecia of the teleomorph state. The perithecia that appeared later were larger than the pycnidia and contained asci with ascospores. Drying was observed in the upper part of the branches, which made it difficult to diagnose from the ground. These results were also confirmed by other authors as Cech et al. (2007) and Kehr (2004; 2008).

According to Kehr, Krauthausen (2004), the warmer weather affects the development of Massaria disease. In the summer months, the temperature on the top of the branches is higher and the water supply to the tissues is lower. In recent years, the Massaria disease has become increasingly important for the urban plane trees (Börker, 2019). The causal agent *Splanchnonema platani* is a weak parasite that initially participates in the death of smaller, physiologically weakened lateral branches (Kehr, Krauthausen, 2004), but damage caused by the pathogen has been found more recently in living large trees in urban areas in Germany (Kehr, 2011) and Britain (Tubby, Pérez-Sierra, 2015).

In recent decades, a clear trend of increasing the frequency of heat waves and lack of rain and snow precipitation has been observed in Bulgaria (Marinova, Bocheva, 2023). Changes in the frequency and intensity of the rainfall have impacted the amount and rate of soil water storage. Most probably, the lack of precipitation during the last growing seasons has led to humidity deficit, physiological weakening of trees and a rise in the number of infected by Massaria disease trees in Southern Bulgaria.

Conclusion

This study reports the occurrence of Massaria disease on *Platanus* × *acerifolia* for the first time in Bulgaria. The fungal pathogen *Splanchnonema platani* was found for the first time in the winter of 2023 on fallen branches of plane trees in 'Rupite' park, southwestern Bulgaria. Additionally, the symptoms of the disease were found on the

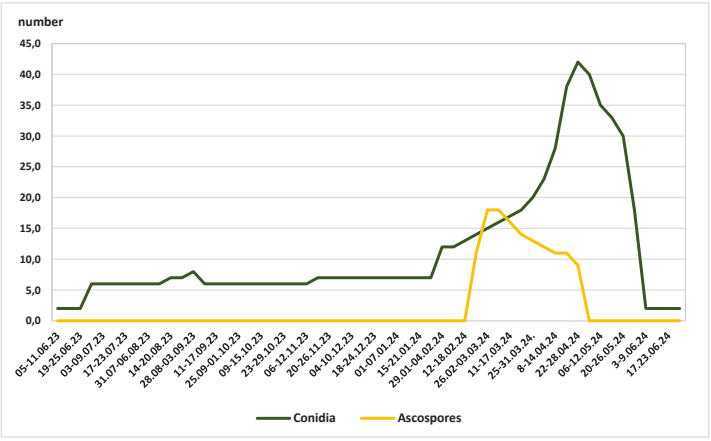

Fig. 2. Life cycle of Massaria disease and dissemination of the conidia of *Macrodiplodiopsis dermazieresii* and ascospores of *Splanchnonema platani* on *Platanus* × *acerifolia* trees planted in the Arboretum of Forest Research Institute in Sofia.

ornamental trees in green city areas of Yambol, Gabrovo, Stara Zagora and Sofia. Most probably, the lack of precipitation during the growing seasons led to physiological weakening of trees and a rise in the number of infected by Massaria disease trees in Southern and Central Bulgaria.

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