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New or Unusual Disease Reports

First report of *Erysiphe elevata* causing powdery mildew on *Catalpa bignonioides* in Montenegro

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Summary. The first record of powdery mildew caused by *Erysiphe elevata* in Montenegro is presented in this paper. The fungus was detected on leaves, fruits and flowers of *Catalpa bignonioides,* causing severe disease of some trees, and was identified on the basis of morphological and molecular characteristics. In pathogenicity tests, inoculation of leaves of healthy young plants of southern catalpa resulted in typical powdery mildew symptoms.

Keywords. Catalpa trees, invasive fungal pathogen, morphological and molecular characterisation.

INTRODUCTION

Catalpa bignonioides is a tree native to the south-eastern United States. It is known as southern catalpa (Olsen *et al*., 2006). These trees are planted as ornamentals in all temperate areas of the world, especially eastern North America and Europe (Olsen and Kirkbride Jr, 2017). The species is an important decorative plant providing urban greenery in central and southern parts of Montenegro. The trees are usually located along footpaths, in parks or allées.

Powdery mildews are common plant diseases, but some of these pathogens have become invasive due to their introduction to, and spread throughout, new territories. This is the case with *Erysiphe elevata* (syn. *Microsphaera elevata* Burrill), a well-known species in North America (Braun, 1987) that appeared recently in Europe and caused severe infections on *Catalpa bignonioides* trees in some European countries (Ale-Agha *et al*., 2004; Kiss, 2005) and Asia (Cho *et al*., 2014). The first report of the pathogen on *Catalpa* trees in Europe was from Hungary in 2002 (Vajna *et al*., 2004). Since then there have been several reports from different European countries: including the

United Kingdom, Slovakia, Slovenia, Romania, the Czech Republic, Germany, Switzerland, Ukraine and Turkey (Ale-Agha *et al.*, 2004; Cook *et al.*, 2004; Milevoj, 2004; Pastirčakova *et al.*, 2006; Heluta *et al.*, 2009; Fodor and Vlad, 2013; Erper *et al.*, 2018).

During 2016 to 2018 in central and southern parts of Montenegro, powdery mildew symptoms were observed on numerous *Catalpa bignonioides* plants in parks, along footpaths and also in a nursery. The aim of the present study was to identify the causal agent of the disease.

MATERIALS AND METHODS

Morphology and pathogenicity tests

In the period of 2016 to 2018, flowers, leaves and fruits of *Catalpa bignonioides* plants were collected from two localities in Podgorica (city in central Montenegro), where powdery mildew symptoms were observed. Samples were examined using a microscope (Axioskop 2 Plus, Zeiss) equipped with a Zeiss Axiocam ERc 5s camera, which was operated using the AxioVision release 4.8.2 software. Morphological features of the anamorph and teleomorph stages of the fungus were studied.

A voucher specimen was deposited in the Plant Pathology Herbarium at the Biotechnical Faculty in Podgorica, Montenegro and in the phyto-pathological herbarium of the Slovenian Institute of Hop Research and Brewing. Pathogenicity tests were performed according to the method described by Cho *et al.* (2014), inoculating the leaves of four healthy young southern catalpa plants with conidia scraped off diseased leaves. Four control plants were not inoculated. All plants were maintained in a laboratory at 24 to 28°C, not bagged, and under natural photoperiod (15h light / 9h dark).

DNA extraction, PCR, sequencing and data analysis

Genomic DNA was extracted using the CTAB method (Weising *et al.*, 1991) from mycelium and conidia obtained from infected leaves of two representative samples (IHPS-F46 and IHPS-F47). PCR was carried out using the internal transcribed spacer (ITS) region primers ITS1/ITS4 (White *et al.*, 1990) and ITS5/P3 (Takamatsu *et al.*, 2009). The PCR products were subjected to both strand direct Sanger sequencing by the commercial sequencing service Eurofins Genomics, Germany. Consensus sequences were assembled using CodonCode Aligner 8.0.1 (United States of America), and submitted to GenBank under the following accession numbers: MK253282 (isolate IHPS-F46, primers ITS1/ITS4), MK253283 (isolate IHPS-F46, primers ITS5/P3), MK253284 (isolate IHPS-F47, primers ITS1/ITS4) and MK253285 (isolate IHPS-F47, primers ITS5/P3). To confirm the identity of the isolates, the BLAST search algorithm was used for sequence comparison in the GenBank nucleotide database. Sequence alignments were made using the MUSCLE algorithm, and phylogenetic analysis was conducted in MEGA6 (Tamura *et al.*, 2013) using the Maximum Likelihood method based on the Jukes-Cantor model performing 10,000 bootstraps.

RESULTS AND DISCUSSION

In the surveyed localities, symptoms on trees (Figure 1) were mostly expressed on leaves, but also on flowers and fruit. Symptoms on leaves included greyish white powdery zones on the upper leaf surfaces, and were especially visible on younger leaves, which were totally covered by ashy coatings. Leaves with powdery mildew became deformed because of growth inhibition in the colonized areas. On older foliage, many chasmo-

Figure 1. Symptoms of powdery mildew on severely-affected catalpa tree.

Figure 2. Brown necrosis of infected catalpa flowers.

Figure 4. Chasmothecium of *Erysiphe elevata* with long appendages.

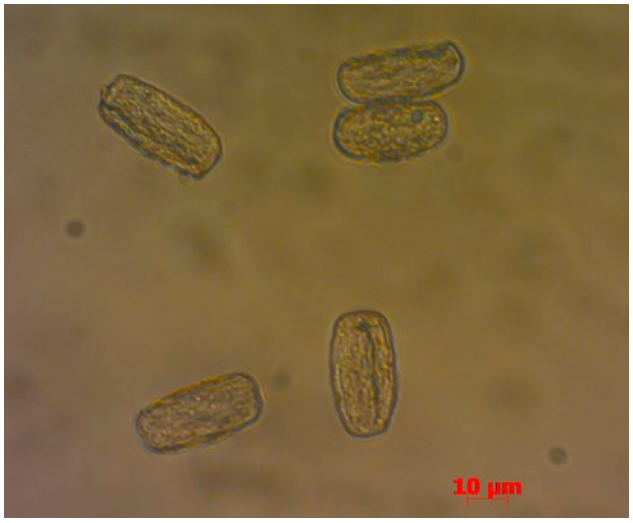

Figure 3. Conidia of *Erysiphe elevata*.

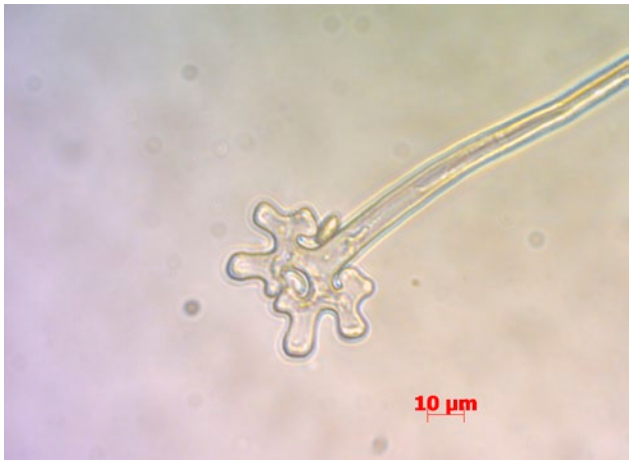

Figure 5. Dichotomously branched tip of appendage in *Erysiphe elevata*.

thecia were seen on the adaxial surfaces of the leaves. The infections led to premature defoliation, leaving bare branches with fruit. Infected flowers were mostly found in June, and they were brownish in colour (Figure 2). Diseased fruit, observed mostly in September, were discoloured, with white, bleached areas, and were cracked if severely affected. The infected plants had reduced growth and reduced decorative value.

Microscopic examinations of the samples revealed the presence of anamorph and teleomorph stages of a powdery mildew fungal pathogen. Mycelium was epiphytic with colourless, branched and septate hyphae. Cylindrical to elliptical conidia (Figure 3) were hyaline and 22 to 39 µm (mean = 29.9 µm) × 12 to 20 µm (mean = 15.0 µm). Conidia were mostly found on the upper leaf surfaces, but were also detected on flowers and fruit of catalpa trees. Chasmothecia were numerous, dark brown to black, globose, 91 to 130 µm (mean = 103.7 µm) in diameter, with several appendages whose lengths were 5-6 times greater than the diameters of the chasmothecia (Figure 4). Chasmothecia were abundant, scattered or in groups on the adaxial leaf surfaces, but rarely on fruit. Appendages were hyaline, slightly thicker towards the bases and ending in dichotomously branched tips (Figure 5). The chasmothecia contained four to seven asci (Figure 6), which were sessile or on short stalks (Figure 7a and b), and measured 29 to 64 µm (mean = 55.0 µm) × 27 to 44 µm (mean = 34.2 µm), and each ascus contained 4 to 6 ascospores. Ascospores were ellipsoid to ovoid, measuring 20 to 29 µm (mean = 24.1 µm) × 11 to 15 µm (mean = 12.8 µm) (Figure 8).

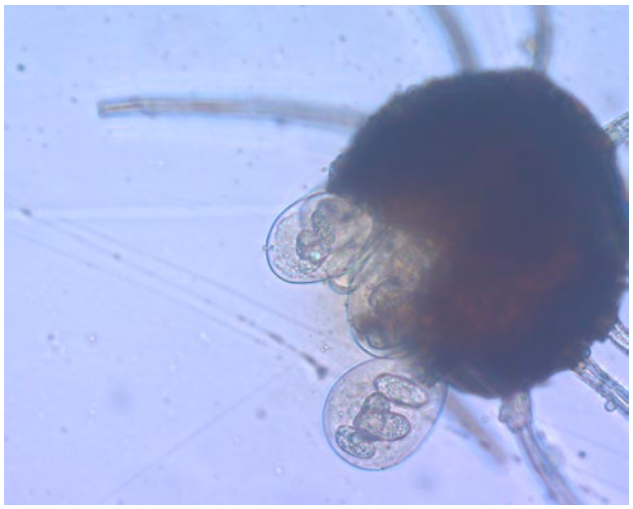

Figure 6. Chasmothecium of *Erysiphe elevata* containing asci with ascospores.

Pathogenicity tests gave positive results, as powdery mildew colonies appeared on the leaves 6 days after inoculation (Figure 9). The leaves of the non-inoculated control plants remained symptomless.

Although seven different species of powdery mildews have been recorded on *Catalpa* spp., only two are host-specific to catalpa: *Erysiphe catalpae* Simonian and *E. elevata* (Burrill) U. Braun & S. Takam. (Olsen *et al.*, 2006). A detailed study of differentiation between these two species was carried out by Ale-Agha *et al.* (2004), who showed that the anamorph and the teleomorph of *E. catalpae* can be distinguished from *E. elevata*. The anamorph of *E. elevata* is sparsely developed, while in *E. catalpae* the anamorph develops abundant conidia. However, for morphological identification, the length and branching of the chasmothecium appendages are key taxonomic characteristics. Ascomata in *E. elevata* are abundant, with long flexuous appendages, which are (1) 2–4 (6) times as long as the ascoma diameter. The appendages have dichotomously branched apices, while ascomata in *E. catalpae* are rarely formed, and their appendages are short, 0.5–1.5 times as long as the ascoma diameter and have unbranched apices.

Based on the morphological features of the fungus studied in our research, the pathogen was tentatively identified as *Erysiphe elevata*. Dimensions of conidia, chasmothecia, asci and ascospores also coincided with the observations of other authors (Braun, 1987; Ale-Agha *et al.*, 2004; Cook *et al.*, 2004; Vajna *et al.*, 2004; Pastirčakova *et al.*, 2006).

Species identity was confirmed by molecular analysis of two representative isolates (IHPS-F46 and IHPS-F47), carried out using the internal transcribed spacer (ITS) region primers ITS1/ITS4 and ITS5/P3. Both primer sets gave PCR products which were subjected to direct sequencing. BLAST analysis of the 646-bp ITS1/ITS4 (MK253282, MK253284) and 715-bp ITS5/P3 (MK253283, MK253285) sequences revealed a 99% similarity with several *E. elevata* sequences (Figure 10) available in GenBank. The four closest Genbank sequences were included in the phylogenetic analysis, together with the sequences derived in this study and sequences of *E. catalpae* and *Neoerysiphe galeopsidis*, which are two additional powdery mildew species pathogenic to *C. bignonioides*. The consensus tree (Figure 10) grouped the Montenegro isolates together with other *E. elevata* isolates (100% bootstrap value). This confirmed the previous findings of differentiation of *E. elevata* from *E. catalpae* and *Neoerysiphe galeopsidis* (Cook *et al.*, 2006).

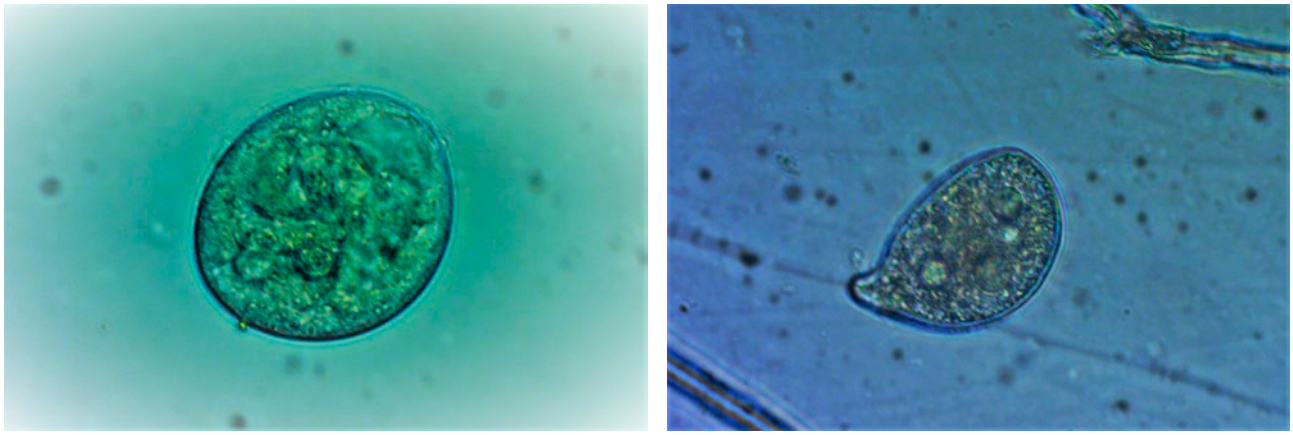

Figure 7. Asci of *Erysiphe elevata*, sessile (a) or on a short stalk (b).

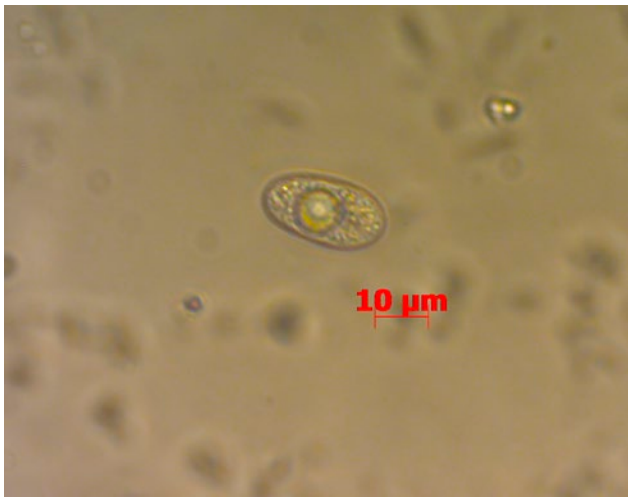

Figure 8. Ascospore of *Erysiphe elevata*.

Figure 9. Powdery mildew colonies developed on inoculated catalpa leaves.

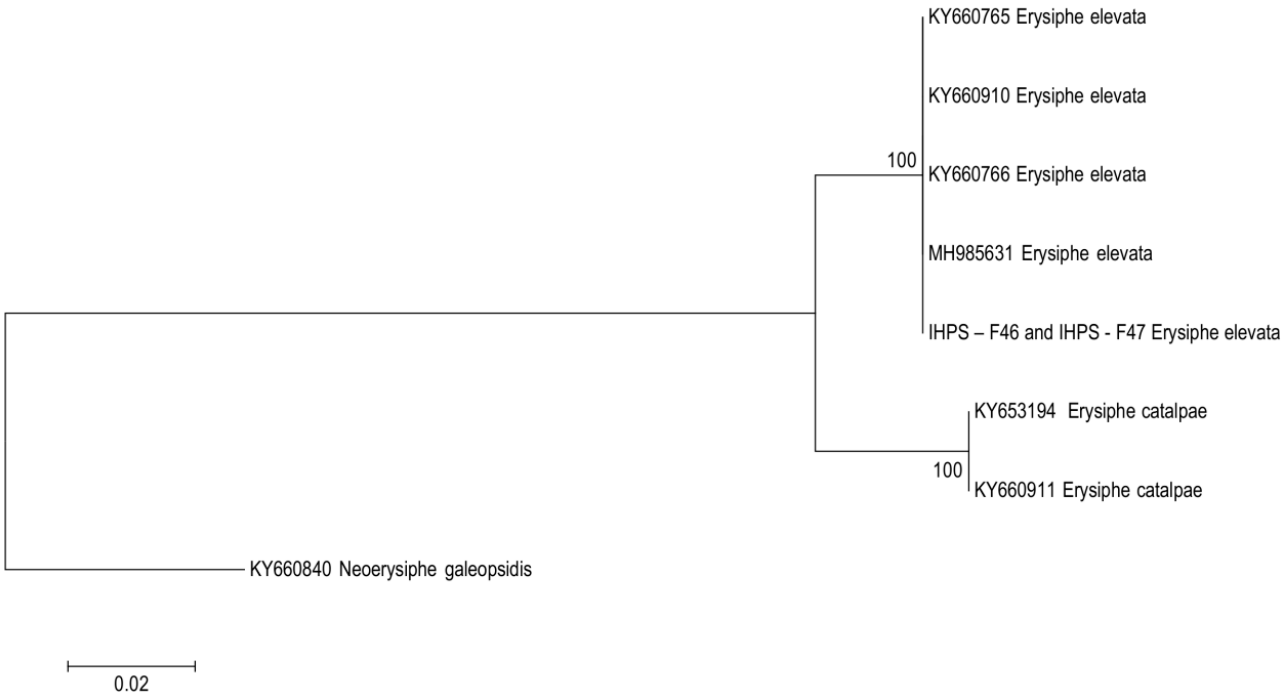

Figure 10. Neighbour-joining phylogenetic tree of *Erysiphe elevata* isolates IHPS-F46 and IHPS-F47, based on rDNA – ITS sequences. Numbers at the nodes indicate bootstrap values generated from 10,000 replicates. The scale bar indicates the number of nucleotide substitutions.

This is the first report of *Erysiphe elevata* causing powdery mildew of *Catalpa bignonioides* in Montenegro. This confirms the further spread of *E. elevata* in Europe. Appropriate control measures need to be taken to prevent or minimize the damage caused by this pathogen to catalpa trees, which are important ornamental trees.

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