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Punica granatum (pomegranate) as new host of *Erysiphe platani* and *Podosphaera xanthii*

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Summary. Pomegranate is important as an ornamental tree with spectacular flowers and delicious fruits, consumption of which has potential health benefits. In 2018 and 2019, pomegranate leaves infected by powdery mildew were collected at two locations in Hungary. One collection of the pathogenic fungi from each location was identified based on morphology and internal transcribed spacer (ITS) region analysis. One sample had pseudoidium-type conidiophores and lobed appressoria, and the other sample had catenate conidiophores and conidia with fibrosin bodies. Chasmothecia were absent in both cases. Based on morphology and ITS sequence analysis one powdery mildew fungus was identified as *Erysiphe platani*, and the other latter as *Podosphaera xanthii*. Pathogenicity tests were conducted with both species. This is the first record of powdery mildew on *Punica granatum* caused by *E. platani* and *P. xanthii*. *Erysiphe platani* has been reported only from *Platanus* species and *Ailanthus altissima*, while *P. xanthii* has a broad host range including more than 12 plant families.

Keywords. Powdery mildew, Erysiphales, *Erysiphe punicae*, host range expansion.

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INTRODUCTION

Pomegranate (*Punica granatum* L.) is a widely cultivated shrub throughout the Middle East and Caucasus region, and in North and tropical Africa, South Asia, Central Asia, the dry regions of Southeast Asia, and parts of the Mediterranean Basin (Holland *et al.*, 2009). Powdery mildew infection on pomegranate was reported first from Azerbaijan in 1964, and the causal agent was described as *Erysiphe punicae* (Braun and Cook, 2012). Later this pathogen was also found in Ethiopia, Greece, India, Iraq and the Ukraine, Crimea (Amano, 1986), and then in Iran (Khodaparast *et al.*, 2000). Recently, a new powdery mildew fungus, with cylindrical conidiophore foot cells and lobed appressoria, was reported on pomegranate in Italy (Pollastro *et al.*, 2016). Conidia were ellipsoid to cylindrical and without fibrosin bodies. Based on the analysis of the ITS sequence the fungus was identified as *Erysiphe* sp., belonging to the unresolved *E. aquilegiae* clade (Pollastro *et al.*, 2016).

The aim of the present study was to identify the causal agent of the powdery mildew on pomegranate plants in Hungary.

MATERIALS AND METHODS

Fungus samples and morphology

In September 2018, severe powdery mildew symptoms were observed on a bonsai pomegranate tree in Budapest and in a nursery garden in Győr (Hungary). Samples from fresh collections were examined by being mounted in 3% KOH solution to determine the presence of fibrosin bodies in the conidia. Dried specimens were rehydrated as described by Shin and La (1993). The morphological characteristics of the fungal structures were examined with bright-field and phase contrast microscopy, using a ZEISS AxioScope2 microscope (Germany) equipped with an AxioCam ICc5 camera (Zeiss). At least 30 measurements were made for each fungus structure.

PCR and sequence analyses

Genomic DNA was extracted from infected pomegranate leaves with the DNeasy Plant Mini Kit (Qiagen) following the manufacturer's instructions. The internal transcribed spacer (ITS) region was amplified using the powdery mildew specific primers PMITS1 and PMITS2 (Kiss *et al*., 2001). One microliter of the first amplification mixture was used for a second amplification using the nested primer set ITS1F (Gardes and Bruns, 1993) and ITS4 (White *et al*., 1990). All PCR amplifications were performed in a final volume of 20 µL. Reaction components included 1 µL of 10 µM forward and reverse primers (Sigma-Aldrich), 1 µL DNA template and 10 µL Phusion Green Hot Start II High-Fidelity PCR Master Mix (Thermo Fisher Scientific). The cycling times and temperatures for both primer pairs were as follows: 98°C for 2 min, followed by 36 cycles of 5 s at 98°C, 5 s at 60°C and 15 s at 72°C, and a final extension step at 72°C for 5 min. The nucleotide sequences of the amplicons were determined with primers ITS1F and ITS4, and were deposited in GenBank under accession numbers MK211158 and MK211159. ITS sequences were compared with accessions in the National Center for Biotechnology Information database (NCBI, http://www.ncbi.nlm.nih.gov/Blast.cgi) by applying the Basic Local Alignment Search Tool (BLAST; Altschul *et al*., 1990) using the nucleotide search option (BLASTn).

Pathogenicity tests

Pathogenicity of the specimens were confirmed through gently pressing infected leaves onto the leaves of four asymptomatic pomegranate seedlings, each with ten fully expanded true leaves. In all pathogenicity tests four non-inoculated plants served as controls.

Plants were maintained in growth chambers and visually evaluated for disease up to 10 d after inoculation.

RESULTS AND DISCUSSION

Light microscopy revealed that the infections of pomegranate in Budapest (Figure 1A) and Győr (Figure 2A) were caused by two morphologically different powdery mildew species. The specimen from Budapest had epiphytic hyphae with lobed hyphal appressoria (Figure 1B), and conidiophores producing single conidia. The foot-cells of the conidiophores measured 76 to 211 × 4 to 8 µm and were slightly sinuous or straight (Figure 1D). Conidia were hyaline, ellipsoid or doliiform, measuring 46 to 53 × 17 to 22 µm. Fibrosin bodies were absent from the conidia, and the conidium germ tubes were terminal (Figure 1C). Examination of the specimen from Győr revealed that the powdery mildew infection of pomegranate was caused by a fungus with catenate conidium development, that produced conidia in chains. Hyphal appressoria are indistinct to slightly nipple-shaped and solitary. The foot-cells of the conidiophores were straight (Figure 2D) and the dimensions were 70 to 140 × 10 to 18 µm. Conidia were ellipsoid, 38 to 45 × 21 to 27 µm, and contained fibrosin bodies (Figure 2B). Conidia each germinated at lateral positions (Figure 2C). No chasmothecia were found in the two specimens.

The BLASTn search of the ITS sequences revealed 100% identity of the pseudoidium (Budapest) sample to *Erysiphe platani*, previously described on *Platanus occidentalis* in China (MG680940), *Platanus* x *hispanica* in the United Kingdom (KY660927) and *Ailanthus altissima* in Switzerland (KX086214). Ninety-nine percent similarity was found with *E. platani* infecting *Platanus occidentalis* in Greece (KM068123) and *Platanus* × *acerifoli* in China (KX611158). The BLASTn analysis of the sample with catenate conidia (from Győr) showed 100% identity to *Podosphaera xanthii*, previously described on *Abelmoschus esculentus* in China (MK439611), *Gynostemma pentaphyllum* in Korea (KP120971), *Senna occidentalis* in Mexico (JQ728480), and on other host plants.

The results of the pathogenicity tests confirmed *E. platani* and *P. xanthii* to be pathogenic to pomegranate. Inoculated plants developed powdery mildew signs and symptoms after 5 d, whereas the control plants remained healthy. The fungi present on the inoculated plants were, respectively, morphologically identical to those originally observed on the diseased pomegranate plants from the two locations.

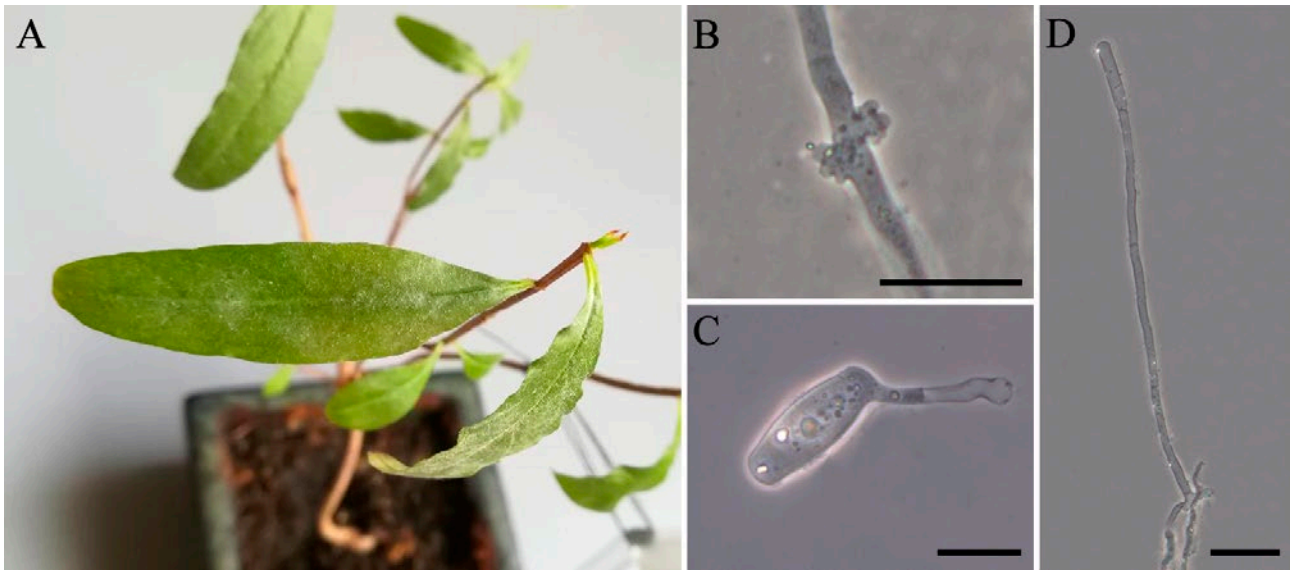

Figure 1. *Erysiphe platani* on *Punica granatum*. A: Symptoms of powdery mildew on *Pu. granatum* leaves. B: hyphal appressoria. C: germinating conidium. D: conidiophore. Bars = 50 μm.

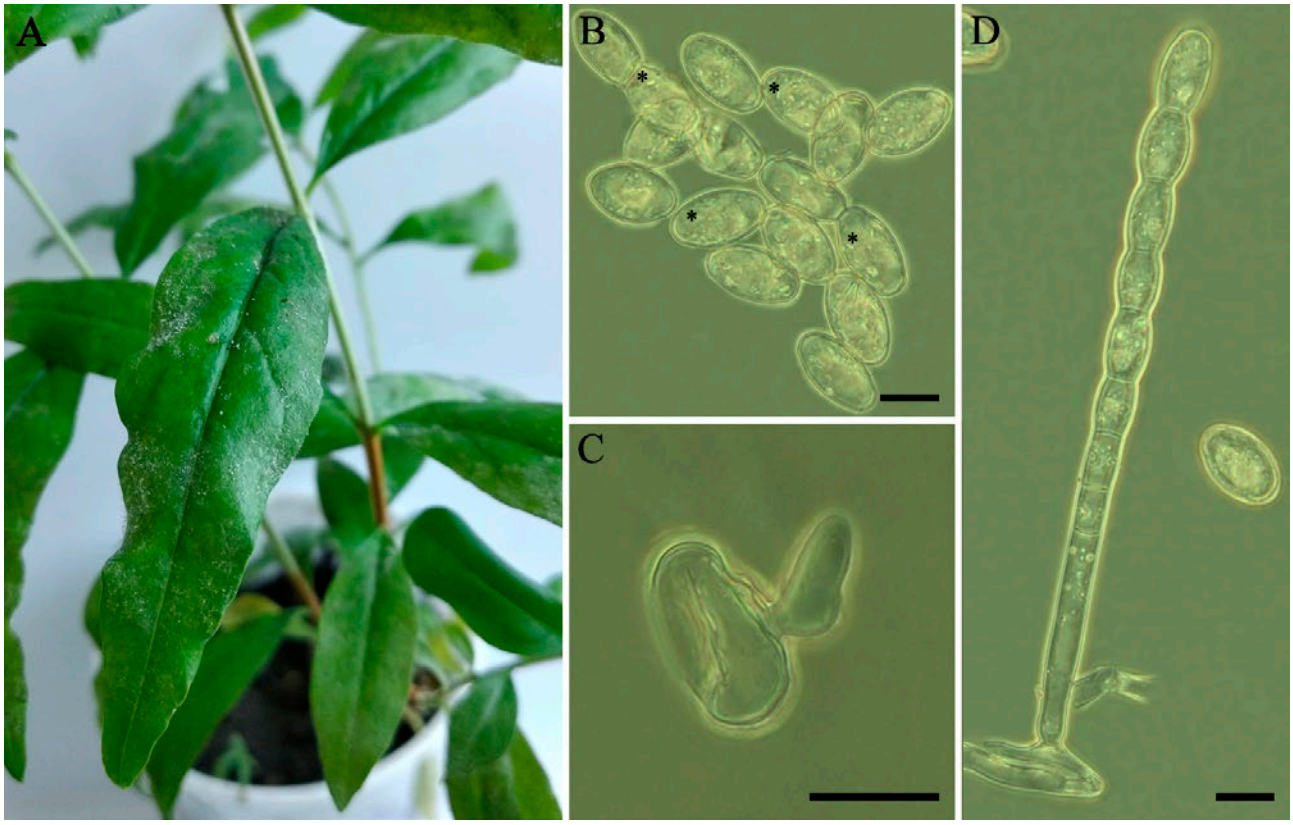

Figure 2. *Podosphaera xanthii* on *Punica granatum*. A: White colonies of *P. xanthii* on *Pu. granatum*. B: conidia, asterisks show fibrosin bodies. C: germinated conidium. D: conidiophore. Bars = 25 μm.

To our knowledge, this is the first report of *E. platani* and *P. xanthii* causing powdery mildew on pomegranate. Both specimens were deposited at the Herbarium of the Hungarian Natural History Museum, Budapest, Hungary, under accession numbers BP110664 (*E. platani*) and BP110665 (*P. xanthii*).

Podosphaera xanthii has a broad host range with worldwide distribution, and is considered a species complex rather than a single species. *Podosphaera xanthii sensu lato* consists of morphologically undistinguishable cryptic species infecting several plant species from at least 12 families (Braun and Cook, 2012). Recently this pathogen was reported from the inflorescence of the carnivorous plant, bladderwort (*Utricularia gibba*; Wu *et al*., 2019) and from *Peperomia tetragona* (Cho *et al*., 2017), which confirms the broad and expanding recorded host range of this fungus. *Erysiphe platani* was first recorded in the United States of America by Howe (1874). This pathogen has a much narrower host range than *P. xanthii*, infecting mainly plants in the genus *Platanus* in the *Platanaceae* (Braun and Cook, 2012). To date, a report of *E. platani* on the invasive tree-of-heaven (*Ailanthus altissima*) has been the only record of *E. platani* on a host that was not in *Platanus*. (Beenken, 2017). The ITS sequence of our sample was identical to that of *E. platani* infecting *A. altissima*. However, the foot cells of conidiophores of our collection were slightly longer than those reported by Beenken (2017).

Our results indicate possible host range expansions or host jumps, of the two powdery mildew species. Expansion of host ranges has been found in many other powdery mildew species (e.g. Ito and Takamatsu, 2010; Takamatsu *et al*., 2013; Vagi *et al*., 2007). To our knowledge, this is the first report of *E. platani* and *P. xanthii* causing powdery mildew on *Punica granatum*.

COMPLIANCE WITH ETHICAL STANDARDS

The authors declare no conflicts of interest. All the experiments undertaken in this study comply with the current laws of the country where they were performed.

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