Phytopathologia Mediterranea
The international journal of the
Mediterranean Phytopathological Union

Firenze University Press
www.fupress.com/pm

Research Papers

Infection of papaya (*Carica papaya*) by four powdery mildew fungi

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Summary. Papaya (*Carica papaya* L.) is an important fruit crop in many tropical and subtropical countries. Powdery mildew commonly affects this host, causing premature leaf loss, reduced yields and poor fruit quality. At least fifteen different fungi have been identified as the causal agents of papaya powdery mildew. Powdery mildew symptoms were detected on potted papaya plants growing in two locations in Hungary. This study aimed to identify the causal agents. Morphology of powdery mildew samples was examined, and sequences of two loci were used for molecular taxonomic identifications. Only anamophs were detected in all samples, and four morphological types were distinguished. Most samples had *Pseudoidium* anamorphs, while some were of the *Fibroidium* anamorph. Based on morphology and molecular taxonomy, the *Fibroidium* anamorph was identified as *Podosphaera xanthii*. The *Pseudoidium* anamorphs corresponded to three different *Erysiphe* species: *E. cruciferarum*, *E. necator* and an unidentified *Erysiphe* sp., for which molecular phylogenetic analyses showed it belonged to an unresolved species complex of *E. malvae*, *E. heraclei* and *E. betae*. Infectivity of *P. xanthii* and *E. necator* on papaya was verified with cross inoculations. A review of previous records of powdery mildew fungi infecting papaya is also provided. *Podosphaera xanthii* was known to infect, and *E. cruciferarum* was suspected to infect *Carica papaya*, while *E. necator* was recorded on this host only once previously. No powdery mildew fungus belonging to the *E. malvae*/*E. heraclei*/*E. betae* species complex is known to infect papaya or any other plants in the Caricaceae, so the unidentified *Erysiphe* sp. is a new record on papaya and the Caricaceae. This study indicates host range expansion of this powdery mildew fungus onto papaya.

Keywords. *Carica*, *Erysiphales*, *Erysiphe necator,* host range expansion, phylogenetic analysis, *Pseudoidium*.

OPEN ACCESS

Citation: D. Seress, G. M. Kovács, O. Molnár, M. Z. Németh (2021) Infection of papaya (*Carica papaya*) by four powdery mildew fungi. *Phytopathologia Mediterranea* 60(1): 37-49. doi: 10.14601/Phyto-11976

Accepted: September 21, 2020

Published: May 15, 2021

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Editor: Thomas A. Evans, University of Delaware, Newark, DE, United States.

INTRODUCTION

Papaya (*Carica papaya* L.) is a tree native to Central America (Carvalho, 2013) that is cultivated for its fruit in many tropical and subtropical countries. In Europe, Spain is the largest papaya producer, with plants grown on

the Canary Islands and the southern regions of mainland Spain (Honoré *et al*., 2020). The most economically important papaya products are edible fruits and the papain enzyme extracted from the fruits (Carvalho, 2013; Carvalho *et al*., 2015). Papain is widely used in beer production, medicines, as a meat tenderizer and for softening textiles and leather (Carvalho, 2013). Additionally, papaya trees are planted for their ornamental value.

Papaya is very susceptible to several diseases (Rawal, 2010). Most of these, such as root and foot rot, damping off, different types of leaf spots, powdery mildew, anthracnose and stem end rot, are caused by fungi or oomycetous pathogens (Ventura *et al*., 2004; Rawal, 2010). Among these, anthracnose and other postharvest diseases are considered the most important, but the significance of these diseases varies with the growing region (Ventura *et al*., 2004). Powdery mildew on papaya is generally regarded as a disease of minor importance, but it has been reported to be severe in some regions (Liberato *et al*., 2004; Ventura *et al*., 2004; Rawal, 2010; Cunningham and Nelson, 2012). Powdery mildew on papaya causes premature leaf drop, reduced yields, poor fruit quality (Cunningham and Nelson, 2012), and may also kill seedlings (Ventura *et al*., 2004). Identification of the causal species of powdery mildews is complicated because the vegetative stages of these fungi are often morphologically similar or indistinguishable (Braun *et al*., 2017).

Braun *et al*. (2017) settled some taxonomic questions concerning powdery mildew fungi infecting papaya, described two new species, and provided a key for identification of the pathogens. At least four *Erysiphe* species commonly occur on papaya (Braun *et al*., 2017). *Erysiphe caricae* was described from Switzerland after it was detected on greenhouse-grown plants of babaco (mountain papaya, *Vasconcellea* × *heilbornii*) (Bolay, 2005). Other *Erysiphe* species infecting papaya include *E. caricae-papayae* (in Thailand and Taiwan), which is newly described, *E. diffusa* (in Brazil, Taiwan and possibly several other countries) and *E. fallax*, also newly described (in the United States of America and Mexico) (Braun *et al*., 2017). In addition, *E. necator* was detected from a sample originating from Hawaii, which was attributed to "accidental infection" (Braun *et al*., 2017). Two *Podosphaera* species were reported on papaya, *P. caricicola* (in Thailand, Taiwan, in the United States of America, and probably also in Australia and Java) (Braun *et al*., 2017), and *P. xanthii* (in Taiwan and Korea) (Tsay *et al*., 2011; Joa *et al*., 2013). Four *Phyllactinia* species are also known to occur on papaya, including *Ph. caricae, Ph. caricicola, Ph. papayae* and *Ph. caricifolia* (Takamatsu *et al*., 2016). Tsay *et al*. (2011) listed three powdery mildew fungi responsible for the disease on papaya. In addition to *E. diffusa* and *P. xanthii, Pseudoidium neolycopersici*, the pathogen associated with tomato powdery mildew (Kiss *et al*., 2001) was found to be widespread in papaya plantations in Taiwan (Tsay *et al*., 2011). *Pseudoidium neolycopersici* was also reported from China, and its identification was verified with cross inoculations onto tomato (Mukhtar and van Peer, 2018). Other species, such as *E. cruciferarum*, *P. macularis*, *Golovinomyces orontii* and *Leveillula* sp. are also listed as powdery mildew fungi infecting papaya, although the status of these species on papaya is lesser known, or the identifications are doubtful (Liberato *et al*., 2004; Braun *et al*., 2017). Altogether, about fifteen different powdery mildew species (including insufficiently known taxa) are thought to infect papaya, based on the data currently available (Table 1). In Europe, *Erysiphe diffusa* was recently reported from papaya plants in Spain (Vielba-Fernández *et al*., 2019), the main papaya producing country on that continent. Three other powdery mildew fungi, *Oidium papayae* (now thought to represent *E. diffusa*) (Liberato *et al*., 2004; Braun *et al*., 2017), *Sphaerotheca caricae-papayae* (now *P. xanthii*) (Braun *et al*., 2017), and *Leveillula taurica*, were reported from Portugal (Sequeira, 1992). Additional reports from Europe include samples identified as *E. caricae* from Switzerland (Bolay, 2005), Ukraine (Takamatsu *et al*., 2015) and Germany (Braun *et al*., 2017).

We have detected powdery mildew symptoms on papaya plants at two locations in Hungary. The aim of the present study was to characterize and identify the causal agents of powdery mildew on the infected plants.

MATERIALS AND METHODS

Samples and morphology

In 2018 and 2019, spontaneous powdery mildew infections were observed on young papaya plants growing in pots as hobby plants in a family yard in Győrújbarát, and in a greenhouse, on plants intended for research purposes in Budapest, Hungary. All these plants were grown from germinated seeds originating from one fruit.

Samples collected during this study are listed in Supplementary Table 1. Fresh powdery mildew colonies were sampled with cellotape and mounted in glycerine on microscope slides. Samples were also prepared using the lactic acid boiling method (Shin and La, 1993). For morphological characterization, a Zeiss Axioskop 2 Plus microscope was used with an AxioCam ICc5 camera. Size, shape and development of conidia (singly or in

Table 1. Powdery mildew species recorded on papaya, with collection data of the corresponding samples. "?" denotes doubtful records because of taxonomic uncertainties and/or lack of molecular-based identifications or other data. Countries where papaya is thought to be indigenous are indicated in *italic* font. Several early reports of '*Erysiphe caricae*' could not be included, as these records cannot be assigned to currently accepted species.

Powdery mildew species	Relevant synonyms or alternative names	Known host plants in Caricaceae	Geographic origin	Form detected on papaya	References
Species reported in this study					
Podosphaera xanthii	*Podosphaera caricae-papayae* *Sphaerotheca caricae-papayae*	*Carica papaya* *Vasconcellea* × *heilbornii* *Vasconcellea pubescens*	Australia, China, Cook Islands, Hungary, India, Japan, New Zealand, Thailand, USA, Portugal(?), Ukraine(?)	anamorph and teleomorph	Miller 1938; Sequeira, 1992; Braun and Cook, 2012; Braun *et al*., 2017 and references therein; this study
Erysiphe sp.		*Carica papaya*	Hungary	anamorph only	this study
Erysiphe cruciferarum		*Carica papaya* *Vasconcellea* × *heilbornii* *Vasconcellea pubescens*	Hungary, New Zealand, South Africa	anamorph only	Boesewinkel, 1982a,b; Gorter, 1993; this study
Erysiphe necator		*Carica papaya*	USA (Hawaii), Hungary	anamorph only	Braun *et al*., 2017; this study
Other species occurring on papaya					
Erysiphe caricae		*Carica papaya* *Vasconcellea* × *heilbornii*	Switzerland, Ukraine, Germany(?), Portugal(?)	anamorph and teleomorph	Bolay, 2005; Takamatsu *et al*., 2015; Braun *et al*., 2017
Erysiphe caricae-papayae		*Carica papaya*	Thailand, Taiwan(?)	anamorph and teleomorph	Tsay *et al*., 2011; Braun *et al*., 2017
Erysiphe diffusa	*Oidium caricae* *Oidium papayae* *Pseudoidium caricae*	*Carica papaya*	*Brazil*, Portugal, Spain, *Venezuela*, Taiwan(?)	anamorph and teleomorph	Liberato *et al*., 2004; Tsay *et al*., 2011; Braun *et al*., 2017; Vielba-Fernández *et al*., 2019
Erysiphe fallax		*Carica papaya*	*Mexico*, USA	anamorph only	Braun *et al*., 2017
Golovinomyces orontii	*Erysiphe cichoracearum*	*Carica papaya*(?) *Vasconcellea* × *heilbornii*(?) *Vasconcellea pubescens*(?)	*Mexico*(?), New Zealand(?), Peru(?)		Boesewinkel, 1982a,b; Braun and Cook, 2012; Braun *et al*., 2017 and references therein
Leveillula taurica s. lat.	*Oidiopsis sicula*	*Carica papaya*	Australia, India, Malawi, Nigeria, Portugal, Zambia, Zimbabwe		Liberato *et al*., 2004 and references therein; Braun *et al*., 2017 and references therein
O. caricae-papayae		*Carica papaya*	India,Taiwan	anamorph only	Braun and Cook, 2012
Phyllactinia caricae	*Ovulariopsis caricae*	*Carica papaya*	Taiwan, Australia(?), India(?)	anamorph only	Braun and Cook, 2012
Phyllactinia caricicola	*Ovulariopsis caricicola* *Streptopodium caricae*	*Carica papaya*	*Brazil*	anamorph only	Liberato *et al*., 2004; Takamatsu *et al*., 2016
Phyllactinia caricifolia	*Phyllactinia caricaefolia*	*Carica papaya*	*Brazil*	anamorph and teleomorph	Liberato *et al*., 2004 and references therein
Phyllactinia papayae	*Ovulariopsis papayae*	*Carica papaya*	Madagascar, Reunion, Rwanda, South Africa, Tanzania	anamorph only	van der Bijl, 1921; Braun and Cook, 2012
Podosphaera caricicola	*Oidium caricicola*	*Carica papaya*	Taiwan, Thailand, USA, Australia(?), Indonesia(?)	anamorph only	Boesewinkel, 1982a; Yen and Wang, 1973; Braun *et al*., 2017

chains), presence of fibrosin bodies in conidia, lengths of conidiophores, size of foot-cells, and morphology of hyphal appressoria were determined. Thirty conidia and all available conidiophores, including foot-cells, were measured from each sample. Type of conidium germination was noted when observed.

Representative herbarium specimens from each morphological type were deposited at the Mycological Collection of the Hungarian Natural History Museum, under accession numbers HNHM-MYC-008079 (111134BP) to HNHM-MYC-008083 (111138BP).

Sequence determinations

Genomic DNA was extracted from powdery mildew material removed from leaf surfaces with cellotape, using the sample boiling method (Pintye *et al.*, 2020), or from powdery mildew-infected leaf fragments using the DNeasy Plant Mini Kit (Qiagen), following the manufacturer's instructions. The internal transcribed spacer (ITS) region of the nuclear ribosomal DNA (nrDNA) was amplified in two fragments (Scholler *et al.*, 2016) using the primer pairs ITS5-PM6 and PM5-ITS4 (Takamatsu and Kano, 2001). A fragment of Minichromosome Maintenance Complex Component 7 encoding gene (*Mcm7*) was amplified with primers Mcm7F2 and Mcm7R8 (Ellingham *et al.*, 2019). For amplifications, Phusion Green Hot Start II High-Fidelity PCR Master Mix (Thermo Fisher Scientific) was used as recommended by the manufacturer, with primer annealing temperatures set to 58°C for ITS and 55°C for *Mcm7* amplifications. The reaction mixture contained 1 µL of template DNA in the ITS and 2 µL in the *Mcm7* amplifications. Amplicons were run on 1% agarose gel, and were sent for sequencing to LGC Genomics GmbH. Sequencing was done with the same primers used for the amplifications. The resulting chromatograms were processed with Staden Program Package (Staden *et al.*, 2000) and CodonCode Aligner version 9.0.1 (CodonCode Corporation). Sequences determined in this study were deposited in GenBank under accession numbers MT658714 to MT658729 and MT755388 to MT755394 (Supplementary Table 1).

Sequence analyses

Three phylogenetic analyses were conducted using ITS sequences (as in Braun *et al.*, 2017): one with sequences of samples belonging to the *Microsphaera* lineage of *Erysiphe* (Takamatsu *et al.*, 2015), the second with *E. necator* sequences, and the third with sequences of *Podosphaera xanthii* and closely related species. These analyses used the determined ITS sequences and sequences from the datasets of Braun *et al.* (2017), supplemented with additional sequences from closely related species obtained from GenBank after a search with Basic Local Alignment Search Tool (BLAST; Altschul *et al.*, 1990). The *E. necator* dataset also contained ITS sequences of isolates originating from non-*Vitaceae* hosts (Fonseca *et al.*, 2019; Pieroni *et al.*, 2020).

ITS alignments were prepared using MAFFT online (Katoh and Standley, 2013) with the E-INS-i algorithm (other settings were used as defaults). Leading and trailing gaps were included as unknown characters.

Mcm7 sequences from fungi of morphological types 2, 3 and 4 determined in this study were aligned with sequences from other *Erysiphe* sp. samples (Ellingham *et al.*, 2019; Shirouzu *et al.*, 2020) with FFT-NS-i algorithm of MAFFT online, and were added to the ITS dataset of the same samples, creating a combined ITS_*Mcm7* alignment.

Two *Cystotheca* species, *E. ornata* and *E. necator* var. *ampelopsidis*, were used as outgroups in the ITS analyses based on the results of Braun *et al.* (2017). For the ITS_*Mcm7* dataset, *Arthrocladiella mougeotii* and *Golovinomyces bolayi* were used as outgroup (Shirouzu *et al.*, 2020).

Phylogenetic analyses were carried out with the maximum likelihood (ML) method using raxmlGUI 1.5 (Silvestro and Michalak, 2012; Stamatakis, 2014). For the analysis of the ITS_*Mcm7* dataset, two partitions were set according to the two loci. Branch supports were calculated from 1000 bootstrap replicates. Phylogenetic trees resulting from analyses were visualized in TreeGraph 2.14.0 (Stöver and Müller, 2010) and were submitted to TreeBASE (study ID 26269).

Cross inoculation experiments

Cross inoculations were conducted with *P. xanthii* and *E. necator*. The two other powdery mildew fungi detected on papaya were not used.

Papaya plants (both infected and healthy) used in these experiments were less than 1 year old, and were 30–50 cm in size. These plants were germinated from the same batch of seeds as the plants originally identified as powdery mildew infected. The seeds were collected from a commercially available papaya fruit of unknown variety, originating from Indonesia. Other plant species used in cross inoculation tests were 1-month-old cucumber plants (*Cucumis sativus* 'Párizsi Fürtös') and 8-month-old grapevine (*Vitis vinifera* 'Chardonnay') plants grown from cuttings in pots.

Table 2. Summary of cross inoculation test results. (+) denotes successful infections and (–) denotes no infection.

Inoculum	To papaya	To cucumber	To grapevine	To *in vitro* grapevine leaves
Podosphaera xanthii ex papaya	–	+		
Podosphaera xanthii ex cucumber	+	+		
Erysiphe necator ex papaya	–		–	+
Erysiphe necator ex grapevine	+		+	+

The first series of experiments were carried out with powdery mildew from *P. xanthii*-infected papaya and cucumber plants onto healthy papaya and cucumber plants, by gently pressing the diseased leaves onto the surfaces of healthy leaves. In the second set of experiments *E. necator*-infected papaya and grapevine plants were used on to similarly inoculate healthy papaya and grapevine plants. All inoculations included two seedlings of each tested plant species to be inoculated, and two plants as positive controls, with the respective powdery mildew-inoculated to the same host plant species. Inoculated seedlings were covered with powdery mildew impermeable transparent plastic foil. Two uninoculated plants from each species were used as negative controls. All experiments were conducted twice.

In addition, transfer of *E. necator* from papaya and grapevine onto grapevine leaves maintained in an *in vitro* system was tested. *In vitro* grapevine plantlets were micropropagated from two-nodal explants grown on Murashige and Skoog (MS) medium (Murashige and Skoog, medium Mod. No. 1B, Duchefa) solidified with 6.5 g L^{-1} phyto agar (Murashige and Skoog, 1962; Aziz *et al.*, 2003). Plants were grown at 22°C with a daily 12 h photoperiod. Grapevine leaves were cut under sterile conditions and cultivated further on the same medium in disposable Petri dishes. Conidia from powdery mildew on papaya were placed on grape leaves using a sterile glass needle under sterile conditions. The Petri dishes were then incubated under the same conditions as the *in vitro* grapevine plantlets.

Inoculated plants and *in vitro* leaves were checked regularly for symptom development. When powdery mildew colonies were observed, the identity of the fungus was verified with microscopic analysis as described above. Cross inoculation experiments are summarized in Table 2.

RESULTS

Small powdery spots, each a few cm^2 in size, were detected on the stems and/or adaxial surfaces of the leaves on all plants investigated (Figure 1). No infection was detected on abaxial leaf surfaces. Some of the infected leaves became necrotic and curled, and later dried and fell off the plants.

According to morphological analysis by light microscopy, infections on some leaves were caused by powdery mildew fungi belonging to the *Fibroidium*, while others belonged to the *Pseudoidium* anamorphs. Four morphological types of powdery mildew fungi occurred in our samples, one *Fibroidium* morphological type and three *Pseudoidium* anamorphs with slightly differing morphology (Figure 2). Chasmothecia were not detected in any sample.

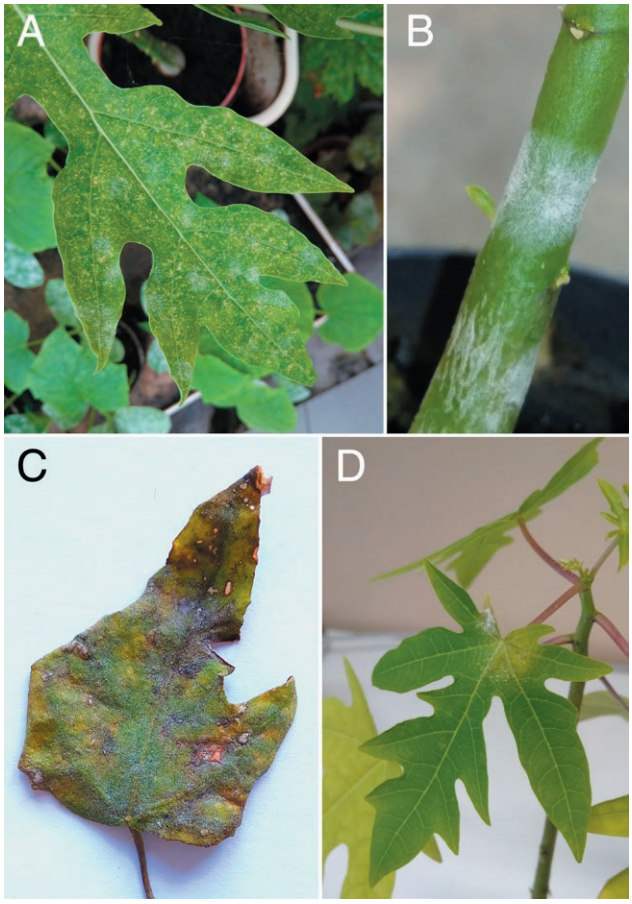

Figure 1. Powdery mildew symptoms on papaya plants. Symptoms caused by: A) *Podosphaera xanthii*, B) *Erysiphe* sp., C) *Erysiphe cruciferarum* and D) *Erysiphe necator.*

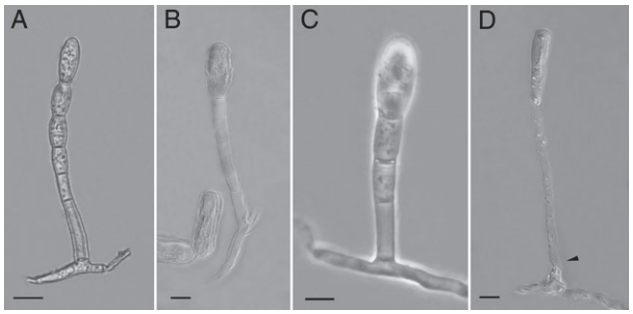

Figure 2. Conidiophore morphology of the four different powdery mildew morphological types designated in this study. A) *Podosphaera xanthii,* B) *Erysiphe* sp., C) *E. cruciferarum*, D) *E. necator*. Arrow in D) indicates the twisted conidiophore foot cell, characteristic of *E. necator.* Bars = 20 µm.

Morphological types

Morphological type 1. This type was detected in samples collected only in Budapest (Supplementary Table 1). Infections in most cases caused small, but well visible colonies on adaxial leaf surfaces. The fungus had *Fibroidium* anamorph, characterized by indistinct hyphal appressoria and production of conidia in chains. Conidium chains usually contained four to six conidia. Conidiophores measured up to 356 µm (including conidium chains), averaging 215 µm. Conidiophore foot cells were 57–94 µm × 8–13 µm and were each usually surmounted by 2-3 shorter cells. Foot cells sometimes showed slight constrictions at the basal septae. Conidia were doliiform, 25–40 µm in length, and 13–25 µm in width and contained fibrosin bodies, which were visible when mounting without boiling. Conidium germination was lateral and germ tubes did not have distinct appressoria. Based on morphological characteristics, the fungi in these samples were tentatively identified as *Podosphaera xanthii*.

Morphological types 2, 3 and 4 were identified as *Erysiphe* spp. based on morphology similar to *Pseudoidium* anamorphs (Braun and Cook, 2012).

Morphological type 2. A morphologically different subset of samples collected in Budapest (Supplementary Table 1) had small, distinct colonies, mainly on stems and petioles of affected host plants. Hyphae formed lobed appressoria. Conidiophores measured 97–152 µm, their foot cells were 26–47 µm × 9–12 µm, and these were each surmounted by two shorter cells. Conidia formed singly, were cylindrical or doliiform with lengths of 35–50 µm and widths of 13–23 µm, and lacked fibrosin bodies. Conidia germinated terminally with short germ tubes which formed lobed to multilobed appressoria.

Morphological type 3. A proportion of samples collected in Budapest and Győrújbarát (Supplementary Table 1) was characterized by thin, evanescent to persistent colonies on petioles and on adaxial leaf surfaces of affected hosts. These fungi developed lobed hyphal appressoria. Conidiophores measured 70–121 µm. Conidiophore foot cells were 18–33 µm × 7–12 µm, and were each surmounted by two shorter cells. Conidiophores each produced single cylindrical conidia, with lengths of 28–45 µm and widths of 10–20 µm, without fibrosin bodies. Conidia germinated terminally and formed moderately lobed appressoria.

Morphological type 4. Powdery mildew of other samples from papaya in Budapest and in Győrújbarát (Supplementary Table 1) developed thin or persistent colonies on host plants. Hyphal appressoria were lobed to multilobed. Conidiophores were highly variable in length, from 109 µm to sometimes slightly longer than 300 µm, averaging 205 µm. Conidiophore foot cells measured 73–154 µm × 5 µm. A portion of foot cells was sinuous or spirally twisted. Foot cells were usually surmounted by two shorter cells. Conidia formed singly, and were ellipsoid-ovoid or doliiform, 30–45 µm long and 15–20 µm wide, and did not contain fibrosin bodies. Conidia germinated terminally and formed lobed appressoria, or germination followed longitubus pattern. Based on these characteristics (Nomura *et al.*, 2003; Braun and Cook, 2012), the fungus was tentatively identified as *E. necator.*

Sequence analyses

Molecular taxonomic analyses of the nrDNA ITS region were carried out for 16 samples, representing all four morphological types. The dataset for the phylogenetic analysis of *Podosphaera* species included 46 sequences (including two newly determined sequences) and had a length of 480 characters, while the dataset with *Erysiphe* species in the *Microsphaera* lineage contained 85 sequences (including six newly sequenced) and the alignment consisted of 530 characters. The alignment of *Erysiphe necator* ITS sequences contained 27 sequences (of which eight were determined in the present study) and had 529 characters. The combined dataset of ITS and *Mcm7* sequences contained newly obtained sequences from seven samples, and altogether 62 samples, with an alignment length of 1068 characters, from which the *Mcm7* partition had 468 characters.

The identical ITS sequences determined from two Hungarian samples representing the *P. xanthii* morphotype formed a clade with three other identical *P. xanthii* sequences from powdery mildews originating from

Cucumis, *Helianthus* and *Saintpaulia* (Supplementary Figure 1).

In the phylogenetic analysis of the *Microsphaera* lineage of *Erysiphe* species, ITS sequences from the various samples from papaya were spread across six different clades (Figure 3). The Hungarian isolates (morphological types 2 and 3) were found in two of the clades. The three samples belonging to morphological type 2 had identical ITS sequences. These clustered in a clade containing identical sequences of other powdery mildew fungi identified as *E. malvae*, *E. heraclei* and *E. betae*, infecting five different plant species. ITS of two other samples labelled as *E. heraclei* and *E. betae* differed in one nucleotide position from the former samples (Figure 3). Three sequences from samples of morphological type 3 clustered in a clade formed by sequences of powdery mildews infecting *Brassicaceae* hosts (Figure 3). The ITS sequences of powdery mildew fungi from our papaya samples from Budapest and Győrújbarát, and *Brassica* sp., *Raphanus sativus* and *Sisymbrium officinale*, were identical.

In the combined ITS_*Mcm7* analysis, samples from morphological type 2 similarly clustered together in a well supported clade with samples labelled as *E. malvae*, *E. heraclei* and *E. betae* (Figure 4). However, sequences of our samples differed at least in one nucleotide position from all currently known *Mcm7* sequences.

The phylogenetic analysis of *E. necator* ITS sequences resulted in two groups, and both groups contained samples from papaya as well as from grapevine (Figure 5). Three of our papaya samples collected in Győrújbarát with identical ITS sequences formed a group with eight other identical sequences of powdey mildews from *Vitis* sp. and one from *Caryocar brasiliense*, and two other sequences differing in one position from the Hungarian samples. ITS sequences of *E. necator* infecting cashew differed in three nucleotides, while the ITS of the isolate infecting rubber tree differed in two nucleotides from our sequences belonging to this group.

Five of our papaya samples with identical sequences from Budapest formed a clade with three *E. necator* samples, including one originating from papaya, and two others from *V. vinifera* (Figure 5). These were all characterized by the same nucleotide sequence. In addition, an Australian *E. necator* sample from grapevine differed in one nucleotide from these sequences.

Cross inoculation tests

Results of cross inoculation tests are summarized in Table 2. Cross inoculations from infected papaya plants to healthy papaya plants were unsuccessful in experiments involving *P. xanthii*. However, healthy cucumber plants, regular hosts of *P. xanthii*, could be infected with the powdery mildew originating from papaya. Cucumber plants developed powdery mildew symptoms after 11 d.

Visible powdery mildew patches developed on the inoculated papaya leaves 11 d after inoculations with *P. xanthii* from cucumber, indicating that infection with powdery mildew from cucumber to papaya was successful (Supplementary Figure 2A). The same inoculum also infected healthy cucumber plants inoculated as controls.

Symptomless papaya plants and grapevine plants became infected with *E. necator* from grapevine, but not with *E. necator* from papaya. However, an *E. necator* sample from papaya (PM198) and another *E. necator* sample from grapevine as a control, were successfully used for starting *in vitro* powdery mildew cultures on *V. vinifera* leaves, causing symptoms 10-12 d after inoculations (Supplementary Figure 2B). The powdery mildews have been maintained on *in vitro* grapevine leaves.

DISCUSSION

Carica papaya and other *Carica* species are hosts of numerous powdery mildew species representing many different lineages of Erysiphales (Table 1). Based on morphological and sequence analyses, we detected *Podosphaera xanthii* and three *Erysiphe* spp. occurring on papaya plants in Budapest and Győrújbarát, Hungary.

Podosphaera xanthii, generally known as cause of powdery mildew on cucurbits, has a broad host range (Pérez-García *et al*., 2009; Braun and Cook, 2012) which is expanding as new hosts are reported (eg. Fan *et al*., 2019; Nayak and Babu, 2019; Nemes *et al*., 2019). Previous cross inoculation studies (Miller, 1938; Alcorn, 1968; Munjal and Kapoor, 1973; all cited in Liberato *et al*., 2004) showed that *P. xanthii* was able to infect papaya. Other studies reported spontaneous infections of papaya by the same species in Taiwan and Korea (Tsay *et al*., 2011; Joa *et al*., 2013). This fungus is a widespread colonizer of papaya in different geographic regions of the world, especially as samples identified earlier as *P. caricae-papayae* also represent *P. xanthii* (Braun *et al*., 2017).

The samples of the morphological type 2 formed a clade with powdery mildew fungi identified as *E. malvae*, *E. heraclei* and *E. betae*. Samples of morphological type 2 differed from *E. malvae* by the longer conidia, and the conidiophores of *E. malvae* arise mostly from towards the ends of mother cells (Braun and Cook, 2012), which was not observed in our samples. However, our samples were morphologically indistinguish-

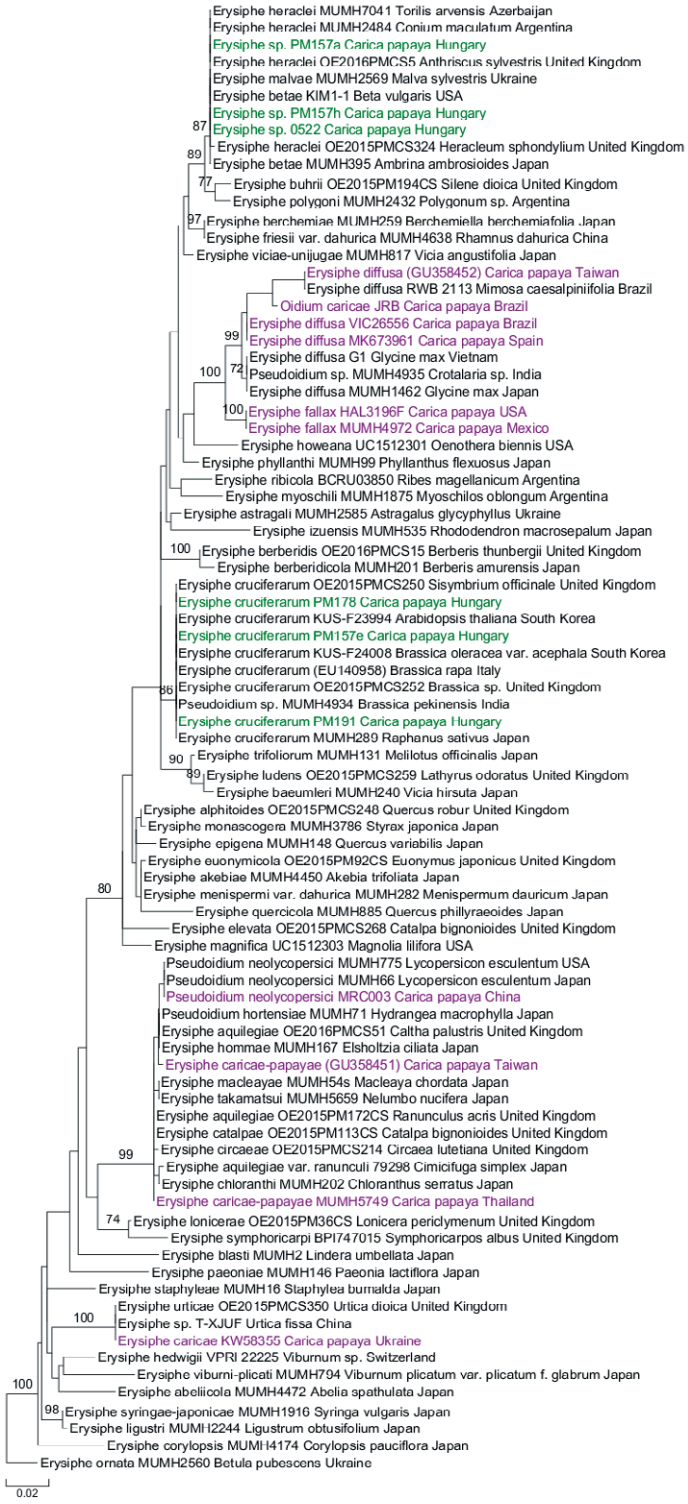

Figure 3. Phylogenetic tree with the greatest likelihood value resulting from the maximum likelihood (ML) analysis of ITS sequences of selected powdery mildew species belonging to the *Microsphaera* lineage of *Erysiphe*. Species names are followed by herbarium accession numbers (or GenBank accession numbers in parentheses if the herbarium accession number is not available), name of host plant, and country of collection. Samples collected in the present study are in green font, while other powdery mildew samples from papaya are in purple. Bootstrap values were calculated from 1000 replicates in the ML analysis (values below 70% and in subclades are not shown). Bar indicates 0.02 expected changes per site per branch.

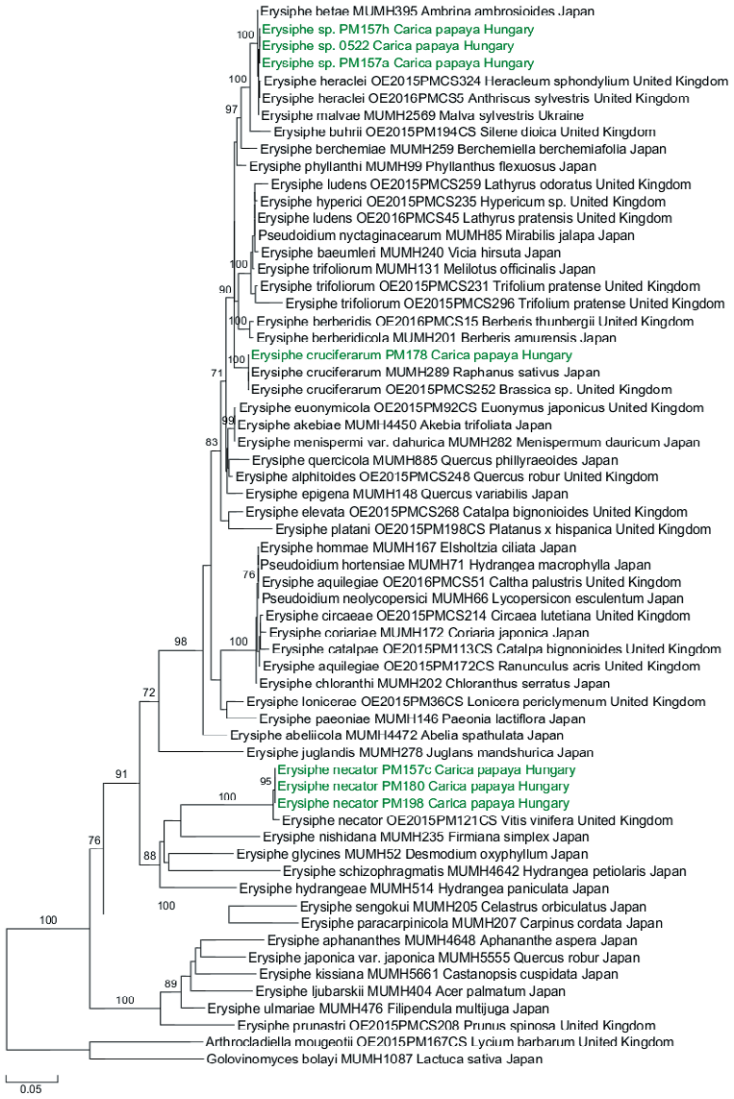

Figure 4. Phylogenetic tree with the greatest likelihood value resulting from the maximum likelihood (ML) analysis of ITS and *mcm7* sequences of selected powdery mildew species belonging to the *Microsphaera* lineage of *Erysiphe*. Species names are followed by herbarium accession numbers (or GenBank accession numbers in parentheses if the herbarium accession number is not available), name of host plant, and country of collection. Samples collected in the present study are in green font. Bootstrap values were calculated from 1000 replicates in ML analysis (values below 70% and in subclades are not shown). Bar indicates 0.05 expected changes per site per branch.

able from *E. betae* and *E. heraclei*. ITS sequences from morphological type 2 belonged to an unresolved complex within the *Microsphaera* lineage (Takamatsu *et al.*, 2015), so this fungus cannot be unambiguously identified to the species level based solely on ITS sequence data. This could be due to the low phylogenetic resolution of nrDNA sequences at the species level for powdery mildew fungi (Takamatsu *et al.*, 2015; Shin *et al.*, 2019). In order to further characterize, and possibly identify, the fungus in this complex, we also determined the sequence of a fragment of *Mcm7*. This locus is known to provide greater resolution than ITS among powdery mildew fungi (Ellingham *et al.*, 2019). This sequence is known to differ between *E. heraclei* and *E. betae*, based on the few sequences obtained to date (Ellingham *et al.*, 2019; Shirouzu *et al.*, 2020). Using *Mcm7* did not give species-level identification of the fungus with morphological type 2, as our sequences differed from both *E. heraclei* and *E. betae*. This indicates that a unique haplotype of an unindentified (or unknown) species infected papaya. Sequencing of additional reference samples could confirm if it belongs to an unknown spe-

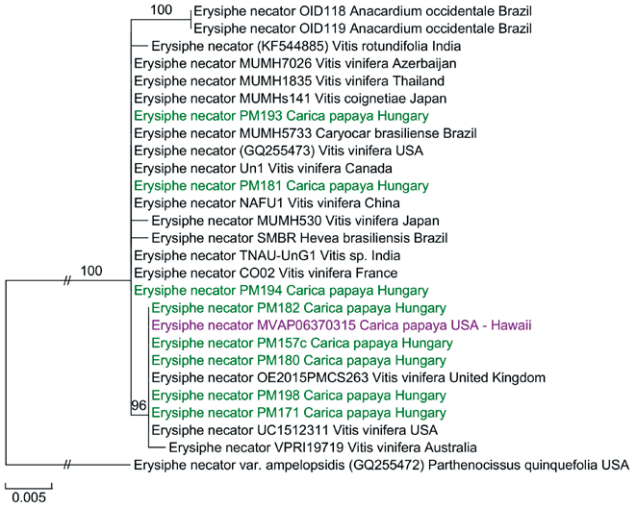

Figure 5. Phylogenetic tree with the greatest likelihood value resulting from the maximum likelihood (ML) analysis of ITS sequences of selected *Erysiphe necator* samples. Species names are followed by herbarium accession numbers (or GenBank accession numbers in parentheses if herbarium accession number is not available), name of host plant, and country of collection. Samples collected in the present study are in green font, while other powdery mildew samples from papaya are in purple. Bootstrap values were calculated from 1000 replicates in ML analysis (values below 70% and in subclades are not shown). Bar indicates 0.005 expected changes per site per branch.

cies. We are not aware of any previous study presenting powdery mildew fungi belonging to this species complex from papaya or any other host plants in the Caricaceae. Our results further indicate that fungi from this lineage infect diverse hosts from different plant families.

The assembly containing morphological type 3 samples can be identified as *E. cruciferarum*. Although this species is found mainly on brassicaceous plants (Braun and Cook, 2012), infectivity of *E. cruciferarum* on papaya is also known from previous results, as the powdery mildew infecting *Brassica napus* was able to cause disease on papaya plants (Boesewinkel 1982a, cited in Braun *et al*., 2007). A checklist of South African powdery mildew fungi also listed papaya as a host of *E. cruciferarum* (Gorter, 1993). Our sequence results provide further evidence of *E. cruciferarum* occurring on papaya. It should be noted that *E. cruciferarum* was detected on papaya leaves collected in the two sampled locations in Hungary, which are more than 100 km apart.

Erysiphe caricae, the first *Erysiphe* species on papaya of which the teleomorph has been detected (Bolay, 2005; Braun *et al*., 2017) is phylogenetically distant from other *Erysiphe* species occurring on papaya (Braun *et al.,* 2017; and see Figure 3). Moreover, as additional sequences were included in our analysis, this showed that *E. caricae* formed a group with two powdery mildew samples from *Urtica* sp., having identical ITS sequences. Further research is required to decipher the relation of *E. caricae* and the fungi causing powdery mildew on *Urtica* sp.

Erysiphe necator is mainly associated with plants in the *Vitaceae* (Braun and Cook, 2012). However, it has been shown recently that this fungus can also infect cashew and rubber tree (Fonseca *et al*., 2019; Pieroni *et al*., 2020). *Erysiphe necator* was also identified from papaya in a single sample from Hawaii (Braun *et al*., 2017). In the present study, infections of papaya were found to be caused by *E. necator* in half of the samples. The fungus was found in two distinct locations in Hungary, and the ITS sequences of samples from the two locations were different, indicating at least two independent occurrences of the fungus on papaya. The two groups differed by a fixed nucleotide difference (T/C at the nucleotide position corresponding to no. 48 of the reference sequence GQ255473), which is known to differ between two genetically differentiated *E. necator* subpopulations (Brewer and Milgroom, 2010). Our results suggest that *E. necator* can readily infect papaya in some circumstances.

Cross inoculations with *E. necator* and *P. xanthii* from papaya onto healthy papaya plants were unsuccessful. On the other hand, *P. xanthii* from papaya infected cucumber, and *E. necator* from papaya infected *in vitro* grapevine leaves. One explanation could be that the powdery mildew growth on papaya is often sparse, providing insufficient inoculum pressure on the healthy plants for successful infections. The respective hosts may also be more susceptible to the corresponding powdery mildew species than papaya. Furthermore, it is also pos-

sible that the growth conditions in our experiments were less conducive to powdery mildew infections on papaya than on the other host plants.

Erysiphe species detected in the present study are different from the species commonly occurring on papaya (see Braun *et al*., 2017). This could be that most of the reports have originated from locations where papaya is widely grown, or commonly found. In Hungary, papaya is present in homes of hobby growers or in greenhouses. Papaya is not native in Europe, but the powdery mildew species detected on papaya are established in Hungary (Sz. Nagy and Kiss, 2006). Fungal pathogens (Thines, 2019), including powdery mildews (Limkaisang *et al*., 2006; Vági *et al*., 2006; Cook *et al*., 2015; Beenken, 2017) have been reported to infect introduced non-local plants. This is considered as host range expansion (Thines, 2019), which is similar to the results from the present study of powdery mildew on papaya.

Our findings and the previous reports show that papaya is a host of several different powdery mildew fungi wherever it is grown. This may indicate that papaya could become a host for locally occurring powdery mildew species when this host is out of its native geographic range. This could lead to repeated occurrences of papaya powdery mildew, as papaya is more widely grown around as a crop or as an ornamental.

ACKNOWLEDGEMENTS

The authors thank Tamás Farkas for providing papaya plants, and for his assistance in sample transport. This research was partly supported by the Széchenyi 2020 Programme, the European Regional Development Fund and the Hungarian Government (GINOP-2.3.2-15-2016-00061), and by the ELTE Institutional Excellence Program by the National Research, Development and Innovation Office (NKFIH-1157-8/2019-DT).

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