

Research Note

PODOSPHAERA XANTHII: AN UPDATE ON THE POWDERY MILDEW OF TROPICAL PUMPKIN (*CUCURBITA MOSCHATA* DUCHESNE) IN PUERTO RICO^{1,2}

Andrea Landrón-García³, Angela Linares-Ramírez⁴, Merari Feliciano-Rivera⁵
and Yanira Miranda-Cortés⁶

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Powdery mildew, usually caused by two obligate biotrophic fungi, *Golovinomyces cichoracearum* (DC.) V.P. Heluta and/or *Podosphaera xanthii* (syn. *P. fusca*) (Castagne) U. Braun y N. Shishkoff, is an important disease in cucurbits causing significant yield loss and reduction of fruit quality (Barickman et al., 2017; Lebeda and Sedláková, 2010; Pérez-García et al., 2009). Initial symptoms of powdery mildew include white mycelium over leaves, stems and petioles (Figure 1) (De Miccolis Angelini et al., 2019). Other symptoms, such as chlorotic leaf tissue and early senescence, can occur under favorable conditions (De Miccolis Angelini et al., 2019). Previous studies, conducted 30 years ago in Puerto Rico indicated that powdery mildew in *Cucurbita moschata* Duchesne is caused by both species, *G. cichoracearum* being the most abundant (Cienfuegos, 1991).

From November 2021 to February 2022, pumpkin cultivars ‘Waltham’ and ‘Ponca’ were sampled under greenhouse and field conditions at Isabela and Lajas Agricultural Experiment Substations (AES) of the University of Puerto Rico. Leaf samples showing white powdery mycelia and chlorotic lesions were collected multiple times during the crop season (Table 1) at both locations (no. of samples = 43) (November [N1] = 8; December [D2] = 10; January [J3] = 12; February [F4] = 13). These symptoms were not observed on fruits. Fungal mycelium and conidia were collected from powdery mildew observed on leaves using a sterile paint brush and placed in a 2 ml microtube. Samples were characterized morphologically and molecularly.

For morphological characterization, fresh powdery mildew samples collected from the leaves of *C. moschata* cultivars ‘Waltham’ and ‘Ponca’ were mounted in sterile-distilled water on a microscope slide and examined under a light microscope (40X, Olympus, Model CX33, Melville, NY)⁷ (Cienfuegos, 1991; Lebeda, 1983). In addition, humid

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³Graduate Student, Department of Agro-environmental Science, University of Puerto Rico, Mayagüez Campus.

⁴Associate Professor, Department of Agro-environmental Science, University of Puerto Rico, Mayagüez Campus.

⁵Professor, Department of Agro-environmental Science, University of Puerto Rico, Mayagüez Campus.

⁶Research Technician, Agricultural Experiment Station of Isabela, University of Puerto Rico, Mayagüez Campus.

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FIGURE 1. Powdery mildew symptoms observed in *Cucurbita moschata* Duchesne ‘Waltham’ leaves.

chambers were prepared with Petri dishes to observe conidial germination tubes (Cook and Braun, 2009). Humid chambers were left in the dark at room temperature for 24 hours before microscopic examination. Images were generated with the Infinity lite microscope installed camera (Lumenera Corporation, Ottawa, ON).

For molecular identification, genomic DNA was extracted using a Qiagen Plan Mini Kit. The internal transcribed spacer (ITS) of the 5.8S rDNA was amplified via PCR with powdery mildew-specific primers PMITS1/PMITS2 (Cunnington et al., 2003), followed by a nested PCR with primers PMITS1/ITS4 (Cunnington et al., 2003; White et al., 1990). Samples were sequenced by Psomagen (Rockville, MD, USA), and nucleotide sequences were verified and deposited in GenBank.

Morphologically, all powdery mildew isolates examined produced hyaline conidia in chains from erected conidiophores on leaf surfaces. Conidia were elliptically shaped and showed the presence of fibrosin bodies (Figure 2a). Conidial ($n=50$) dimensions were 26.3 to 35.8 (31.3) μm x 14.3 to 23.6 (17.8) μm , similar to those previously described for *P. xanthii* (Bojórquez-Ramos et al., 2012; Lebeda, 1983). Conidial germination occurred laterally with germination tubes either broad and bifurcated or apically swollen (Figures 2b and 2c). Germination tube development was slow (+5h). Sexual reproduction structure or chasmothecia was not observed during this study.

TABLE 1.—*GenBank accession numbers for Podosphaera xanthii sequences of internal transcribed space (ITS) obtained in this study.*

	Isolate code ¹	Location	Month of collection	GenBank accession numbers
<i>P. xanthii</i>	N1	Isabela	November 2021	OP882307
	D2	Lajas	December 2021	OP882308
	J3	Lajas	January 2022	OP882309
	F4	Lajas	February 2022	OP882310

¹Isolates obtained from surveys at Isabela and Lajas AES from November (N1) and December (D2) 2021; January (J3) and February (F4) 2022.



FIGURE 2. Morphological identification of *Podosphaera xanthii*, causing powdery mildew of *Cucurbita moschata* Duchesne. (40X magnification) (a) Elliptical shape conidia showing fibrosin bodies (arrows) produced in chain from erected conidiophores. Conidial germination tubes were (b) broad and bifurcated or (c) apically swollen.

Molecular analysis of the ITS sequences of four isolates (N1, D2, J3 and F4, GenBank Accession No. OP882307, OP882308, OP882309 and OP882310, respectively) showed 99 to 100% homology with *Podosphaera xanthii* (Accession No. KX369541 and KX842351) and *P. fusca* (Accession No. KJ698669). Nucleotide sequences were deposited in GenBank (Table 1). A phylogenetic tree was generated using Molecular Evolutionary Genetics Analysis X (MEGA X) on powdery mildew samples collected (N1, D2, J3 and F4), *Podosphaera xanthii* (Accession No. KX369541 and KX842351) and *Podosphaera fusca* (Accession No. KJ698669) as reference sequences. Other *Podosphaera* spp. reference sequences used from GenBank were *P. clandestina* (Accession No. MW168800), *P. longiseta* (Accession No. MK530460) and *P. pannosa* (Accession No. OM278384). Nucleotide sequences of *G. cichoracearum* (Accession No. EF010914) and *Leveillula taurica* (Accession No. MH698492) were added as outgroups (Figure 3) (Kumar et al., 2018).

Based on our findings, we concluded that the primary causal agent of powdery mildew in *C. moschata* at Lajas and Isabela substations was *P. xanthii*, not *G. cichoracearum*. *Golovinomyces cichoracearum* conidia does not show fibrosin bodies, and germination

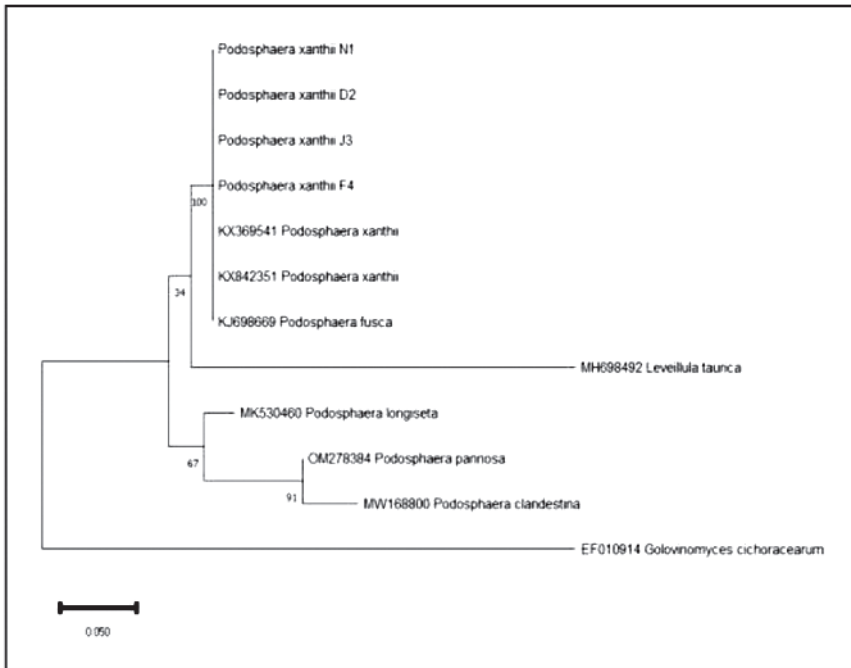


FIGURE 3. Phylogenetic tree generated with the "Test Maximum Likelihood Tree" method using the products of ITS sequences of four powdery mildew samples (N1 = November; D2 = December; J3 = January; F4 = February), *Podosphaera xanthii* reference sequences (KX369541 and KX842351), *Podosphaera fusca* reference sequence (KJ696669) *Podosphaera longiseta* (MK530460), *Podosphaera pannosa* (OM278384), *Podosphaera clandestina* (MW168800), *Leveillula taurica* (MH698492) and *Golovinomyces cichoracearum* sequence (EF010914) from GenBank. Bootstrap parameter was set to 1000 repetitions

tubes are simple, not bifurcated or swollen at the tip, as in *P. xanthii*. Nonetheless, *G. cichoracearum* might be present in other cucurbit species or in other areas of Puerto Rico not sampled during this study.

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