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Fungal species that cause powdery mildew in greenhouse-grown cucumber and melon in Paraná State, Brazil

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ABSTRACT. The powdery mildew caused by *Oidium* spp. is an important disease for several crops of the Cucurbitaceae family. Although the teleomorphs, *Podosphaera xanthii* and *Golovinomyces cichoracearum*, currently have already been described as the causal agents of powdery mildew in Brazil, only *P. xanthii* is considered the main causal agent of powdery mildew field epidemics. The objective of this work was to identify and determine the prevalence of the species causing powdery mildew in cucumber (*Cucumis sativus*) and melon (*Cucumis melo* var. *reticulatus*) grown in greenhouses in the State of Paraná in Brazil. The morphological traits of the conidial stages, such as the presence of fibrosin bodies and a germinative tube, were used to identify the species. Leaves exhibiting high severity of powdery mildew were collected from plants of 13 plastic greenhouses during different seasons in 2003/2004 and in different regions of Paraná State. In all environments, a significant prevalence of *P. xanthii* (80-100%) was observed affecting parthenocarpic or ordinary cucumber and melon. *Golovinomyces cichoracearum* was observed in six greenhouses, with up to 20% of conidia of this species on the samples.

Keywords: morphological characterization, grafting, *Podosphaera xanthii*, *Golovinomyces cichoracearum.*

Espécies de fungos que causam oídio em casas de vegetação cultivadas com pepino e melão no Estado do Paraná, Brasil

RESUMO. O oidio, causado por *Oidium* sp. é uma importante doença para espécies de plantas cultivadas da família das cucurbitáceas. Apesar das espécies teleomórficas *Podosphaera xanthii* e *Golovinomyces cichoracearum* já terem sido citadas como causadoras de oídio no Brasil, geralmente em trabalhos publicados atualmente tem-se referenciado somente a *P. xanthii* como agente causal dessa doença em cucurbitáceas em cultivo convencional. Por isso, este trabalho teve como objetivo identificar e quantificar a freqüência de ocorrência dessas duas espécies causadoras de oídio nas culturas de pepino (*Cucumis sativus*) e melão nobre (*Cucumis melo* var. *reticulatus*) conduzidas em estufas plásticas no Estado do Paraná. Para a identificação de *P. xanthii* e *G. cichoracearum* utilizaram-se as características morfológicas dos conídios (fase anamórfica), presença de corpos de fibrosina no interior dos conídios e características do tubo germinativo dos conídios. A amostragem foi realizada coletando-se folhas das plantas apresentando alta severidade de oidio em 13 estufas plásticas em 2003/2004 em diferentes regiões do estado do Paraná. Em todas as 13 estufas plásticas ocorreu à presença de *P. xanthii* em alta prevalência (80-100%). Entretanto, a presença de *G. cichoracearum* foi observada em seis estufas plásticas, apresentando freqüência de conídios característicos da espécie de até 20% nas amostras avaliadas.

Palavras-chave: caracterização morfológica, enxertia, *Podosphaera xanthii*, *Golovinomyces cichoracearum.*

Introduction

Powdery mildew is a common disease of both domesticated and wild species of cucurbits; it is one of the most destructive leaf diseases, affecting mainly melon (*Cucumis melo* L.) and cucumber (*Cucumis sativus* L.) among the several cucurbits. In the absence of chemical or biological control (ISHIDA et al., 2001; KUROZAWA et al., 2005; ZATARIN et al., 2005) or the use of resistant varieties (TEIXEIRA et al., 2008), the disease can significantly reduce yield and quality of the crop.

According to Reifschneider et al. (1985), six different fungal species have been reported to be associated with powdery mildew of cucurbits. *Sphaerotheca fuliginea* (Schlecht) Pollacci and *Erysiphe cichoracearum* DC. Ex Mérat, currently named *Podosphaera xanthii* and *Golovinomyces cichoracearum*, respectively (KUZUYA et al., 2006), are the most common and damaging species. *P. xanthii* can be represented by more than one strain and causes powdery mildew predominantly in tropical and subtropical climates (NARUZAWA et al., 2011;

COHEN et al., 2004). Conversely, *G. cichoracearum* is restricted to temperate climates (VAKALOUNAKIS et al., 1994), and less important in semi-arid climates.

Ballardin et al. (1997) have also reported that more than one species may occur simultaneously in the same area and even on the same plant. In their anamorph stage, *P. xanthii* and *G. cichoracearum* are called *Oidium* spp., the only stage reported in Brazil (STADNIK et al., 2001). The distinction between the two species may be made according to the morphology of the conidia, the morphology and localization of the germinative tube of the conidia, and the presence of fibrosin bodies in their interior (STADNIK et al., 2001; VAKALOUNAKIS et al., 1994).

Dry environmental conditions are the most favorable for the development and sporulation of *G. cichoracearum,* whereas *P. xanthii* requires higher humidity, and it is more commonly found in protected cultivation in regions of temperate climate (VAKALOUNAKIS et al., 1994). In Brazil, *P. xanthii* is mostly found under the conventional cropping conditions of cucurbits (outside of a greenhouse) (REIFSCHNEIDER et al., 1985). However, there are no studies reporting the existence of these two fungal species that cause powdery mildew in protected cultivation system such as plastic greenhouses, which is common in all the Brazilian territory, producing either an "umbrella effect" in regions with hot and rainy periods or a warming effect that accumulates heat in regions of cold periods (CARDOSO, 2002; CARDOSO; SILVA, 2003).

According to Vida et al. (2007), melon (*C. melo* var. *reticulates* and *C. melo* var. *cantalupensis*) in the summer and cucumber parthenocarpic in any season are the most-widely cultivated cucurbits in plastic greenhouses in these regions. Therefore, the warming effect of the plastic greenhouses during periods of lower temperature also creates another factor that interferes with the meteorological conditions, reducing the gradients of these temperatures during the day and night. Moreover, the minimum and maximum temperatures are much higher in plastic greenhouses compared to those in conventional cultivation. Thus, it is hypothesized differences in the presence of *G. cichoracearum* and *P. xanthii* in protected cultivation of melon and cucumber may be different from that found in conventional cultivation in Brazil.

In more recent studies regarding the powdery mildew in melon and cucumber in conventional or protected cultivation published in Brazil, only *P. xanthii* is cited as the etiological agent and represents the pathogen in the perfect form (teleomorfic state). (KUROZAWA et al., 2005; REIS; BUSO, 2004). For this reason, the objective of this work was to identify and assess the frequency of the fungal species that cause powdery mildew under the protected cultivation of melon and parthenocarpic cucumber in the State of Paraná, Brazil.

Material and methods

Leaf samples were collected from melon (*Cucumis melo* var. *reticulatus*, hybrid Bonus II), ungrafted parthenocarpic cucumber (*Cucumis sativus*, hybrids Hokushin, Natsubayashi and Samurai), grafted parthenocarpic cucumber (rootstock Shelper pumpkin, hybrid graft Hokushin) and ordinary cucumber (*C. sativus*, hybrid Safira) plants grown in plastic greenhouses (high-tunnel model) in the State of Paraná, Brazil. A total of 13 plastic greenhouses were visited during different periods of the year in 2003/2004 (Table 1).

Table 1. Summary information of the greenhouse cucurbit crops sampled for powdery mildew fungi. Maringá/Paraná State/Brazil, 2011.

Greenhouse code	Host type	Collection date	Municipality
1	[1] Natsubayashi	30/10/2003	Marialva
2	[1,2] Hokushin	31/10/2003	Maringá
3	[1] Hokushin	20/11/2003	Maringá
4	[3] Bonus II	04/02/2004	Marialva
5	[1] Natsubayashi	04/02/2004	Marialva
6	[4] Safira	02/02/2004	Sto. Antônio da Platina
7	[1] Natsubayashi	04/02/2004	Sto. Antônio da Platina
8	[1] Hokushin	29/03/2004	Maringá
9	[1] Samurai	27/08/2004	Maringá
10	[1] Samurai	30/09/2004	Marialva
11	[1] Samurai	30/09/2004	Marialva
12	[1] Hokushin	26/10/2004	Maringá
13	[1] Hokushin	13/12/2004	Maringá

[1]parthenocarpic cucumber; [2]grafted on Shelper pumpkin; [3]melon; [4]ordinary cucumber.

For each environment (plastic greenhouse), five plants showing well-developed pustules of *Oidium* spp. on the leaves were randomly chosen at the beginning of the harvesting. From each plant, the leaf exhibiting the highest severity of powdery mildew was selected, and the five leaves represented one sample. From each leaf, the conidia of several pustules were removed for evaluation.

The identification of the fungal species associated with powdery mildew was based on the characteristics of the conidia. To verify the shape of the conidia, microscope slides were prepared, and 100 conidia from each sample were analyzed. Conidia with parallel lateral walls and round borders were considered cylindrical, and those with some thinning at the edges were considered ovoid.

To confirm the presence of fibrosin bodies and their morphology, the conidia from the pustules on each leaf were transferred to a drop of KOH solution (pH 3.0) on a microscope slide, covered with a cover glass and observed under an optical

microscope, at 400 x magnification. A total of 100 conidia on each slide were analyzed, and the results are expressed as a percentage.

To characterize the type of germination and the germinative tube, the conidia removed from the pustules on the leaves were transferred to 2.0% agar-water medium in Petri dishes. The Petri dishes were then placed in a growth chamber at 26°C for 12 hours under fluorescent light. Then, visualization under an optical microscope was performed to evaluate approximately 100 germinated conidia for each sample. The characteristics noted were the position of the germinative tube (apical or lateral) and the type of germinative tube (simple or bifurcated). The results were expressed as a percentage. Approximately 100 conidia were observed under the microscope for the germination rate.

For the measurements, the conidia were transferred to a drop of liquid glycerin and observed under an optical microscope that contained a micrometric slide; length and width measurements were then performed. Fifty conidia retrieved from the pustules of each leaf were measured. To identify the fungal species found in the samples, the observed structures were compared with those described in the literature (ADAM et al., 1999; LEBEDA et al., 2002; STADNIK, 2001; VAKALOUNAKIS et al., 1994).

Results and discussion

Ovoid conidia accounted for 80% or more of the total number of conidia (Table 2) in each pustule of the melon, parthenocarpic cucumber, ordinary cucumber and grafted parthenocarpic cucumber leaves. In seven of the plastic greenhouses used to cultivate cucumber (greenhouses 5, 6, 7, 8, 10, 12 and 13), 100% of the conidia were ovoid. However, the percentages of the cylindrical-shaped conidia were low (2% maximum) in the samples from four of the plastic greenhouses (numbers 1, 3, 9 and 11). In the greenhouse cultivated with parthenocarpic cucumber grafted on Shelper pumpkin (number 2), cylindrical conidia accounted for 20% of the conidia.

There was no apparent prominence of either the conidium shape or the presence of fibrosin bodies associated with any specific hybrid. A similar lack of correlation was observed for the period of the year and the site of conidium collection.

The presence of yellowish to bluish fibrosin bodies was observed in the interior of most of the ovoid conidia (between 97 and 100%), and the presence of fibrosin bodies was not observed in only a few ovoid conidia. According to Stadnik et al. (2001), fibrosin bodies are evident only in young conidia. Because the sampling was carried out from several sites and different stages were collected, it is possible that some of the material was dry and unviable, thus fibrosin bodies in the interior of the conidia were not found.

The presence of fibrosin bodies was not observed inside of any of the cylindrical conidia. According to Vakalounakis et al. (1994) and Stadnik et al. (2001), the presence or absence of fibrosin bodies inside the conidia of *Oidium* spp. is an indicator of the telemorphic forms of *P. xanthii* and *G. cichoracearum*, respectively. According to these authors, *P. xanthii* also has ovoid conidia, whereas *G. cichoracearum*, in general, has cylindrical conidia.

Table 2. Shape of conidia of *Oidium* sp. from cucumber and melon cultivated in plastic greenhouses. Maringá/Paraná State/Brazil, 2011.

Greenhouse number	Host of origin	Shape of conidia			
		Ovoid (%)		Cylindrical (%)	
		T	CF	T[1]	CF[1]
1	[1] Natsubayashi	98	98	2	0
2	[1,2] Hokushin	80	100	20	0
3	[1] Hokushin	98	100	2	0
4	[3] Bonus II	98	100	2	0
5	[1] Natsubayashi	100	97	0	0
6	[4] Safira	100	100	0	0
7	[1] Natsubayashi	100	97	0	0
8	[1] Hokushin	100	100	0	0
9	[1] Samurai	99	90	1	0
10	[1] Samurai	100	95	0	0
11	[1] Samurai	99	98	1	0
12	[1] Hokushin	100	96	0	0
13	[1] Hokushin	100	100	0	0

[1]parthenocarpic cucumber; [2]grafted on Shelper pumpkin; [3]melon; [4]ordinary cucumber; T - conidia per shape; CF - conidia with fibrosin bodies.

In relation to the position of the germinative tube and its morphology, we observed that among the samples with conidia germinated in an ovoid shape, those from the greenhouses number 1, 2 and 3 presented predominantly lateral germination (93.3, 84.4 and 99%, respectively), and a low percentage germinated in the apical position (6.7, 15.6 and 1.0%, respectively) (Table 3). For conidia with the germinative tube in the lateral position, a high percentage of both simple and bifurcated tubes were found. For the other 10 plastic greenhouses, ovoid conidia germinated with an apical germinative tube were not observed. For these plastic greenhouses, most of the germinated ovoid conidia presented a lateral germinative tube. However, the sample from plastic greenhouse number 6 was an exception, as 100% of the conidia had a lateral germinative tube.

According to Stadnik et al. (2001) small variations may occur in the morphological characteristics of conidia and of germinative tubes of *Oidium* spp. used for the identification of the telemorphic forms *G. cichoracearum* and *P. xanthii*, and the dominant frequency of these characteristics in the samples of conidia must be taken into account.

Table 3. Germination of conidia of *Oidium* spp. and characterization of the germinative tube. Maringá/Paraná State/Brazil, 2011.

Plastic greenhouse	Host of origin	Ovoid							Cylindrical						
		% G[5]	% TA[5]			% TL[5]			% G[5]	% TA[5]			% TL[5]		
			T[5]	S[5]	B[5]	T[5]	S[5]	B[5]		T[5]	S[5]	B[5]	T[5]	S[5]	B[5]
1	[1] Natsubayashi	21.8	6.7	100.0	0.0	93.3	57.1	42.9	50.0	100.0	100.0	0.0	0.0	0.0	0.0
2	[1,2] Hokushin	14.3	15.6	100.0	0.0	84.4	85.2	14.8	63.2	100.0	100.0	0.0	0.0	0.0	0.0
3	[1] Hokushin	14.4	1.0	100.0	0.0	99.0	42.9	57.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	[3] Bonus II	5.8	0.0	0.0	0.0	100.0	50.0	50.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	[1] Natsubayashi	4.0	0.0	0.0	0.0	100.0	75.0	25.0	⋆	⋆	⋆	⋆	⋆	⋆	⋆
6	[4] Safira	2.0	0.0	0.0	0.0	100.0	0.0	100.0	⋆	⋆	⋆	⋆	⋆	⋆	⋆
7	[1] Natsubayashi	8.1	0.0	0.0	0.0	100.0	87.5	12.5	⋆	⋆	⋆	⋆	⋆	⋆	⋆
8	[1] Hokushin	14.7	0.0	0.0	0.0	100.0	64.3	35.7	⋆	⋆	⋆	⋆	⋆	⋆	⋆
9	[1] Samurai	12.8	0.0	0.0	0.0	100.0	72.7	27.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	[1] Samurai	14.6	0.0	0.0	0.0	100.0	86.7	13.3	⋆	⋆	⋆	⋆	⋆	⋆	⋆
11	[1] Samurai	9.7	0.0	0.0	0.0	100.0	53.9	46.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
12	[1] Hokushin	1.4	0.0	0.0	0.0	100.0	47.1	52.9	⋆	⋆	⋆	⋆	⋆	⋆	⋆
13	[1] Hokushin	8.5	0.0	0.0	0.0	100.0	55.6	44.4	⋆	⋆	⋆	⋆	⋆	⋆	⋆

[1]parthenocarpic cucumber; [2]grafted on Shelper pumpkin; [3]melon; [4]ordinary cucumber, [5]G- germinated conidia; TA- apical germinative tube; TL- lateral germinative tube; T- total of germinative tubes in position; S- simple germinative tube; B- bifurcated germinative tube; ⋆Absence of cylindrical conidia.

Stadnik et al. (2001) reported that the species causing powdery mildew with simple-shaped conidia with a germinative tube in the apical position correspond to the telemorphic species, *G. cichoracearum*. For plastic greenhouse number 3 (hybrid Hokushin grafted onto Shelper pumpkin), the sample of germinated conidia is consistent with this pattern. The authors also stated that the species of *Oidium* that present conidia with a bifurcated germinative tube in the lateral position correspond to the telemorphic species, *P. xanthii*.

The germination of cylindrical conidia was much higher than the germination of ovoid conidia during the 12 hours (incubation period). According to Stadnik et al. (2001), the conidia of the anamorphic form of *P. xanthii* germinated faster than the conidia of the anamorphic form of *G. cichoracearum* (Figure 1).

Concerning the dimensions of the samples (Table 4), the average value for the length and width of the ovoid conidia are consistent with those described in the literature (30.6 x 18.6 μm) for *P. xanthii* (VAKALOUNAKIS et al., 1994) for all of the samples from the 13 plastic greenhouses. The length/width ratio may be compartmentalized into the following three groups: values up to 1.6 for the conidia of the samples from plastic greenhouse numbers 5, 9, 10, 12 and 13; values from 1.6 to 1.85 for the conidia from plastic greenhouse numbers 1, 3, 4 and 11; and values above 1.85 for the conidia of all of the other plastic greenhouses.

For the conidia sampled from plastic greenhouse number 2, the average value of the length was standard for *G. cichoracearum* (29.9 μm). The average width values and the length/ width ratio of the conidia were 12.7 and 2.49 μm, which were above those cited in the literature, 15 and 2.0 μm, respectively (ADAM et al., 1999). It is possible that the cultivation conditions in the protected environment influenced the dimensions of the conidia.

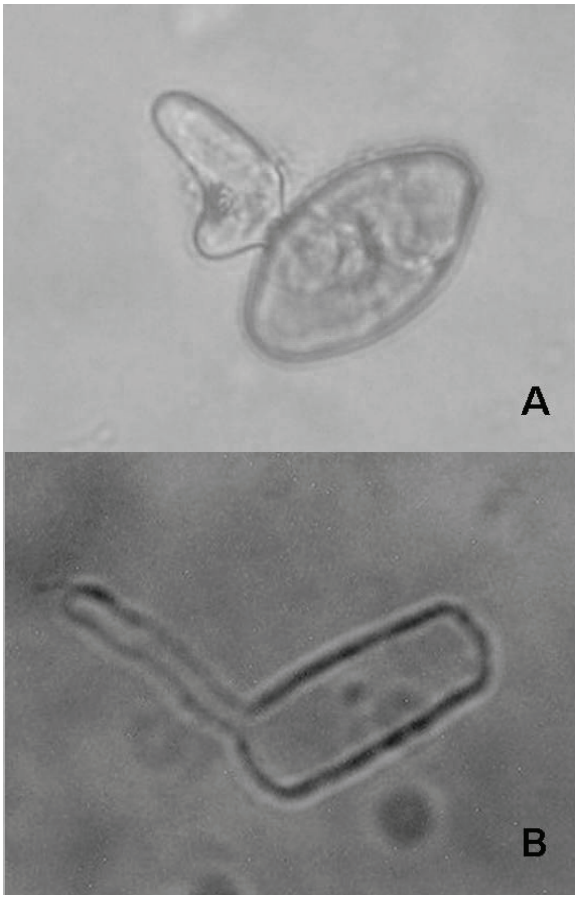

Figure 1. Ovoid conidia with a bifurcated lateral germinative tube (A), and cylindrical conidia with a simple apical germinative tube (B).

Table 4. Dimensions of *Oidium* spp. conidia originating from parthenocarpic cucumber, ordinary cucumber and melon. Maringá/Paraná State/Brazil, 2011.

Greenhouse number	Host of origin	Length (μm)	Width (μm)	Length/width ratio
1	[2] Natsubayashi	29.50[6] ± 1.55[7] (31.00[8] – 27.90[9])	16.79[6] ± 1.47[7] (18.60[8] – 15.50[9])	1.77[6] ± 0.19[7] (2.00[8] – 1.50[9])
2-C[1]	[2,3] Hokushin	31.60 ± 3.23 (38.75 – 24.80)	12.74 ± 1.14 (15.50 – 10.85)	2.49 ± 0.25 (3.00 – 1.90)
2-O[1]	[2] Hokushin	31.36 ± 2.18 (34.10 – 27.90)	17.05 ± 1.63 (20.15 – 15.50)	1.85 ± 0.18 (2.20 – 1.50)
3	[2] Hokushin	30.28 ± 1.71 (34.10 – 27.90)	16.53 ± 1.43 (18.60 – 15.50)	1.85 ± 0.20 (2.20 – 1.50)
4	[3] Bônus II	30.02 ± 1.70 (34.10 – 27.90)	18.29 ± 1.93 (24.80 – 15.50)	1.66 ± 0.22 (2.20 – 1.25)
5	[2] Natsubayashi	27.95 ± 1.93 (31.00 – 24.80)	17.67 ± 1.61 (21.70 – 15.50)	1.59 ± 0.13 (1.90 – 1.36)
6	[5] Safira	30.74 ± 2.61 (37.20 – 24.80)	16.38 ± 1.81 (18.60 – 12.40)	1.90 ± 0.29 (2.63 – 1.50)
7	[2] Natsubayashi	34.77 ± 2.56 (43.40 – 31.00)	18.65 ± 1.84 (21.70– 15.50)	1.88 ± 0.17 (2.30 – 1.57)
8	[2] Hokushin	35.08 ± 3.00 (43.4 – 31.00)	18.65 ± 1.84 (21.70 – 15.50)	1.89 ± 0.19 (2.33 – 1.57)
9	[2] Samurai	30.23 ± 1.94 (34.10 – 27.90)	19.12 ± 2.24 (27.90 – 15.50)	1.59 ± 0.15 (1.90 – 1.22)
10	[2] Samurai	35.08 ± 4.04 (52.70 – 31.00)	22.37 ± 1.85 (27.90 – 18.60)	1.57 ± 0.16 (1.92 – 1.25)
11	[2] Samurai	31.05 ± 2.32 (37.20 – 27.90)	18.50 ± 2.03 (21.70 – 12.40)	1.7.0 ± 0.20 (2.25 – 1.29)
12	[2] Hokushin	33.33 ± 2.11 (37.20 – 31.00)	23.35 ± 1.72 (27.90 – 21.70)	1.44 ± 0.15 (1.71 – 1.25)
13	[2] Hokushin	31.74 ± 3.03 (40.30 – 27.90)	20.96 ± 2.57 (27.90 – 15.50)	1.53 ± 0.18 (1.90 – 1.13)

[1]2-C: cylindrical conidia; 2-O: ovoid conidia; [2]parthenocarpic cucumber; [3]grafted on Shelper pumpkin: [4]melon; [5]ordinary cucumber; [6]average value; [7]standard deviation; [8]higher value observed; [9]lower value observed.

Based on the results obtained for the morphological characteristics of the conidia, the type of germination and germinative tube and the dimensions of the conidia, it was possible to identify the presence of teleomorphical forms of *Oidium* spp., *P. xanthii* and *G. cichoracearum*. *Podosphaera xanthii* was found in all 13 of the plastic greenhouses, infecting ungrafted and grafted parthenocarpic cucumber, ordinary cucumber and melon, in much higher frequencies than *G. cichoracearum*. In plastic greenhouse number two, *G. cichoracearum* was found at a frequency of 20%, probably because it was the only with cucumber grafted on Shelper pumpkin. In plastic greenhouse number one, three, four, nine and eleven, the frequency of this species was up 2.0%.

Conclusion

Two fungal species causing cucurbit powdery mildew, *P. xanthii* and *G. cichoracearum,* occur in greenhouses in the State of Paraná, Brazil, with a much larger predominance of the former species.

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