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Identification and variability of *Monilinia* spp. isolates from peach

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ABSTRACT

The brown rot caused by the fungus *Monilinia* spp. is the main disease of peach (*Prunus persica*) that occurs from flowering to post-harvest fruit. The objective of this study was to identify the species that causes brown rot in Rio Grande do Sul state using molecular method based on specific *primers* and study the morphological variability of *Monilinia* isolates using classic characterization methods. The isolates were collected in the main producing regions of peaches and determination of species was performed by using specific *primers* to the genus *Monilinia*. The culture media BDA®, peach juice and Pagnocca were used for the morphological characterization of isolates. Molecular study showed that all isolates from Rio Grande do Sul are the species *M. fructicola*. In the morphological characterization, such as mycelial diameter, color, margin, shape and elevation of the colonies and dry mass of mycelium, there are variability in the analyzed characteristics of the isolates in the three-culture media used. In the comparing the culture media, the higher dry matter values and diameter of colony isolates were obtained in Pagnocca and peach juice means.

Key words: phenotypic characterization; brown rot; *Prunus persica*

Identificação e variabilidade de isolados de Monilinia spp. de pessegueiro

RESUMO

A podridão parda causada pelo fungo *Monilinia* spp. é a principal doença que ocorre desde a floração até a pós-colheita de frutos de pessegueiro (*Prunus persica*) no Brasil. O objetivo deste trabalho foi identificar a espécie causadora da podridão parda no Rio Grande do Sul usando método molecular baseado em *primers* específicos e estudar a variabilidade morfológica de isolados de *Monilinia* através dos métodos clássicos de caracterização. Os isolados foram coletados nas principais regiões produtoras de pêssegos e a determinação da espécie foi realizada através da utilização de *primers* específicos para o gênero *Monilinia*. Os meios de cultura BDA®, Suco de pêssego e Pagnocca foram utilizados para a caracterização morfológica dos isolados. Pelo estudo molecular verificou-se que todos os isolados do Rio Grande do Sul são da espécie *M. fructicola*. Na caracterização morfológica, como diâmetro micelial, coloração, margem, forma e elevação das colônias e massa seca de micélio, existem variabilidade nas características analisadas dos isolados nos três meios de cultura utilizados. Na comparação entre os meios de cultura, os maiores valores de massa seca e diâmetro de colônia dos isolados foram obtidos nos meios Pagnocca e suco de pêssego.

Palavras-chave: caracterização fenotípica; podridão parda; *Prunus persica*

Introduction

The brown rot caused by the fungus *Monilinia fructicola* (Wint.) Honey is the most destructive disease of the peach tree *Prunus persica* (L.), causing infection in the flowers, cancer of branches and rotting of the green and ripe fruits (Ogawa et al., 1995; Gell et al., 2007).

Three species of *Monilinia* cause brown rot in the world peach producing regions: *M. fructicola* (Wint) Honey, *M. laxa* (Aderh & Ruhl.) Honey and *M. fructigena* (Aderh & Ruhl.) Honey. In Brazil, the only species reported causing severe damage and identified through morphological characteristics was *M. fructicola* (Ogawa et al., 1995). However, in recent years *M. laxa* has been reported in peach orchards in the state of São Paulo (Souza et al., 2008); until then, this species was reported only in orchards in Europe and South Africa (De Cal & Melgarejo, 1999; Gibert et al., 2009). Currently, *M. fructicola* has occurred in Asia (China, India, Japan, the Republic of Korea, Taiwan and Yemen), in Africa (Nigeria and Zimbabwe), in the Americas (Argentina, Bolivia, Brazil, Canada, Guatemala, Ecuador, Mexico, Panama, Paraguay, Peru, Uruguay, the USA and Venezuela) and in Oceania (Australia, New Caledonia and New Zealand) (CABI/EPPO, 2010; EFSA, 2011).

The phenotypic and genotypic characterization techniques are crucial for the identification and knowledge of the variability among isolates of a given pathogen. Currently, the morphological characterization, combined with the use of specific primers for the identification of *Monilinia spp.*, has allowed verifying phenotypic variations among isolates. This, in addition to facilitating the identification at the species level of fungi causing brown rot, makes possible the planning of control strategies (De Cal & Melgarejo, 1999; Côté et al., 2004; Poniatowska et al., 2013).

The methods for identifying *Monilinia* species include morphological characterization and cultural characteristics (De Cal & Melgarejo, 1999) allied to molecular techniques (Côté et al., 2004). According to Lane (2002), there is no specific morphological character that will reliably identify species of this genus, but the three species of the genus can be discriminated by investigating several morphological characteristics simultaneously, such as conidial size, formation of the germ tube, color, margin and rate of colony growth, among others. On the other hand, molecular techniques allow the rapid and reliable identification of *Monilinia* species, mainly the PCR technique (Côté et al., 2004).

Studies using molecular techniques make it possible to verify the variations that occur in the phytopathogenic species. Villarino et al. (2012) verified genetic diversity of *M. fructicola* in French and Spanish isolates, compared to isolates from California, Uruguay and New Zealand. Fan et al. (2010) found in *M. fructicola* isolates 93% of genetic variance within the regional populations and about 7% of the total genetic variation was due to geographical separations between the regional populations.

The determination of *Monilinia* species using specific primers is a useful, fast and reliable technique that guarantees the precise identification of the species causing brown peach rot in a given region. Therefore, the objective of this work was to identify the species responsible for brown rot in Rio Grande do Sul by using a molecular method based on specific primers and to study the morphological variability of *Monilinia* isolates through classical characterization methods.

Material and Methods

Obtaining the isolates

The isolates of *Monilinia* spp. were carried out from intact or mummified infected fruit of different peach cultivars with typical symptoms of brown rot from the producing regions of Rio Grande do Sul - Brazil, as described in Table 1.

For isolation, the fungal structures were aseptically transferred using a platinum loop to Petri dishes containing potato-dextrose-agar (PDA) culture medium, and thereafter incubated under a 12-hour light photoperiod at 22° C for seven days.

Table 1. Identification of *M. fructicola* isolates obtained from different peach cultivars, collection site in the state of Rio Grande do Sul, and isolated plant organ.

No.	Code/Cultivar	City	Isolated plant organ
1.	Aldrighi (A)	Pelotas	Fruit
2.	Amarelo (AR)	Pelotas	Fruit
3.	Amarelo (1AR)	Colônia Maciel	Fruit
4.	Amarelo Molar (ARM)	Monte Bonito	Fruit
5.	Bolinha (1B)	Pelotas	Fruit
6.	Branco Molar (1BM)	Morro Redondo	Fruit
7.	Branco Molar (4BM)	-	Fruit
8.	Branco Molar (2BM)	Caxias do Sul	Fruit
9.	Branco Molar (5BM)	Canguçu	Fruit
10.	Branco Molar (6BM)	Monte Bonito	Fruit
11.	Cardeal (CD)	Canguçu	Fruit
12.	Chimarrita (1C)	Colônia Maciel	Fruit
13.	Chiripá (1CP)	-	Mummified Fruit
14.	Chiripá (2CP)	Colônia Maciel	Fruit
15.	Diamante (D)	Pelotas	Fruit
16.	Eldorado (1EL)	Pelotas	Fruit
17.	Eldorado (2EL)	Canguçu	Fruit
18.	Esmeralda (1E)	Pelotas	Fruit
19.	Esmeralda (2E)	Canguçu	Fruit
20.	Granada (1G)	Pelotas	Fruit
21.	Granada (2G)	Vila Nova	Fruit
22.	Granada (3G)	-	Fruit
23.	Granada (4G)	Canguçu	Fruit
24.	Granada (5G)	Caxias do Sul	Fruit
25.	Granada (6G)	-	Fruit
26.	Jade	Pelotas	Fruit
27.	Jade (JR)	Piratini	Fruit
28.	Jade (2JR)	Cerrito	Fruit
29.	Maciel (3MV)	Colônia Maciel	Fruit
30.	Molar (M)	Colônia Maciel	Fruit
31.	Molar (1M)	Pelotas	Fruit
32.	Molar (2M)	Caxias do Sul	Fruit
33.	Molar (3M)	Porto Alegre	Fruit
34.	Pit	Pelotas	Fruit
35.	Turquesa (T)	Pelotas	Fruit

Genomic DNA extraction and PCR

The mycelium of 34 isolates of *Monilinia* spp. grown in BDA® medium was macerated in liquid nitrogen and then the DNA was purified with the CTAB method, as described by Doyle & Doyle (1991). At the end of the procedure, the DNA precipitates were dissolved in 100 µl TE (Tris-

HCl, 10 mM, pH 8.0; 1 mM EDTA). After 24 hours, the purified genomic DNA was quantified by 1% agarose gel electrophoresis compared to the Lambda Phage DNA marker digested with *Hind* III (Invitrogen). For use in the Polymerase Chain Reaction (PCR), the samples were diluted to the final concentration of 10 ng $\mu l^{-1}$of DNA. The primers used were: MO368-5 (5'-GCAAGGTGTCAAAACTTCCA–3') as a 3'-5' sense primer, universal for *Monilinia* spp. and the three primers 5'-3 ': MO368-8R (5'–AGATCAAACATCGTCCATCT–3') specific for *M. fructigena*, MO368-10R (5'-AAGATTGTCACCATGGTTGA–3') specific for *M. fructicola* and Laxa-R2 (5'–TGGACATCATATCCCTCGAC–3') specific for *M. laxa*.

The reactions were performed in a PTC-100 thermocycler (MJ Research), with the following parameters: five cycles at 95° C for 30 s (denaturation), 60° C for 60 s (annealing), 72° C for 30 s (initial extension), followed by 35 cycles at 95° C for 30 s (denaturation), 58° C for 60 s (annealing), 72° C for 30 s (initial extension) and a final extension of 5 minutes at 72° C. The horizontal electrophoresis of the PCR products was performed in 1% agarose gel, stained with ethidium bromide and visualized in ultraviolet (UV) light transilluminator. As standard for comparison of the size of the amplified bands, the molecular weight marker DNA Ladder 100pb (Invitrogen) was used.

Mycelial growth and morphological characterization

For the studies of mycelial growth, shape and color of the 35 isolates of *Monilinia* spp., mycelial disks were transferred to Petri dishes containing PDA culture medium. For each isolate (Table 1), an 8 mm diameter disk containing the actively growing mycelium was individually deposited in the center of the Petri dish containing 15 mL $plate^{-1}$ of the PDA® culture medium (40g of PDA and 1000 mL of distilled water), peach juice + agar (200 mL of peach juice, 3g of $CaCO_3$, 15 g of agar and 800 mL of distilled water (Xiao & Sitton, 2004) and Pagnocca (10g glucose, 5g of NaCl, 5g of peptone, 10g of yeast extract, 17g of agar, 20g of hydrolyzed casein, 20g of soybean flakes, 20g of oat flakes and distilled water to obtain the final volume of 1000 mL) (Silva-Pinhati et al., 2005).

The experimental design for the study of these variables was entirely randomized in a 3 x 35 factorial scheme (three culture media x 35 isolates), each plate being considered as a repetition, totaling six plates/isolate. Subsequently, the plates were maintained at 22° C with 12-hour of light photoperiod.

In the morphological characterization, the color of the mycelium on the back of the plate was evaluated according to the color notation system described by Munsell Color Company (1954); the shape, the margin and the elevation of the colonies, according to the classification of Capuccino & Sherman (1998); and the topographic aspect of the colonies. The diameter of each mycelium colony was also determined, which was evaluated on the seventh day of incubation, whereas colony coloration and elevation and margin type and color were performed when the first isolate of each treatment reached the border of the plate. The results were submitted to ANOVA and Tukey and Scott-Knott averages comparison test at 5% of error probability through the GENES program (Cruz, 2013).

Quantification of the dry mass of the mycelium

For quantification of the dry mass of the mycelium of each of the 35 isolates, two 8 mm diameter disks containing the actively growing mycelium (Table 1) were transferred to 125 ml Erlenmeyer flasks containing 50 ml of the liquid culture media of potato-dextrose, Pagnocca and peach juice with no agar, and then they were kept under stirring (160 rpm) at 22° C for five days. At the end of this period, the cultures were filtered on sterilized filter paper and dried in an oven for three days at 40° C. Subsequently, the mycelial dry mass of each isolate was determined.

The experimental design was completely randomized in the 3 x 35 factorial scheme (three culture media x 35 isolates) with four replicates. The results were submitted to ANOVA and to the Tukey test at 5% of error probability through the GENES program (Cruz, 2013).

Results and Discussion

The identification of the species of the isolates of *Monilinia* spp. was confirmed with the specific primers MO368-5 and MO368-10R, as a PCR fragment of 535 base pairs was observed for all the studied isolates (Figures 1 and 2). This fragment size is expected only for *Monilinia fructicola* specimen, as described by Côté et al. (2004). In a study by Pizzuolo et al. (2006), the authors, using the RAPD technique, confirmed the identification of species of *Monilinia* isolates performed by morphological criteria. This fact was also verified by Hu et

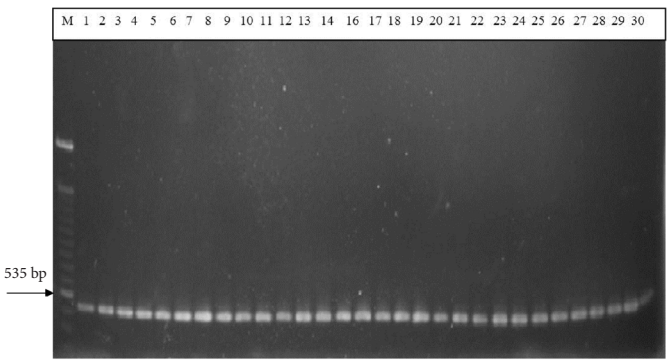

Figure 1. Fragments of PCR products on 1% agarose gel, obtained by amplification of the DNA samples of the isolates of *Monilinia* spp. with the primer MO368-5 specific for *Monilinia fructicola*; M = 100 bp Lambda Phage DNA marker (Invitrogen); 1-30 = *Monilinia isolates*.

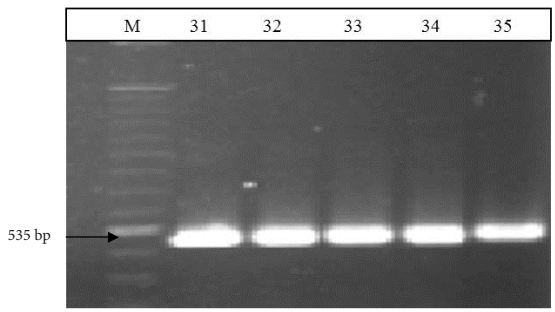

Figure 2. Fragments of PCR products on 1% agarose gel, obtained by amplification of the DNA samples of the isolates of *Monilinia* spp. with the primer MO368-5 specific for *Monilinia fructicola*; M = 100 bp Lambda Phage DNA marker (Invitrogen). 31-35 = *Monilinia isolates*.

al. (2011) with *Monilinia* spp. isolates causing brown rot in peaches in China.

The *M. fructicola* isolates collected in the peach orchards of Rio Grande do Sul and identified by PCR have a wide morphological variation, besides that the appearance of the colonies is variable in relation to the culture medium used, and there is a significant interaction between isolates and culture media. According to Poniatowska et al. (2013), there is variation of the colony appearance between the different isolates of *Monilinia* species when they were evaluated in three different culture media.

For the mycelial growth evaluations there was a significant interaction between culture media and isolates. Of the 35 isolates, eight (2M, D, AR, B, 1CP, 4G, JADE and 6BM) had higher mycelial diameter values in the peach juice medium, followed by Pagnocca medium, with seven isolates (1E, 1BM, 6G, 2E, 3, PIT and 1AR) with larger diameter and five isolates (2CP, 2EL, A, 5BM and 1C) with higher mycelial diameter in the PDA medium (Table 2).

When evaluating differences of mycelial diameter between the isolates in the culture media, ten different groups were formed in the PDA medium, with growth diameters between 0.63 and 5.95 cm; the isolates 2CP, 2EL and A had the largest diameters, whereas the isolates 2M, 6BM, 2G, 5G and M had the lowest mycelial growth. In the peach juice medium five different groups were formed, with values between 2.03 and 4.27 cm, in which the isolates A, B, D and 2M had the largest diameter and the isolate 3G had the smallest diameter. For the Pagnocca medium of the five clusters formed (1.97> diameter <4.14 cm), the isolates with the largest diameter were A, 3M, 1BM and 1E and the lowest values were found in the isolates 2EL, B, 1CP, 4G and D (Table 2). However, for the isolates 2JR, JR and 3MV there were no significant differences in mycelial diameter between the three culture media used.

Developmental differences between *Monilinia laxa* isolates in culture media were reported by Tian & Bertolini (1999), with peach juice being the best medium for mycelial growth. However, Poniatowska et al. (2013) found that the highest mean of mycelial growth for the *M. fructicola* species was obtained in the PDA culture medium, followed by the cherry juice medium. In general, in the present study of the 35 isolates, 17 of them had a larger mycelial diameter in the peach juice medium when compared to the PDA medium.

Significant interaction between culture media and *Monilina* spp. isolates was also verified for the measurements of dry mass of mycelium, and all 35 isolates evaluated had the highest values of dry mass of mycelium in the Pagnocca medium in comparison to the other media. In the peach juice medium, only the isolate CD had a similar dry mass value as that obtained in the Pagnocca medium. In the PDA culture medium, the isolates with the lowest dry mass of mycelium were: 1EL, 1E, 2G, B, M, 2EL, T, 1C, 2E, 1AR and 6G (Table 3).

Table 2. Mycelial diameter (cm) of *Monilinia fructicola* isolates in PDA, peach juice and Pagnocca culture media incubated for seven days at 22 ± 3 °C.

Isolates	PDA	Peach juice	Pagnocca
2CP	5.95 Aa	3.84 Bb	3.52 Bb
2EL	4.98 Ab	3.83 Bb	2.26 Ce
A	4.84 Ab	4.27 Ba	4.12 Ba
2JR	3.97 Ac	3.58 Ab	3.66 Ab
5BM	3.95 Ac	2.87 Bc	3.55 Ab
1C	3.93 Ac	2.75 Cc	3.33 Bc
3M	3.90 Ac	2.76 Bc	4.14 Aa
1M	3.77 Ac	3.78 Ab	3.12 Bc
2BM	3.61 Ac	3.65 Ab	2.55 Bd
JR	3.50 Ad	3.76 Ab	3.57 Ab
CD	3.40 ABd	3.10 Bc	3.52 Ab
B	3.18 Be	3.98 Aa	1.97 Ce
6G	3.17 Be	3.21 Bc	3.68 Ab
4BM	3.00 Ae	2.56 Bd	3.34 Ac
3G	2.73 Bf	2.03 Ce	3.56 Ab
1CP	2.65 Bf	3.78 Ab	2.29 Be
AR	2.62 Bf	3.81 Ab	2.72 Bd
3MV	2.53 Af	2.57 Ad	2.72 Ad
2E	2.43 Bg	2.47 Bd	3.59 Ab
JADE	2.27 Bg	3.69 Ab	2.49 Bd
1EL	2.25 Bg	2.94 Ac	3.08 Ac
1BM	2.22 Cg	3.04 Bc	3.76 Aa
1E	1.95 Ch	3.12 Bc	4.05 Aa
4G	1.93 Bh	3.74 Ab	2.12 Be
D	1.90 Bh	4.10 Aa	2.12 Be
T	1.90 Bh	2.37 ABd	2.59 Ad
1AR	1.90 Bh	2.36 Bd	3.46 Ab
1G	1.83 Bh	3.42 Ab	3.20 Ac
ARM	1.48 Bi	3.39 Ab	3.28 Ac
PIT	1.30 Ci	3.06 Bc	3.53 Ab
2M	1.07 Cj	4.18 Aa	2.95 Bd
6BM	1.00 Cj	3.54 Ab	2.97 Bd
2G	0.93 Bj	3.59 Ab	3.17 Ac
5G	0.92 Bj	2.53 Ad	2.81 Ad
M	0.63 Bj	3.00 Ac	3.31 Ac

* Means followed by the same capital letter in the row and lowercase in the column do not differ significantly at the 5% probability level, respectively, by the Tukey and Scott-Knott test.

Table 3. Dry mass (g) of mycelium of the *Monilinia fructicola* isolates in PDA, peach juice and Pagnocca culture media (Pelotas, RS).

Isolates	PDA	Peach juice	Pagnocca
ARM	0.30 Ba	0.31 Ba	0.78 Ab
CD	0.30 Ba	0.52 Aa	0.55 Ad
JADE	0.29 Ba	0.29 Ba	0.56 Ad
3MV	0.29 Ba	0.20 Bb	0.66 Ac
2BM	0.28 Ba	0.16 Cb	1.09 Aa
3G	0.27 Ba	0.28 Ba	0.53 Ad
PIT	0.26 Ba	0.28 Ba	0.76 Ab
4G	0.26 Ba	0.14 Cb	0.88 Ab
6BM	0.26 Ba	0.26 Ba	0.59 Ad
1G	0.26 Ba	0.16 Bb	0.85 Ab
5G	0.25Ba	0.25 Ba	0.86 Ab
3M	0.24 Ba	0.30 Ba	0.50 Ad
T	0.24 Ca	0.32 Ba	0.90 Ab
A	0.23 Ba	0.28 Ba	0.64 Ac
D	0.23 Ba	0.15 Cb	0.74 Ac
2CP	0.23 Ba	0.18 Bb	0.74 Ac
1CP	0.23 Ba	0.18 Bb	1.11 Aa
2JR	0.23 Ba	0.28 Ba	0.46 Ad
1M	0.21 Ba	0.27 Ba	0.82 Ab
1C	0.21 Ca	0.32 Ba	1.02 Aa
AR	0.18 Ba	0.21 Bb	0.83 Ab
JR	0.18 Ba	0.15 Bb	0.72 Ac
5BM	0.16 Bb	0.19 Bb	0.64 Ac
2M	0.16 Bb	0.23 Bb	0.59 Ad
4BM	0.16 Bb	0.19 Bb	0.80 Ab
1BM	0.15 Bb	0.20 Bb	0.58 Ad
1AR	0.14 Cb	0.26 Ba	0.84 Ab
1EL	0.13 Cb	0.36 Ba	0.66 Ac
1E	0.11 Cb	0.29 Ba	0.87 Ab
2EL	0.09 Cb	0.25 Ba	0.68 Ac
M	0.07 Cb	0.13 Bb	0.64 Ac
2G	0.06 Cb	0.31 Ba	0.98 Aa
2E	0.04 Cb	0.12 Bb	0.59 Ad
B	0.02 Cb	0.24 Ba	0.66 Ac
6G	0.02 Cb	0.21 Bb	0.77 Ab

* Means followed by the same capital letter in the row and lowercase in the column do not differ significantly at the 5% probability level, respectively, by the Tukey and Scott-Knott test.

In the comparison of means between isolates for measurements of dry mass of mycelium, in the PDA culture medium the isolates were separated into two groups, of which the ones with greater mass were the isolates ARM, CD, JADE, 3MV, 2BM, 3G, PIT, 4G, 6BM, 1G, 5G, 3M, T, A, D, 2CP, 1CP, 2JR, 1M, 1C, AR and JR. In the Pagnocca culture medium, four different groups were formed with values between 0.46 and 1.11 g, and the isolates with the highest dry mass of mycelium were: 2BM, 1CP, 1C and 2G; and the ones with the lowest mass were the isolates: JADE, 3G, 6BM, CD, 3M, 2JR, 2M, 1BM and 2E. For the peach juice medium, two groups were formed, in which the isolates ARM, CD, JADE, 3G, PIT, 6BM, 5G, 3M, T, A, 2JR, 1M, 1C, 1AR, 1EL, 1E, 2EL, 2G and B had the largest dry mass of mycelium. The isolate CD in the Pagnocca and peach juice culture media behaved equivalently for the production of dry mass of mycelium when compared to the PDA culture medium (Table 3). These morphological differences among isolates are associated to the genetic factor, which promotes phenotypic variability among the isolates of a species, such as that observed with *M. laxa* isolates (Fazekas et al., 2014).

In addition, differences in the evaluated variables of the *Monilinia* isolates were observed in relation to the three culture media used. According to Dhingra & Sinclair (1995), culture media containing high carbohydrate concentration may stimulate mycelial growth whereas low-carbohydrate media, but with plant extracts, may stimulate fungal sporulation. These differences in nutrient utilization explain the differences in the variables evaluated among the *Monilinia* spp. isolates. According to Pereira et al. (2006), this response is related to the ability of some fungal isolates to adapt to the substrate in function of their physiological requirements.

The composition of the culture media also influenced the coloration and the margins of the colonies of the isolates, in which the highest color variability was recorded in the PDA medium with ash, brown and olive tones and the margins of the colonies with lighter shades, such as white and yellow (Figure 3). According to Lane (2002), variations in colony coloring help distinguishing the three *Monilinia* species. This assertion is also supported by Poniatowska et al. (2013), who state that the coloration, crop growth and the colony margin characteristics are particular to each *Monilinia* species. According to these authors, the shades of gray are the most characteristic color of *M. fructicola*, similar to what was observed in this work for most of the studied isolates.

According to the results of Poniatowska et al. (2013), differences in colony coloration between *M. fructicola* isolates were observed in three culture media, ranging from shades of gray, for the PDA culture medium, to white and gray, for cherry juice and acidified PDA media. In the present study, in the Pagnocca medium, the predominant coloration of the isolates was white whereas in the peach juice medium there was a small variation, from white to rosy, according to the Munsell Color table (1954) (Figure 4). Lichtemberg et al. (2014) also found that *M. fructicola* isolates showed differences in coloration, varying in shades of gray, yellow/cream and white. These differences, according to Pereira et al. (2006), indicate that most of the fungi differ in their morphological characteristics according to the modifications in the development substrate.

As for the shape of the colonies, most of the isolates presented a circular shape regardless of the culture medium. For the margin of the colonies, all the isolates cultivated in peach juice medium had wavy margin and in the PDA and Pagnocca media the isolates presented lobed and wavy margins. Similar results were obtained by Poniatowska et al. (2013), who reported that the margins of *M. fructicola* and *M. frutigena* colonies in PDA are whole and the margins of *M. laxa* are lobed, but in the cherry juice medium the three species presented whole margin and in acidified PDA medium the *M. frutigena* isolates presented whole margin and the *M. fructicola* and *M. laxa* isolates presented lobed margin. According to Lane (2002), the presence of the lobed margin in the colony helped identifying the *M. laxa*, *M. fructicola* and *M. fructigena* isolates.

Concerning the topography of the colonies, the isolates cultivated in peach juice medium presented flat elevation, except for the isolate CD. The isolates grown in PDA and Pagnocca media presented variations in elevation between flat and elevated, as reported by Capuccino & Sherman (1998). For Teixeira et al. (2004), the morphophysiological variability may be influenced by some factor intrinsic to the pathogen, to the host genotype, or even to the interaction between them. According to the data obtained in the present study, the morphological characterization presents many variations in the topography of colonies of isolates between the culture media used, a result also verified by Lichtemberg et al. (2014) when evaluating *Monilinia* spp. isolates in acidified PDA culture medium.

Several studies have already reported variability among isolates of the same *Monilinia* species, such as that performed by Gril et al. (2008), who used the AFLP technique and verified variability among 67 isolates of *M. laxa*. Villarino et al. (2012) found genetic differences among isolates of *M. fructicola* from French and Spanish orchards. Similar result was also reported by Jänsch et al. (2012) with *M. fructicola*

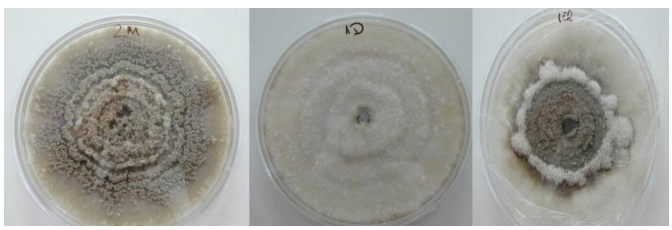

Figure 3. *Monilinia fructicola* colonies grown in PDA culture medium.

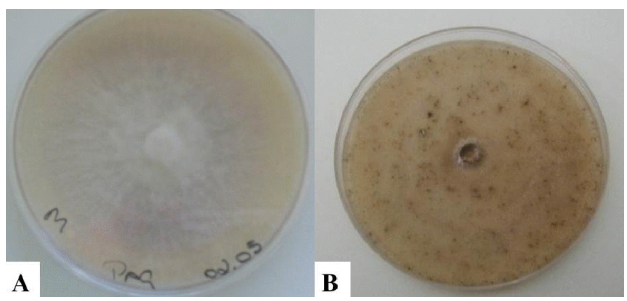

Figure 4. *Monilinia fructicola* colonies grown in Pagnocca (A) and peach juice (B) culture media.

isolates from the United States and Europe. For these authors, the variability among isolates may have occurred due to sexual reproduction, accumulation of random mutations or through gene flow. According to Grindle (1979), genetic variability is generally correlated with the morphological and physiological variability of the fungus.

Conclusions

The isolates associated with brown peach rot, collected in southern Rio Grande do Sul, are of the *Monilinia fructicola* species, which was confirmed by PCR using the primer MO368-10R specific for this species.

The best media for mycelial growth and dry mass of mycelium of the *Monilinia fructicola* isolates were peach juice and Pagnocca.

There is variability in the morphological characteristics of dry mass of mycelium, mycelial growth, coloration, margin, shape and elevation of the colonies among the *Monilinia fructicola* isolates.

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