

## First report of *Diaporthe paranensis* causing peach fruit rot

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### Abstract

The production of peaches in Brazil is concentrated in the states of Rio Grande do Sul and São Paulo. Peach is a fruit rich in sugars and water, and these characteristics, combined with wounds in the harvesting and post-harvest stages, are determinants of shelf life of the fruit. Several fungi were isolated from peaches with rot spots obtained at the central fruit and vegetables distribution center of São Paulo (CEAGESP). Fungal identification was performed by sequencing of the ITS region genes. Twenty isolates were identified as *Monilinia fructicola*, two as *Botrytis cinerea*, and two as *Diaporthe* spp. (*D. cf. beveae* and *D. paranensis*), the last two never before reported as causing postharvest rot in any fruit. Pathogenicity of *D. paranensis* was confirmed by observation of rot symptoms on inoculated peach fruits, reisolation and molecular identification of the fungus. This is the first report of *D. paranensis* causing postharvest peach fruit rot.

**Keywords:** *Phomopsis* sp., *Prunus persica*, Koch's postulates, fruit rot

### 1. Introduction

Peach tree [*Prunus persica* (L.) Batsch] is the third most important fruit crop species of temperate climate in the world, after apple and pear tree (BYRNE et al., 2012). According to the Brazilian Institute of Geography and Statistics (IBGE), in 2019, Brazil was responsible for the production of 183.1 thousand tons of peach, in about 16 thousand hectares harvested. The largest producing state is Rio Grande do Sul, with production of 110.2 thousand tons, in 11.8 thousand hectares. São Paulo is in second place, with 32.9 thousand tons, in 1.5 thousand hectares.

The peach, among temperate climate fruits, is one of the most perishable, as it presents high post-harvest metabolism, which causes rapid loss of firmness of the pulp, incidence of rot and withering. The accelerated ripening of the peach is responsible for its reduced shelf life, which results in serious restrictions for efficient handling and transportation (NAVA and BRACKMANN, 2001). Among the most common rot in peach fruits are those caused by *Monilinia fructicola*, *Penicillium*, *Rhizopus*, *Fusarium*, *Colletotrichum*, *Cladosporium*, and *Geotrichum*. (FORCADA et al., 2013).

Huang et al., 2021, isolated *Diaporthe* species from ten different genus of hosts in Yunnan in China and found three new species and five others already known of the fungus and when comparing the morphology and phylogeny, based on DNA, proved the high diversity of species of *Diaporthe* and a wide range of hosts, causing disease and also acting as an endophytic.

In Brazil, species of the genus *Diaporthe* have never been reported as pathogenic to peach cultivation.

### 2. Material and Method

In the period from September to December 2016, 24 fruits with rot symptoms of different cultivars and origins were collected at the Companhia de Entrepósitos e Armazéns Gerais de São Paulo (CEAGESP), which is the central fruit and vegetables distribution center of the city of São Paulo, and then sent to the Phytosanitary Laboratory of the Integral Cantareira Faculty, SP. For isolation of the fungi, small tissue fragments from the transition region of the rot lesion were cut, followed by disinfection in 70% alcohol solution for 15 seconds and sodium hypochlorite solution (0.5%) for 30 seconds. The material was subsequently rinsed in sterile water and left on sterile filter paper to remove excess water.

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They were then transferred under aseptic conditions to Petri dishes containing potato-dextrose-agar (PDA) culture medium, incubated for 72 hours at 25 °C in the dark until growth of the fungi. After this period, the colonies obtained were isolated and transferred to new PDA medium in order to obtain pure cultures.

The isolates were then sent to the Laboratory of Phytopathological Biochemistry of the Biological Institute of São Paulo. DNA was extracted according to the method described by Doyle and Doyle (1987), from the mycelium grown in culture medium. Genomic DNA was submitted to polymerase chain reaction (PCR) for amplification of the rDNA internal transcribed spacer (ITS) region using primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCITCCGCTTATTGATATGC-3') (WHITE et al., 1990).

The PCR mixture consisted of 1.0 µL of DNA, 1 µL of each primer at 10 µM, 10 µL of PCR buffer of 5.0X, 1.0 µL of dNTPs at 10 mM, 0.2 µL of GoTaq DNA polymerase 5U. µL<sup>-1</sup> (Promega) and 35.8µL autoclaved MilliQH<sub>2</sub>O, to a final volume of 50µL. The amplification program consisted of initial denaturation at 94°C for 2 minutes followed by 40 cycles of denaturation at 94°C for 10 seconds, annealing at 54°C for 30 seconds, extension at 72°C for 45 seconds, and final extension at 72 °C for 4 minutes. The amplified products were verified by means of 0.8% agarose gel electrophoresis stained with ethidium bromide. The amplified products were purified by precipitation with polyethylene glycol (SCHMITZ & RIESNER, 2006), submitted to sequencing reaction by chain termination method using Big Dye 3.1 reagent (Applied Biosystems) and analyzed in an automatic capillary sequencer 3500 xL (Applied Biosystems). Sequences similar to those obtained for the isolates of the present study were searched in GenBank using the Blastn tool. Phylogenetic tree was constructed by the Neighbor Joining method with 1000 bootstrap replications using MEGA 6.0 (TAMURA et al., 2013)

### 3. Results and Discussion

Molecular identification of the fungal agents causing rot in fruits (Table 1) resulted in *Monilinia fruticola* in 20 samples (100% identity to strain CBS 203.25, GenBank MH854846), *Botrytis cinerea* in two samples (100% identity to strain CBS 261.71, GenBank MH860108), *Diaporthe cf. heveae* in one sample (98.5% identity to strain CBS 852.97, GenBank KC343116), and *Diaporthe paranensis* in one sample (97.3% identity to strain CBS 133184, GenBank KC343171), these last two never before described as etiological agents of postharvest diseases in peaches in Brazil or elsewhere in the world. *Monilinia fruticola* and *Botrytis cinerea* are known agents of peach fruit rot (WILSON & OGAWA, 1979).

Table 1. Cultivate, origin and identification of fungi causing rot in peaches collected at the Companhia de Entrepostos e Armazéns Gerais de São Paulo (CEAGESP).

SAMPLE	CULTIVATE	ORIGIN	FUNGUS
1A	Douradão	Atibaia- SP	<i>Botrytis cinerea</i>
1B	Douradão	Atibaia - SP	<i>Monilinia fruticola</i>
1C	Douradão	Ibiúna - SP	<i>Monilinia fruticola</i>
1D	Douradão	Paranapanema - SP	<i>Monilinia fruticola</i>
1G	Rubimel	Toledo- MG	<i>Monilinia fruticola</i>
1J	Rubimel	Jarinú - SP	<i>Monilinia fruticola</i>
1K	Rubimel	Paranapanema - SP	<i>Monilinia fruticola</i>
1L	Coral	Jarinú - SP	<i>Diaporthe paranensis</i>
1M	Kampai	Paranapanema - SP	<i>Monilinia fruticola</i>
1N	Douradão	Paranapanema - SP	<i>Diaporthe cf heveae</i>
1T	Douradão	Paranapanema - SP	<i>Monilinia fruticola</i>
1V	Chimarrita	Bento Gonçalves - RS	<i>Botrytis cinerea</i>
1X	Chimarrita	Botucatu - SP	<i>Monilinia fruticola</i>
1Z	Douradão	Botucatu - SP	<i>Monilinia fruticola</i>
I2	Chimarrita	Botucatu - SP	<i>Monilinia fruticola</i>
N10	Douradão	Atibaia - SP	<i>Monilinia fruticola</i>
N12	Granada	Farroupilha - RS	<i>Monilinia fruticola</i>
N15	Fascínio	Pilar do Sul - SP	<i>Monilinia fruticola</i>
N16	Chimarrita	Apiáí - SP	<i>Monilinia fruticola</i>
N18	Chimarrita	Apiáí - SP	<i>Monilinia fruticola</i>
N20	Granada	Farroupilha - RS	<i>Monilinia fruticola</i>
E	Douradão	Paranapanema - SP	<i>Monilinia fruticola</i>
I	Granada	Farroupilha - RS	<i>Monilinia fruticola</i>
P	Douradão	Paranapanema - SP	<i>Monilinia fruticola</i>

The phylogenetic tree constructed with sequences of *Diaporthe* spp. isolates of the present study with sequences of other related *Diaporthe* species or that has been reported to occur on fruits shows the close relationship with *D. cf. heveae* (isolate 1N) and *D. paranensis* (isolate 1L) (Figure 1). The ITS sequence of *D. paranensis* isolate 1L has been deposited in the GenBank with assigned number MK216796.

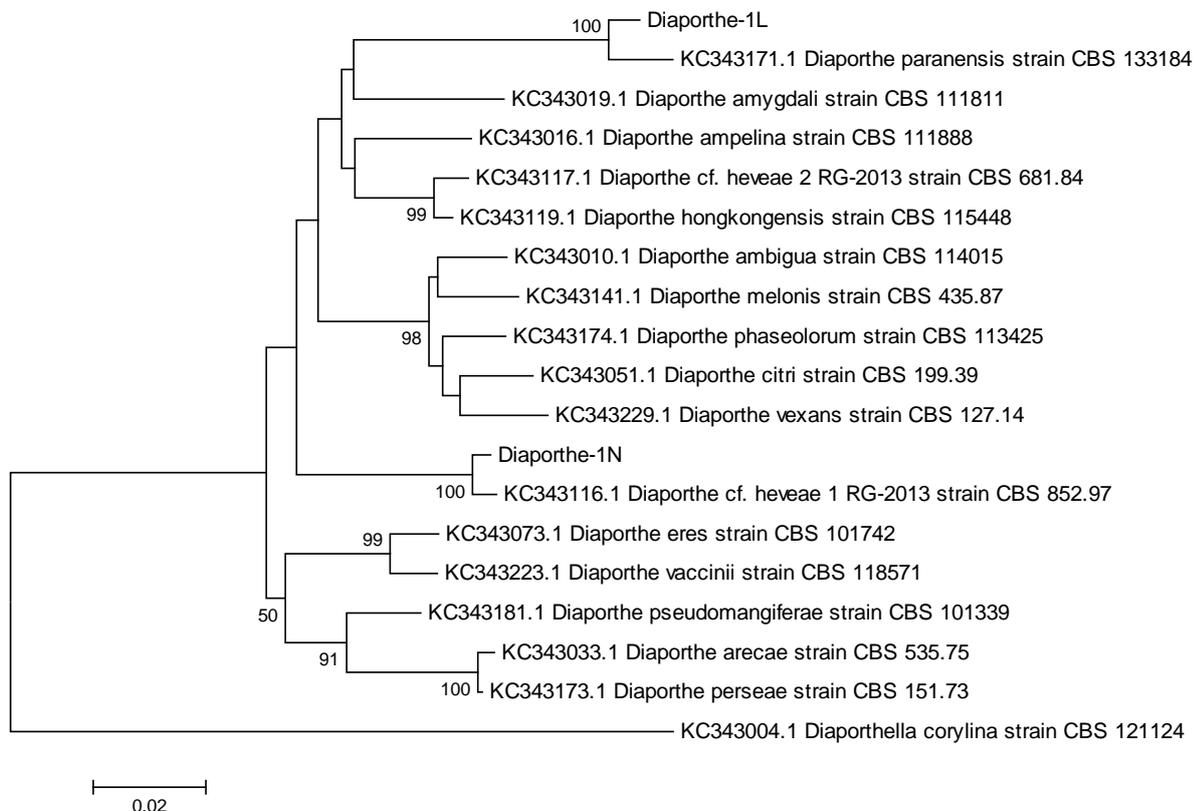


Figure 1. Phylogenetic relationship of *Diaporthe-1L* and *-1N* of the presents tudy with other closely related *Diaporthe* species or that has been reported to occuron fruits. Neighbor Joining tree constructed with ITS sequences with 1000 bootstra preplifications (values equal or above 50% only are shown on the tree).

Confirmation of pathogenicity of *D. paranensis* was performed by inoculation of mycelial disks with 7.0 mm diameter on the surface of 40 healthy fruits (completely randomized design). Peaches were kept under room temperature, with average temperature and humidity of 25 °C and 70%, respectively. Control treatment consisted of mock-inoculated fruits. The incidence of rot was evaluated by number of fruits with symptoms seven days after inoculation. The pathogen was reisolated in BDA medium, followed by molecular identification, completing the Koch postulate. Confirmation of pathogenicity of *D. cf. heveae* will be performed in a future study.

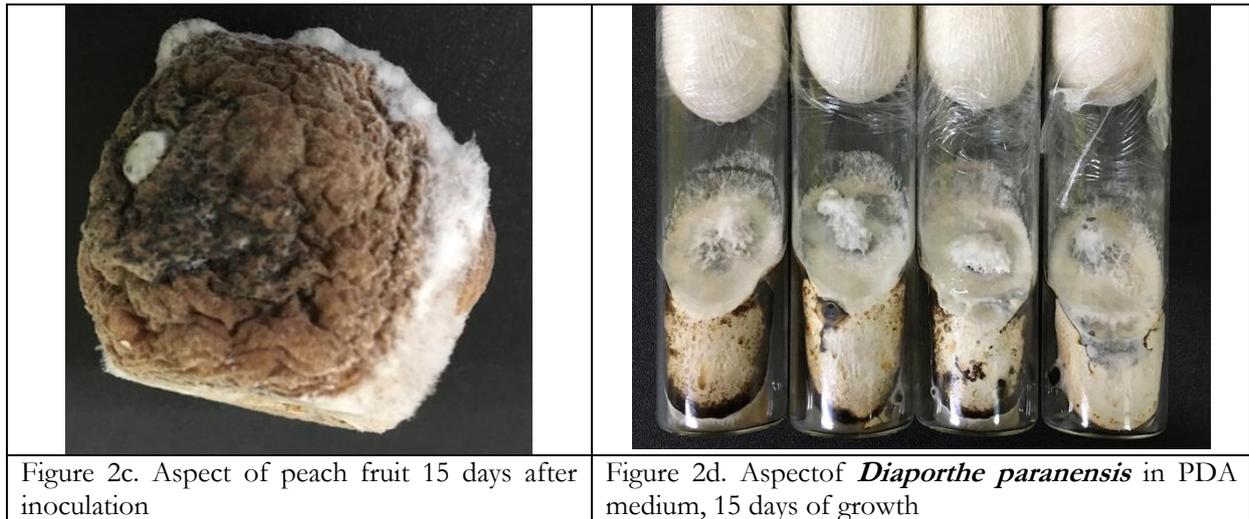
In the pathogenicity bioassay, *D. paranensis* developed in 100% of the inoculated fruits, reproducing symptoms of rot identical to those observed in the original fruits (Figures 2a, b, c). Molecular identification of the reisolated fungus confirmed it as *D. paranensis*.



Figure 2a. Aspect of peach fruit, 2 days after inoculation



Figure 2b. Aspect of peach fruit 4 days after inoculation



In culture medium, *D. paranensis* forms a colony with gray-white coloration, visible aerial mycelium (Figure 2d), and produces hyaline, smooth, and slightly curved beta conidium (Figure 3), that is in accordance with the characteristics described by Gomes et al. (2013).



Figure 3. Optical microscopy of beta conidia of *Diaporthe paranensis*. Scale bar: 50 $\mu$ m

The genus *Diaporthe*, anamorph *Phomopsis*, belongs to the phylum Ascomycota, subphylum Pezizomycotina, class Sordariomycetes, subclass Sordariomycetidae, order Diaporthales, family Diaporthaceae (HAWKSWORTH et al., 2011). The species *D. paranensis* was so named because it was isolated for the first time in the city of Colombo, Paraná, Brazil, as endophytic in the petiole of *Maytenus ilicifolia* (popular name espinheira-santa) (GOMES et al., 2013). This species has never been reported as a disease-causing in peach or any other fruit.

The genus *Diaporthe* is characterized by a large phenotypic variability, and because of its generalized morphology the identification is difficult (WEHMEYER, 1933). *Diaporthe* spp. can infect a wide range of plant species causing diseases such as root and fruit rots, dieback, cankers, leaf spots, blights, decay and wilt (GOMES et al., 2013). On peach, *Diaporthe* has been reported causing stem canker in Italy and Greece (PRENCIPE et al., 2017; THOMIDIS & MICHAILIDES, 2009). *Diaporthe (Phomopsis) amygdali* causes shoot blight of peach in the southeastern United States and fruit rot of peach in Greece (UDDIN et al., 1998; FARR et al., 1999; MICHAILIDES & THOMIDIS, 2006). The identification of the pathogenic species of a certain host, as well as its viability, is of fundamental importance for the development of more efficient strategies of control, besides providing a better understanding of the epidemiology of the disease. Future studies are needed to determine the epidemiology and strategies for control of the fruit rot caused by *D. paranensis* on peaches.

## Conclusion

From the observations made and molecular identification, it is concluded that the symptoms of rot found on peach fruits in the state of São Paulo, are caused by the fungus *Diaporthe paranensis*.

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