Klover International Journal of Food Science and Nutrition
Volume 11 Issue 2, April-June 2023
ISSN: 2995-4517
Impact Factor: 6.37
https://kloverjournals.org/journals/index.php/fsn

A RACE AGAINST TIME: COMBATING DIAPORTHE PARANAENSIS IN PEACH ORCHARDS

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Abstract
Peach tree (Prunus persica L. Batsch) stands as the third most significant fruit crop in temperate climates globally, trailing behind apple and pear trees (Byrne et al., 2012). As per the Brazilian Institute of Geography and Statistics (IBGE), Brazil held sway over the production of 183.1 thousand tons of peaches in 2019, harvested across approximately 16 thousand hectares. Rio Grande do Sul emerged as the foremost producing state, contributing 110.2 thousand tons from 11.8 thousand hectares. São Paulo secured the second position with 32.9 thousand tons sourced from 1.5 thousand hectares.

Keywords: Peach Tree, Prunus Persica, Fruit Crop, Brazilian Agriculture, Crop Production Statistics.

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 Entrepostos 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.
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'-TCCTCCGCTTATTGATATGC-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^{-1} (Promega) and 35.8µL autoclaved MilliQH_2O, 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 fructicola* 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 fructicola* 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	**Botrytis cinerae**
1B	Douradão	Atibaia - SP	**Monilinia fructicola**
1C	Douradão	Ibiúna - SP	**Monilinia fructicola**
1D	Douradão	Paranapanema - SP	**Monilinia fructicola**
1G	Rubimel	Toledo- MG	**Monilinia fructicola**
1J	Rubimel	Jarinú - SP	**Monilinia fructicola**
1K	Rubimel	Paranapanema - SP	**Monilinia fructicola**
1L	Coral	Jarinú - SP	**Diaporthe paranensis**
1M	Kampai	Paranapanema - SP	**Monilinia fructicola**
1N	Douradão	Paranapanema - SP	**Diaporthe cf heveae**
1T	Douradão	Paranapanema - SP	**Monilinia fructicola**
1V	Chimarrita	Bento Gonçalvez - RS	**Botrytis cinerea**
1X	Chimarrita	Botucatu - SP	**Monilinia fructicola**
1Z	Douradão	Botucatu - SP	**Monilinia fructicola**
I2	Chimarrita	Botucatu - SP	**Monilinia fructicola**
N10	Douradão	Atibaia - SP	**Monilinia fructicola**
N12	Granada	Farroupilha - RS	**Monilinia fructicola**
N15	Fascinio	Pilar do Sul - SP	**Monilinia fructicola**
N16	Chimarrita	Apiaí - SP	**Monilinia fructicola**
N18	Chimarrita	Apiaí - SP	**Monilinia fructicola**
N20	Granada	Farroupilha - RS	**Monilinia fructicola**
E	Douradão	Paranapanema - SP	**Monilinia fructicola**
I	Granada	Farroupilha - RS	**Monilinia fructicola**
P	Douradão	Paranapanema - SP	**Monilinia fructicola**

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.

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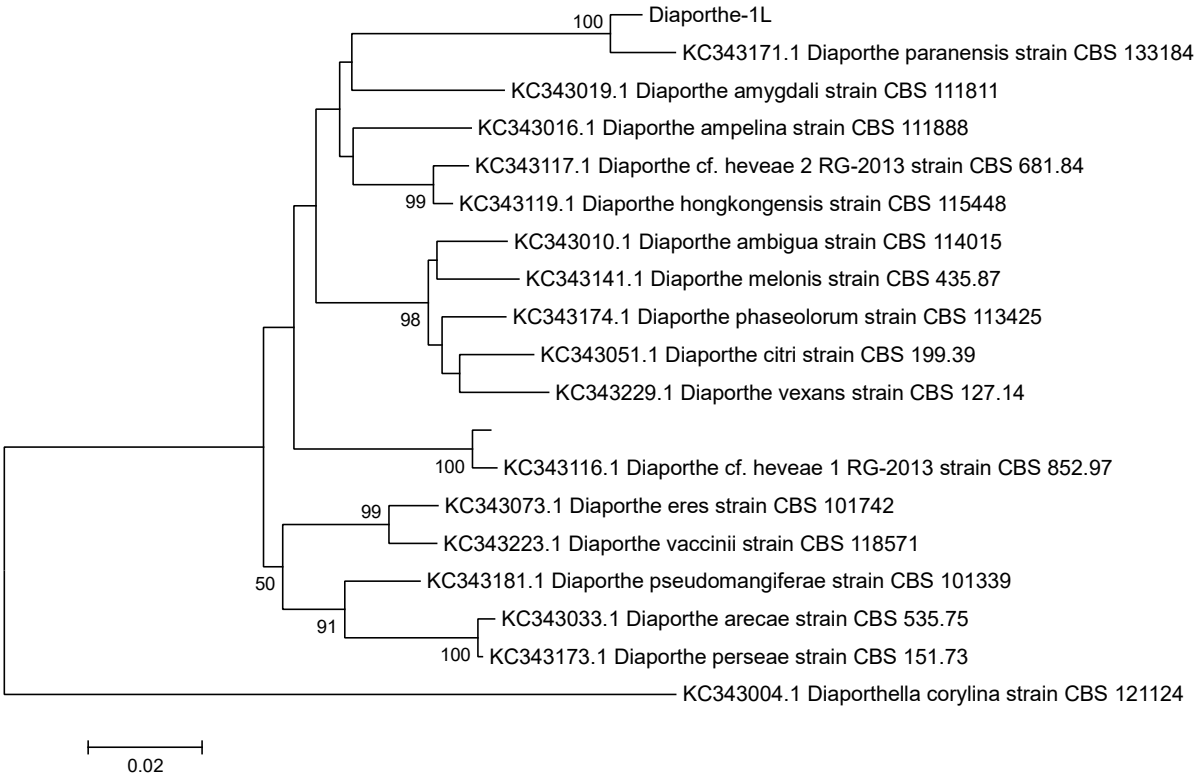

Figure 1. Phylogenetic relationship of *Diaporthe*-1L and -1N of the presents tudy with other closely related *Diaporthe* species or that has been reported to occuron fruits. Neighbor Joining tree constructed with ITS sequences with 1000 bootstra preplications (values equal or above 50% only are shown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

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

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

Figure 2c. Aspect of peach fruit 15 days after inoculation

Figure 2d. Aspectof **Diaporthe paranensis** in PDA medium, 15 days of growth

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).

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Figure 3. Optical microscopy of beta conidia of **Diaporthe paranensis.** Scale bar: 50µm

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 *Maytenusilicifolia*(popular name espinheirasanta) (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, *Diaportheeres* 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*.

Acknowledgements

The authors would like to thank Companhia de Entrepostos e Armazéns Gerais de São Paulo (CEAGESP) for granting access to symptomatic fruits and the Phytosanitary Laboratory of the Integral Cantareira Faculty, SP, for its financial support.

References

BYRNE, D. H. et al. Peach. In: BADENES, M. L.; BYRNE, D. H. **Fruit Breeding**: Handbook of Plant Breeding. New York: Springer Science+Business Media, 2012. p. 505-569.

DOYLE, J.J.; DOYLE, J.L. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. **Phytochemical Bulletin**, v.19, p.11-15, 1987.

FARR, D.F. et al.*Phomopsis amygdali* causes peach shoot blight of cultivated peach trees in the southeastern United States. **Mycologia**, v.91, n.6, p.1008-1015, 1999.Available from: <https://doi.org/10.2307/3761632>. Accessed: Mar. 18, 2019. doi.org/10.2307/3761632.

FORCADA, C. F. et al. Population structure and marker-traid associations for pomological traits in peach and nectarine cultivars. **Tree Genetics and Genomes**, v. 9, n.2, p. 331-349. 2013. Available from: <http://dx.doi.org/10.1590/0100-29452017166>. Accessed: Mar. 15, 2019.doi.org/10.1590/010029452017166.

GOMES, R.R. et al. *Diaporthe*: a genus of endophytic, saprobic and plant pathogenic fungi. **Persoonia**, v.31, p.141, 2013.Available from: <https://*doi*:10.3767/003158513X666844>. Accessed: Apr. 18, 2019. *doi*:10.3767/003158513X666844

HAWKSWORTH D.L. et al. The Amsterdam Declaration on Fungal Nomenclature. **IMAFungus,**v.2,n.1, p.105– 112, 2011.Available from: <https://*doi*:10.3767/003158513X666844>. Accessed: Apr. 18, 2019. *doi*:10.3767/003158513X666844

HUANG D, XIA J, ZHANG X, SUN W (2021) Morphological and phylogenetic analyses reveal three new species of *Diaporthe* from Yunnan, China. **Mycokeys** 78: 49-77. https://doi.org/10.3897/mycokeys.78.60878

BRAZILIAN INSTITUTE of GEOGRAFHY and STATISTICS – IBGE.Produçãoagrícola municipal. Rio de Janeiro: IBGE, 2019. Disponívelem:<https://sidra.ibge.gov.br/>. Acessoem: fev. 2021.

MICHAILIDES, T.J., THOMIDIS, T. First report of *Phomopsis amygdali* causing fruit rot on peaches in Greece. **Plant Disease**, v.90, n.12, p.1551, 2006. Available: <https://*doi*.org/10.1094/PD-90-1551C>. Acessed: Apr. 18, 2019.*doi*.org/10.1094/PD-90-1551C.

NAVA, Gilmar Antônio; BRACKMANN, Auri. Efeito do pré-resfriamento e da umidade relativa da câmara sobre a qualidade pós-colheita de pêssegos (*Prunuspersica* (L.) Batsch), cv. 'Chiripá'. **Revista Científica Rural**, Bagé, v.6, n.2, p.142-146, 2001. Disponívelem:

<https://periodicos.ufpel.edu.br/ojs2/index.php/CAST/article/viewFile/302/297>. Acessoem: 9 mar. 2021.

PRENCIPE S, et al. First report of *Diaportheeres* causing stem canker on peach (*Prunus persica*) in Italy. **Plant Disease**, v.101, n.6, p.1052, 2017. Available from: <https://apsjournals.apsnet.org/doi/10.1094/PDIS-12-16-1770-PDN>. Accessed: Mar.16, 2019. doi.org/10.1094/PDIS-12-16-1770-PDN

SCHMITZ, A.; RIESNER, D. Purification of nucleic acids by selective precipitation with polyethylene glycol 6000. **Analytical Biochemistry**, v. 354, n.2, p. 311-313., 2006. Available from: < https://doi.org/10.1016/j.ab.2006.03.014>. Accessed: Mar. 16, 2019. doi.org/10.1016/j.ab.2006.03.014.

TAMURA, K. et al. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0.**Molecular Biology and Evolution**v.30, n.12, p.2725-2729, 2013. Available: <https://*doi*.org/10.1093/molbev/mst197>. Acessed: Apr. 18, 2019.*doi*.org/10.1093/molbev/mst197.

THOMIDIS, T.; MICHAILIDES, T. J. Studies on *Diaportheeres* as a new pathogen of peach trees in Greece. **Plant Disease**,v.93, n.12, p.1293, 2009. Available: <https://*doi*.org/10.1094/PDIS-93-12-1293>.Acessed: Apr. 18, 2019.*doi*.org/10.1094/PDIS-93-12-1293.

UDDIN, W.; STEVENSON, K.L.Pathogenic and molecular characterization of three *Phomopsis* isolates from peach, plum and Asian pear. **Plant Disease**, v.82, n.7, p.732-737, 1988. Available: <https://*doi*.org/10.1094/PDIS.1998.82.7.732.>. Acessed: Apr. 18, 2019. *doi*.org/10.1094/PDIS.1998.82.7.732.

WEHMEYER, L. E. **The genus DiaportheNitschke and its segregates**. University of Michigan Press: Ann Arbor, p.1-349, 1933. Available: <https://pdfs.semanticscholar.org/2e5d/66e153b226d6bd45bbce221d0fb04745bbe6.pdf>. Acessed: Apr. 18, 2019.

WHITE, T. J. T. et al. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. p. 315-322. In: INNIS, M. A. et al. **PCR Protocols**: A Guide to Methods and Applications.New York: Academic PressInc., 1990.

WILSON, E.E., OGAWA, J.M. **Fungal, bacterial and certain nonparasitic diseases of fruit and nut crops in California**. California: University of California, 1979, 190 p.