Online Perspectives Journal: Biological & Health
March/2021, v.11, n.36, p.26-40
ISSN: 2236-8868 (Online)
DOI: 10.25242//8868113620212233

IS THE ENDOPHYTIC FUNGUS *Neofusicoccum kwambonambiense* ANOTHER BRICK IN THE WALL OF THE POST-HARVEST PATHOGENIC FRUIT FUNGI SYSTEM?

Vicente Mussi-Dias[2]*, Pedro Henrique Dias dos Santos[2]; Beatriz Murizini Carvalho[2]; Adão Valmir dos Santos[3] & Maria das Graças Machado Freire[1]

MUSSI-DIAS, V.; SANTOS, P.H.D.; CARVALHO, B.M.; SANTOS, A.V. & FREIRE, M.G.M. Is the endophytic fungus *Neofusicoccum kwambonambiense* another brick in the wall of the post-harvest pathogenic fruit fungi system? **Online Perspectives: Biological & Health, v.11, n.36, p.26-40, 2021.**

RESUMO

Os fungos endofíticos são caracterizados como micro-organismos que colonizam os tecidos de plantas sem causar sintomas de infecções. Estes organismos são fontes de diversidade genética e bioquímica, com inúmeras aplicações, desde a produção de compostos farmacêuticos à alimentação. Entretando, muitos destes fungos podem se tornar patogênicos às plantas, caso encontrem substrato adequado para sua infecção e desenvolvimento. *Neofusicoccum* spp. tem sido considerado um fungo fitopatogênico para inúmeras culturas agrícolas e agressivo para algumas delas, tanto nas partes lenhosas quanto nos frutos ainda nos pomares e na comercialização, sendo a identificação e patogenicidade dos isolados dois aspectos importantes para a sua caracterização. Assim, o objetivo deste trabalho foi caracterizar a patogenicidade de dois isolados endofíticos do fungo *Neofusicoccum* sp., sobre diferentes frutos em processo de pós-colheita. Para tanto, a confirmação da identidade dos isolados foi obtida utilizando a reconstrução bayesiana das regiões *internal transcribed spacer region* (ITS) e *translation elongation factor 1-alpha* (TEF 1-α). Posteriormente, os isolados foram inoculados em frutos de abobrinha, banana, caqui, feijão vagem, goiaba, laranja, maçã, melão, mamão e tomate, avaliados durante 10 dias, e as áreas abaixo da curva de progresso da doença foram calculadas. A reconstrução filogenética possibilitou a identificação dos dois isolados do fungo endofítico de folhas de pitangueira como *Neofusicoccum kwambonambiense*, mostrando-se patogênicos para frutos de pitanga, mamão, tomate, banana e maçã, com variações na agressividade. No entanto, em abobrinha, melão e goiaba não ocorreram infecções por nenhum dos dois isolados. Acredita-se que esta seja a primeira ocorrência *N. kwambonambiense* isolado de plantas de *Eugenia uniflora*, com patogenicidade em diferentes frutos pós-colheita.

Palavras-chave: Fungos endofíticos; Pós-colheita; *Eugenia uniflora*; Patogenicidade.

ABSTRACT

Entophytic fungi are characterized as microorganisms that colonize plant tissues without causing infection symptoms. These organisms are sources of genetic and biochemical diversity for numerous applications, from the production of pharmaceutical compounds to foods. However, many of these fungi can become pathogenic to plants if they find suitable substrate for their infection and development. *Neofusicoccum* spp. has been considered a phytopathogenic fungus for many agricultural crops, being aggressive for some of them both in their woody parts or fruits, either while still in the orchards or after being harvested. The identification and pathogenicity of the isolates are two important aspects for their characterization. Thus, the objective of this work was to characterize the pathogenicity of two endophytic isolates of the fungus *Neofusicoccum* sp. in different fruits in the post-harvest process. Therefore, isolates identity confirmation was obtained using Bayesian reconstruction of the internal transcribed spacer region (ITS) and translation elongation factor 1-alpha (TEF 1-α). Subsequently, the isolates were inoculated in zucchini, banana, persimmon, green beans, guava, orange, apple, melon, papaya and tomato, followed by a 10-day evaluation in order to calculate the areas under the disease progress curve. Phylogenetic reconstruction allowed identification of two isolates of the endophytic fungus of Brazilian cherry leaves as *Neofusicoccum kwambonambiense*, which were confirmed as pathogenic for the following fruits: Brazilian cherry, papaya, tomato, banana and apple, with variations in aggressiveness. However, no infections were found in zucchini, melon and guava by any of the isolates. This is believed to be the first occurrence of *N. kwambonambiense* isolated from plants of *Eugenia uniflora*, with pathogenicity in different post-harvest fruits.

Keywords: Endophytic fungi; Post-harvest; *Eugenia uniflora*; Pathogenicity**.**

[1]Laboratory of Chemistry and Biomolecules (LAQUIBIO), CENSA - ISECENSA Superior Education Institutes, Rua Salvador Corrêa, 139, Centro, Campos dos Goytacazes, RJ, CEP: 28035-310, Brazil.
[2]Laboratory of Entomology and Plant Pathology LEF/CCTA, Northern Rio de Janeiro State University Darcy Ribeiro – UENF, Av. Alberto Lamego, 2000, Parque Califórnia, Campos dos Goytacazes, RJ, CEP: 28013-602, Brazil.
[3]Laboratory of Biotechnology - LBT/CBB, Northern Rio de Janeiro State University Darcy Ribeiro – UENF, Av. Alberto Lamego, 2000, Parque Califórnia, Campos dos Goytacazes, RJ, CEP: 28013-602, Brazil.
(*) e-mail: vimdias@yahoo.com.br

Received: 05/10/2020. Accepted: 16/12/2020. Published: 02/03/2021.

1. INTRODUCTION

Pioneering works developed in the decade of 2010 aimed the prospecting of endophytic fungi from restinga ecosystems in the northern state of Rio de Janeiro, Brazil (FREIRE et al., 2016; FREIRE et al., 2017; MUSSI-DIAS et al., 2018). Although many groups of fungi have different associations with plants, whether symbiotic or parasitic, endophytes comprise an inexhaustible and little explored mycological flora (YAN et al., 2019).

Endophytic fungi, with the exception of mycorrhizal associations, are defined as those capable of colonizing plants' healthy internal tissues without causing apparent damage throughout their life cycles, and never originating disease symptoms (WHITE el at., 2019). Some fungus might be endophytic for some plant species and pathogenic for others (CARVALHO et al., 2016; SANTOS et al., 2016). However, there is a threshold for such species' pathogenicity characterization that has a dual behavior, that is, they grow and develop as endophytic and, later, at a certain moment or under favorable conditions, they become pathogenic. Hence, they are considered latent pathogens (SLIPPERS and WINGFIELD, 2007).

When evaluating post-harvest rot, many latent species may be present in marketed products. In Brazil there is a great diversity of fruit species intended for both domestic consumption and for export (FAO, 2020). The production chain, however, faces great losses, mainly in the post-harvest phase, largely due to fungal diseases (SILVA et al., 2016), such as rotting caused by *Colletotrichum* sp. and *Lasiodiplodia* sp., (MUSSI-DIAS and FREIRE, 2016). These losses occur during fruits storage and commercialization processes, even if they were infected before harvesting, still in the field.

Regarding fungi isolated from healthy plant tissues, it cannot be said that such organisms are strictly endophytic, latent/quiescent or pathogenic. One of the ways to assess the ability of a fungus to cause disease in another host species is through inoculation in healthy plants or parts of them. Many fruits pathogenic fungi are characterized by the wide range of hosts they attack, as well as by the extent of their worldwide distribution (LIU et al., 2018). Therefore, it is necessary to know the diversity of pathogenic species present in a given area or geographic region, as well as their host range, for the purpose of creating management strategies for the diseases caused by these fungi, becoming essential to know the severity of the species in each host (SILVA et al., 2016).

Neofusicoccum is a cosmopolitan and polyphagic fungus belonging to the Botryosphaeriaceae family, possibly including pathogenic, saprophytic or endophytic species (DENMAN et al., 2000; MORAL et al., 2017). Hence, it is important to identify and characterize the pathogenic species involved in the different pathosystems (SAKALIDIS et al., 2013; MACHADO et al., 2014).

Therefore, the objective of this study was to evaluate the pathogenicity of two isolates of *N. kwambonambiense* obtained from Brazilian restinga cherry leaves to different post-harvest fruits.

2. MATERIAL AND METHODS

2.1. Isolation of fungi

Fungi were isolated from healthy leaves of Brazilian cherry (*Eugenia uniflora* L.) collected in areas of restinga in the northern state of Rio de Janeiro, Brazil. The leaves were washed with tap water and cut into 0.6 cm diameter pieces, which were surface-sterilized according to Freire et al. (2017). The leaf fragments were subsequently transferred to Petri dishes containing potato dextrose agar (PDA) (DHINGRA and SINCLAIR, 1995) and incubated at 28 ^{o}C under 12 h light/dark cycles. After growth, colonies were separated and restreaked until reaching the point of axenic cultures. Subsequently, tips of hyphae were streaked in order to obtain pure cultures.

2.2. DNA extraction pcr amplification, sequencing and identification

For DNA extraction, we used the methodology reported by Santos et al. (2019). The internal transcribed spacer (ITS) was amplified with primers ITS1 and ITS4 (WHITE et al., 1990) and translation elongation factor 1-alpha (TEF 1-α) with primers EF1-728F and EF2 (JACOBS et al., 2004). PCR conditions were the same as those reported by Santos et al. (2017). Samples were sent for sequencing to ACTGene Análises Moleculares Ltda. (Biotechnology Center, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil). Nucleotide sequences were edited in DNA Dragon software (HEPPERLE, 2011). Other sequences of ITS and TEF-1α regions were downloaded from GenBank. Sequences in the GenBank database were compared to check for molecular identification through the nucleotide BLAST algorithm (ALTSCHUL et al., 1990). MUSCLE was used in the alignments (EDGAR, 2004), implemented in MEGA v.7 (KUMAR et al., 2016). The assessed gene regions were concatenated in the Mesquite v.3.40 software (MADDISON and MADDISON, 2018). Bayesian inference (BI) analysis was carried out based on the Monte Carlo Markov chain method (MCMC). The MrMODELTEST v. 3.04 (POSADA and BUCKLEY, 2004) was adopted to select the nucleotide substitution model for BI analysis. Likelihood values were calculated, and the model was selected according to the Akaike information criterion (AIC). Evolution models selected for each gene region were HKY + G for ITS and GTR + I + G for TEF1-α. The BI analysis was carried out in MrBayes v.3.1.1 (RONQUIST and HUELSENBECK, 2003). The consensus tree was obtained after 10 million generations of a Markov chain were applied to two runs, four chains each, with a 25% burn-in. Likelihood log convergence was analyzed in TRACER v. 1.4.1 software (RAMBAUT et al., 2018). The tree was visualized and edited in ITol v.4 (LETUNIC and BORK, 2019). Tree was rooted in *Neofusicoccum pennatisporum* MUCC 510.

2.3. Inoculation in fruits and evaluation of pathogenicity

Fruits of Brazilian cherry (*E. uniflora*), zucchini (*Cucurbita pepo* L.), dwarf Cavendish banana (*Musa* L.), persimmon (*Diospyros kaki* Thunb.), green beans (*Phaseolus vulgaris* L.), guava (*Psidium guajava* L.), orange (*Citrus sinensis* (L.) Osbeck), apple (*Malus domestica* (Suckow) Borkh.), melon (*Cucumis melo* L.), papaya (*Carica papaya* L.) and tomato (*Lycopersicon esculentum* Mill.) were inoculated with the two endophytic isolates of *Neofusicoccum* sp. and evaluated every 48 h for 10 days. For banana and tomato fruits this evaluation was carried out every 24 h. Ripe and healthy fruits were obtained in the market, washed with water and neutral soap, rinsed under tap water and placed on paper towels until dry. The inoculations were made by perforating the fruit surface 1 cm deep with a sterile needle. At this site was placed a disk of 0.6 cm of diameter of the fungus colony or mycelium,

previously growing for 10 days. No inoculations were made in the negative control treatments and for positive control, the fungus *Alternaria solani* was used. The fruits were kept in a humid chamber for 24 h.

The experiment was carried out following a completely randomized design (CRD), with three replications, and the evolution of the disease was obtained by measuring the diameter of the lesions for plotting and calculating the area under the disease progress curve (AUDPC) in function of time (CAMPBELL and MADDEN, 1990). The results were subjected to analysis of variance and the means compared by Tukey's test at 5% probability.

3. RESULTS AND DISCUSSION

The two fungi isolates obtained from *E. uniflora* leaves, referred as 6C and 6D, when cultivated in culture medium, initially showed a whitish mycelium becoming cottony-like colonies with a dark and brown aspect (Figure 1A and 1B). These two colonies presented aspects similar to colonies of the *Alternaria solani* fungus (Figure 1C), genus frequently isolated from restinga plants (FREIRE et al., 2017), or from lesions in post-harvest fruits. Comparisons of aggressiveness for both isolates were made through tests of plant pathogenicity in a diverse variety of post-harvest fruits. Such comparisons allowed us to identify plant species susceptible to isolates 6C and 6D. Comparison with an *Alternaria solani* isolate was made by similarity between the colonies and the absence of sporulation.

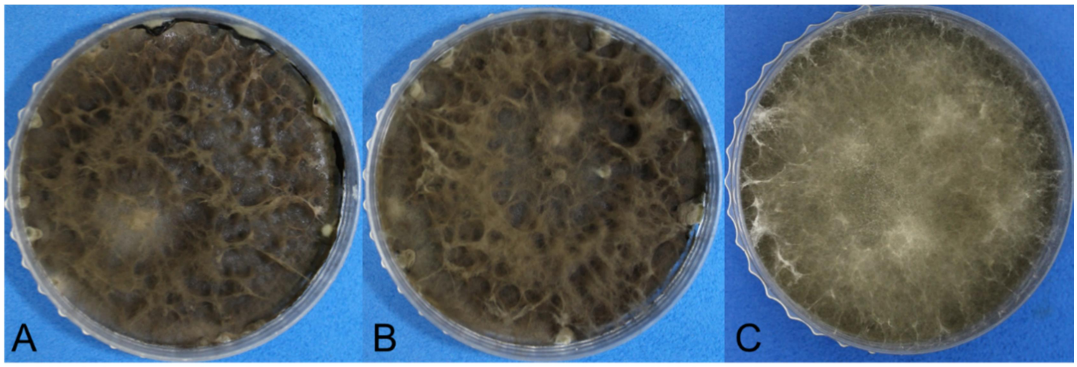

Figure 1: Colonies of *Neofusicoccum* sp. endophytes of *Eugenia uniflora* (A and B) and *Alternaria solani* (C) leaves, used for inoculation in post-harvest fruits.

In the tests of fruits inoculation, the two endophytic isolates and the isolate of *A. solani* did not present any differences among themselves in the initial stages of lesions growth. However, the aggressiveness was distinctly greater for the 6C and 6D isolates. Although it appears that *A. alternata* is repeatedly the most frequent pathogen causing post-harvest fruit decay and stem-end rots in persimmon and in different types of fruits (PALOU et al. 2012), other pathogens can also occur with equal importance, such as *Lasiodiplodia theobromae* and different species of *Neofusicoccum* spp. (PALOU et al., 2013) that have colonies morphological similarities in their initial stages of growth, before sporulation.

Due to the absence of sporulation or formation of fruiting bodies that would enable the morphological identification of the isolates, the molecular identification provided the grouping of the two isolates (6C and 6D) to the fungi of the Botryosphaeriaceae family, which includes a range of species that can be found latent in healthy plant tissues (CROUS et al.,

2006; SLIPPERS and WINGFIELD, 2007). The new sequences were deposited in GenBank and the access numbers, together with the species used for the comparison, are shown in Table 1.

The internal transcribed spacer region (ITS) and translation elongation factor 1-alpha (TEF 1-α), sequenced for the two isolates studied, resulted in 567 bp and 433 bp respectively. The multiloccus phylogenetic analysis was performed with 36 taxa, and sequence alignment resulted in a total of 1246 characters, 151 of which were informative for parsimony, 200 were variable and 968 were conserved. By the phylogenetic analysis, it was possible to identify the isolates at the species level, which were allocated to the *Neofusicoccum kwambonambiense* clade, which was well supported with a posterior probability (pp) = 0.95 (Figure 2).

Our phylogenetic results show that the isolates in this study share the same most recent common ancestor (MRCA) with the known species *N. kwambonambiense*, allowing us to identify the isolates 6C and 6D as same species, since the branch length of the *N. kwambonambiense* shows low phylogenetic signal.

Table 1. Collection details and GenBank accession numbers of isolates included in the present study

Species of *Neofusicoccum*	Strain	Host	Origin	GenBank Accession no.	
				ITS	Tef1- α
N. algeriense	CBS 137504	*Vitis vinifera*	Argelia	KJ657702	KJ657715
	CAA 322	*Eucalyptus globulus*	-	KX505906	KX505894
N. andinum	CBS 117453	*Eucalyptus* sp.	Venezuela	AY693976	AY693977
	CBS 117452	*Eucalyptus* sp.	Venezuela	DQ306263	DQ306264
N. arbuti	CBS 116131	*Arbutus menziesii*	USA	AY819720	KF531792
	CBS 117090	*Arbutus menziesii*	USA	DQ306263	KF531791
N. batangarum	CBS 124924	*Terminalia catappa*	Africa	FJ900607	FJ900653
	CBS 124923	*Terminalia catappa*	Africa	FJ900608	FJ900654
N. brasiliense	CMM 1338	*Mangifera indica*	Brazil	JX513630	JX513610
	CMM 1269	*Mangifera indica*	Brazil	JX513629	JX513609
N. cordaticola	CBS 123634	*Syzygium cordatum*	South Africa	EU821898	EU821868
	CBS 123635	*Syzygium cordatum*	South Africa	EU821903	EU821873
N. eucalypticola	CBS 115766	*Eucalyptus rossii*	Australia	AY615143	AY615135
	CBS 115679	*Eucalyptus rossii*	Australia	AY615141	AY615133
N. grevilleae	CBS 129518	*Grevillea aurea*	Australia	JF951137	-
N. illicii	CGMCC 3.18312	*Illicium verum*	Guangxi, China	KY350150	KY817756
	CGMCC 3.18311	*Illicium verum*	Guangxi, China	KY350151	KY817757
N. kwambonambiense	CBS 123639	*Syzygium cordatum*	South Africa	EU821900	EU821870
	CBS 123641	*Syzygium cordatum*	South Africa	EU821919	EU821889
	6C	***Eugenia uniflora***	**Brazil**	**MT295308***	**MT576130***
	6D	***Eugenia uniflora***	**Brazil**	**MT295309***	**MT576131***
N. macroclavatum	WAC 12444	*Eucalyptus globulus*	Australia	DQ093196	DQ093217
	WAC 12446	*Eucalyptus globulus*	Australia	DQ093197	DQ093218
N. mangiferae	CBS 118531	*Mangifera indica*	Australia	AY615185	DQ093221
	CBS 118532	*Mangifera indica*	Australia	AY615186	DQ093220
N. nonquaesitum	CBS 126655	*Umbellularia*	Chile	GU251164	GU251296
N. occulatum	CBS 128008	*Eucalyptus grandis hybrid*	Australia	EU736947	EU339511
	MUCC 286	*Eucalyptus pellita*	New Zealand	AY236943	AY236888
N. parvum	CMW 9081	*Actinidia deliciosa*	New Zealand	AY236943	AY573221
N. pennatisporum	MUCC 510	*Allocasuarina fraseriana*	Australia	EF591925	EF591976
N. ribis	CBS 115475	*Ribes* sp.	USA	AY236935	AY236877
	CBS 121.26	*R. rubrum*	USA	AF241177	AY236879
N. sinense	CGMCC 3.18315	*unknown woody plant*	Guizhou, China	KY350148	KY817755
N. umdonicola	CBS 123645	*Syzygium cordatum*	South Africa	EU821904	EU821874
	CBS 123646	*Syzygium cordatum*	South Africa	EU821905	EU821875

*Isolates and newly generated sequences are in bold.

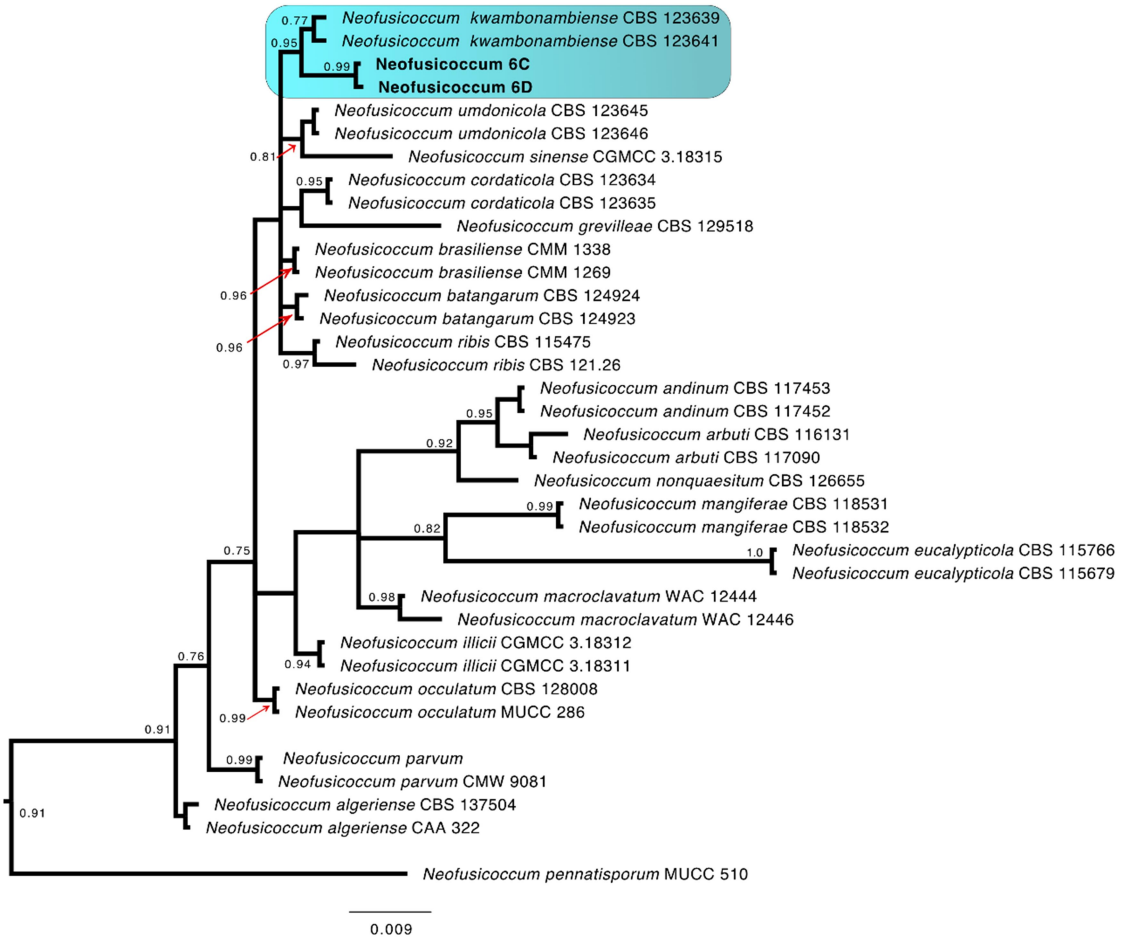

Figure 2. Phylogenetic tree based on the Bayesian Inference of two combined sequences (ITS and TEF 1-α) of *Neofusicoccum* spp. The posterior probability is indicated near the branches nodes and values under 0.75 are not shown. The tree was rooted in *Neofusicoccum pennatisporum* MUCC 510.

Different species of *Neofusicoccum* spp. are known to be pathogenic to a wide variety of plants. *N. kwambonambiense*, however, had already been reported in forest species in other countries (SAKALIDIS et al., 2011; FARR and ROSSMAN, 2013) although it was first identified in Brazil, causing mummification and post-harvest rot in strawberry fruits (LOPES et al., 2014). The presence of this asymptomatic fungus, internally in *E. uniflora* plants, should be seen as an alert, since important diseases have been frequently reported by *Neofusicoccum* sp. in plants of the Myrtaceae family, such as eucalyptus (*Eucalyptus grandis* W. Hill) and Malabar plum (*Syzygium cumini* (L.) Skeels) (PILLAY et al., 2013; HANIN and FITRIASARI, 2019). They can be considered asymptomatic hosts in the spread of the pathogen, mainly by means of seedlings or cuttings for regions where the disease does not occur yet. In addition, because this fungus is capable of infecting different botanical families, epidemics can occur, such as those caused by *N. parvum* in loquat (*Eriobotrya japonica* (Thunb.) Lindl, where rotting symptoms were observed in ripened fruits, still in the orchard, before they had been harvested (ZHAI and ZHANG, 2019). Other species of *Neofusicoccum* spp. are also important pathogens causing rot of olive fruit (SERGEEVA et al. 2009), kiwifruit (PENNYCOOK and SAMUELS, 1985) and post-harvest fruit rot of persimmon (PALOU et al., 2013) and other agricultural products.

When the two isolates of *N. kwambonambiense* were inoculated in post-harvest fruits,

there was a variation in pathogenicity and aggressiveness under controlled conditions. The isolates were not pathogenic in fruits of zucchini, guava and melon. Although some post infection lesions had occurred in persimmon, orange and green beans, there was no statistical difference between the inoculated and uninoculated fruits (data not shown). Regarding apple, banana, tomato and papaya, the lesions evolved over time, being similar in the first 48 h and changing afterwards, according to the type of fruit and inoculated isolate (Figures 3 and 4). Brazilian cherry inoculated fruits exhibited soft rot lesions, followed by wilting and mummification after four days of inoculation (Figure 5). Fruits that were perforated but not inoculated (Control groups) did not show symptoms of disease and those inoculated only with *A. solani* showed mild lesions when compared to the injuries caused by *N. kwambonambiense*. The fungi were re-isolated from internal lesions of the fruits from both 6C and 6D isolates, confirming the pathogenicity. When they were inoculated in *E. uniflora* leaves, with and without injuries, there were no symptoms. Pathogenicity tests through cross-inoculations in several hosts are used as a way of pathogenic characterization among isolates, aiming to demonstrate the specificity or the quantity of possible and probable hosts for pathogens (PERES et al., 2003; BONETT et al., 2010), as in the case of *N. kwambonambiense* isolates used in this work.

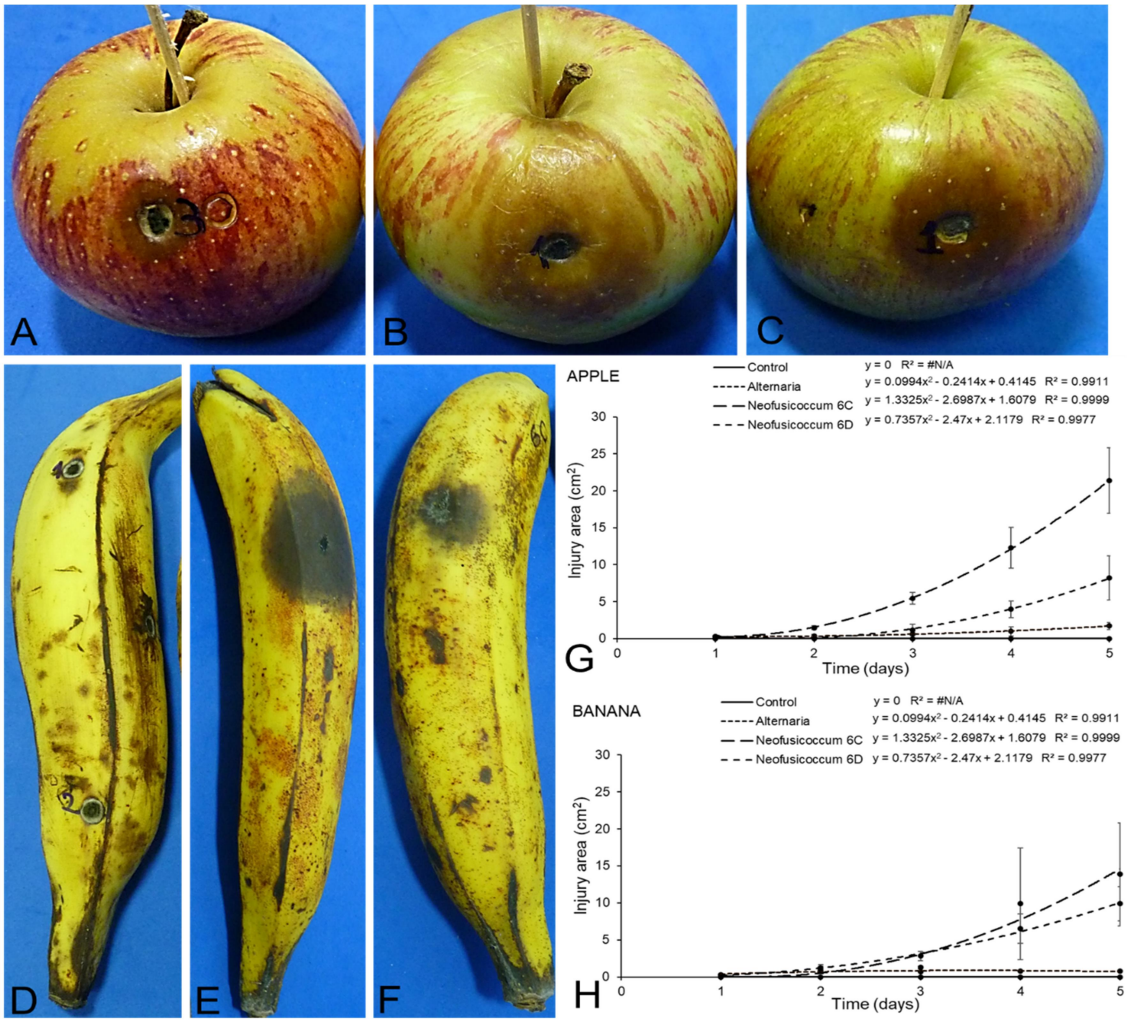

Figure 3: Injuries caused by artificial inoculations in apple and banana fruits. Symptoms of *Alternaria solani* infections (A and D), *Neofusicoccum kwambonambiense* isolated 6C (B and E), *N. kwambonambiense* isolated 6D (C and F) and Disease Progress Curves as a function of Time (G and H) for apple and banana, respectively.

Apple and banana fruits' lesions were greater for the two isolates of *Neofusicoccum* when compared with lesions caused by *Alternaria solani*, in which the isolate 6C was more aggressive than isolate 6D (Figure 3B and 3E). The same could be observed when comparing the area under the disease progress curve (AUDPC) for both isolates (Figure 3G and 3H). The variations that occur in the symptomatology of the infection vary according to the different hosts, either in terms of morphological characteristics or in terms of pathogenicity (BONETT et al., 2010), as well as according to the presence of pathogen isolates' physiological races and to the fungus's genetic adaptability when in contact with a new host (FREEMAN et al., 1998).

Evaluations of tomato infections showed that the isolate *Neofusicoccum* 6D was almost twice as aggressive as the isolate *Neofusicoccum* 6C, with a statistical difference between them and the *A. solani* control. Lesions grew fast on tomatoes, causing soft rot in the fruits and presenting a symptomatic feature that was distinct from those caused by infections of *Alternaria* sp. (Figures 4A-C and 4G).

On the other hand, evaluations of papaya inoculations showed that the aggressiveness of the two isolates of *N. kwambonambiense* was the opposite of those occurred in tomatoes, that is, isolate 6C was twice as aggressive as isolate 6D (Figure 4D-F). The largest areas below the disease progress curve (ABDPC) were observed for papaya above all the other susceptible fruit species used in this work. In these fruits, lesions had an average of 50 cm^2 of the surface area (Figure 4H). Peres et al. (2003) reported that papaya is very susceptible to isolates of the fungus *Colletotrichum gloeosporioides* originated from other hosts and cross-inoculation can separate distinct populations of different pathogen species. Bonett et al. (2010) also reported papaya's greater susceptibility among several other species used in cross-inoculations. The same could be observed in this work, where the two isolates of *N. kwambonambiense* presented the highest average of lesions among tested fruits and among different isolates of the fungus.

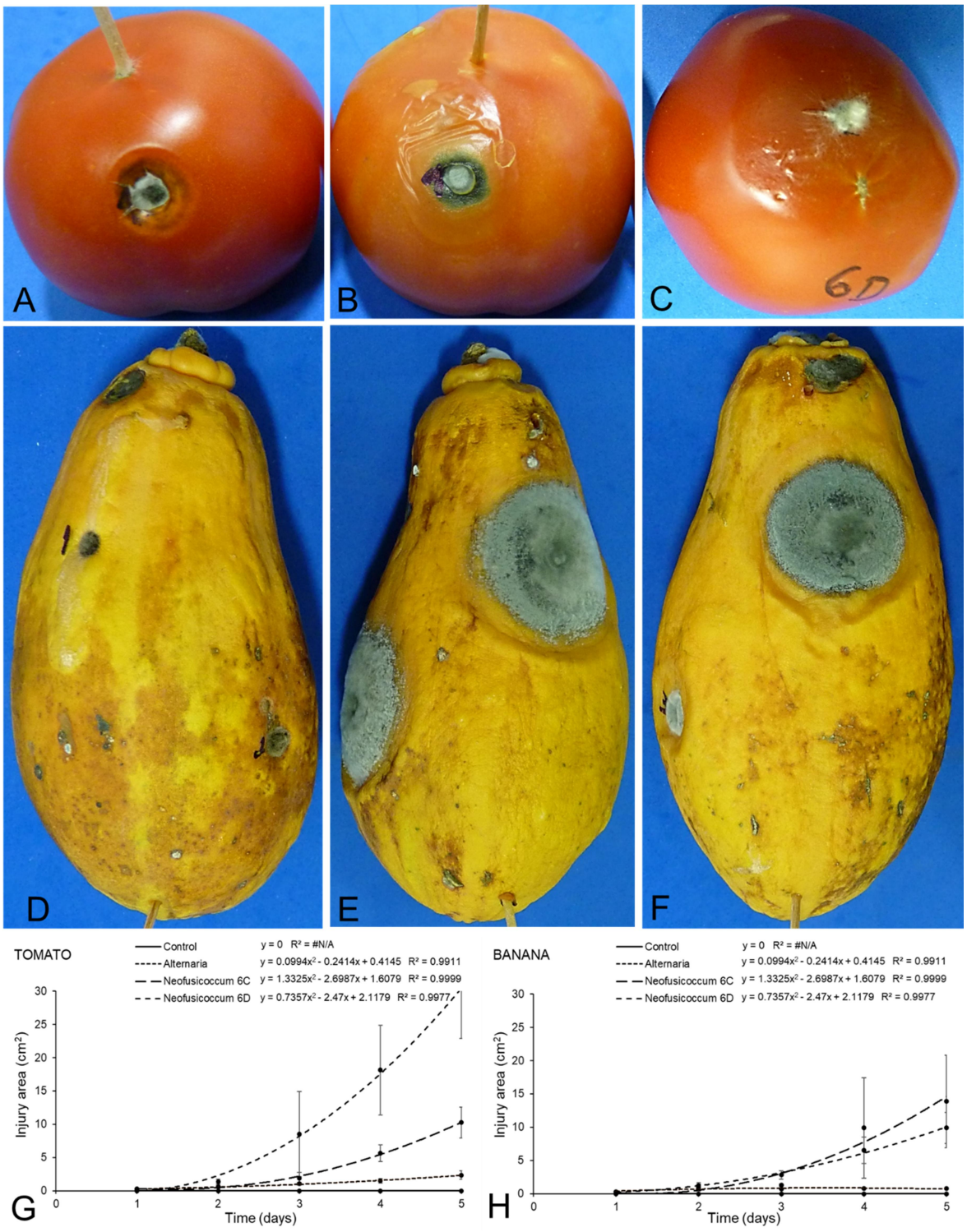

Figure 4: Injuries caused by artificial inoculations in tomato and papaya fruits. Infection symptoms of *Alternaria solani* (A and D), *Neofusicoccum kwambonambiense* isolated 6C (B and E), *N. kwambonambiense* isolated 6D (C and F) and Disease Progress Curves as a function of Time (G and H) for tomato and papaya, respectively.

Figure 5: Infection in Brazilian cherry fruits (Eugenia *uniflora*) artificially inoculated with *Neofusicoccum kwambonambiense*. Symptom evolution after four days of inoculation, showing absence of injury, soft rot and mummification, respectively, from left to right.

As the two endophytic isolates of *N. kwambonambiense* induced extensive lesions in the fruits of these five plant species (apple, banana, tomato, papaya and Brazilian cherry), they can be considered latent pathogens in *E. uniflora* and can also become aggressive pathogens for other species. The growing number of host species and the lack of pathogenic specialization of *Neofusicoccum* sp. combined with the endophytic character or latent infections, certainly impose great obstacles for the control of diseases caused by this organism. It is evident that genetic diversity, combined with host-pathogen co-evolution, lead to different responses in each pathosystem. This may be one of the factors for the wide range of hosts that this family encompasses. In this sense, molecular studies to identify and characterize species in different hosts and geographic locations are of paramount importance in epidemiological studies that aim to prevent the introduction of diseases in areas where they do not occur (CARDOSO et al., 2009). Molecular studies comprise important information in the post-harvest integrated disease management strategy.

4. CONCLUSIONS

Two isolates of the fungus *Neofusicoccum* sp. obtained endophytically from *Eugenia uniflora* leaves were identified as *N. kwambonambiense*. There was variation in pathogenic characteristics between these isolates in the assessment of pathogenicity. Among 11 different species of inoculated fruits, most of them developed lesions that evolved over time. Papaya, tomato, banana and apple fruits were the most susceptible to infections by *N. kwambonambiense*, respectively, as well as Brazilian cherry fruits. However, in zucchini, melon and guava no infections by the fungi could be seen.

5. REFERENCES

ALTSCHUL, S.F., GISH, W., MILLER, W., MYERS, E.W., LIPMAN, D.J.. Basic Local Alignment Search Tool. **Journal of Molecular Biology**. 1990. https://doi.org/10.1016/S0022-2836(05)80360-2.

BONETT, L.P., ALMEIDA, M., GONÇALVES, R.G.A., AQUINO, T.F., BERNARDI-WENZEL, J. Caracterização morfocultural e infecção cruzada de *Colletotrichum gloeosporioides* agente causal da antracnose de frutos e hortaliças em pós-colheita. **Ambiência**, v.6 n.3 p. 451 – 463, 2010.

CAMPBELL, C.L., MADDEN, L.V. **Introduction to plant disease epidemiology**. New York NY. John Wiley & Sons. 1990.

CARDOSO, J.E., BEZERRA, M.A., VIANA, F.M.P., SOUSA, T.R.M., CYSNE, A.Q., FARIAS, F.C. Ocorrência endofítica de *Lasiodiplodia theobromae* em tecidos de cajueiro e sua transmissão por propágulos. **Summa Phytopathologica**, v.35, n.4, p.262-266, 2009.

CARVALHO, B.M., SANTOS, P.H.D., MUSSI DIAS, V., FREIRE, M.G.M. Identificação de espécie fitopatogênica de *Colletotrichum* endofítico. **VIII Congresso Brasileiro de Micologia**. Florianópolis - SC, p. 106, 2016.

CROUS, P.W., SLIPPERS, B., WINGFIELD, M.J., RHEEDER, J., MARASAS, W.F.O., PHILIPS, A.J.L., ALVES, A., BURGESS, T., BARBER, P., GROENEWALD, J.Z. Phylogenetic lineages in the Botryosphaeriaceae. **Studies in Mycology,** v. 55, p. 235–253, 2006.

DENMAN, S., CROUS, P.W., TAYLOR, J.E., KANG, J.C., PASCOE, I., WINGFIELD, M.J. An overview of the taxonomic history of Botryosphaeria, and a re-evaluation of its anamorphs based on morphology and ITS rDNA phylogeny. **Studies in Mycology**, v. 45:, p.129–140, 2000.

DHINGRA, O.D., SINCLAIR, J.B. **Basic plant pathology methods**. 2 ed. CRC Press. 434p. 1995.

EDGAR, R.C. Muscle: Multiple sequence alignment with high accuracy and high throughput. **Nucleic Acids Research,** v. 32, p. 1792–1797, 2004.

FAO - Food and Agriculture Organization of the United Nations. FAOSTAT. **Summary of food and agriculture statistics**. Disponível em: <http://faostat.fao.org/site/339/default.aspx>. Acesso em: 12 abril 2020.

FARR, D.F., ROSSMAN, A.Y. **Fungal Databases, Systematic Mycology and Microbiology Laboratory, ARS, USDA**. Disponível em: http://nt.ars-grin.gov/fungaldatabases/. Acesso em: 05 mai 2013.

FREEMAN, S., KATAN, T., SHABI, E. Characterization of Colletotrichum species responsible for anthracnose diseases of various fruits. **Plant Disease**, v.82, n.6 p. 596-605, 1998.

FREIRE, M.G.M., MUSSI-DIAS, V., COUTINHO, H.S., HERNÁNDEZ, G.A.F., TAVARES, L.P.S. Bioprospecção da flora fúngica endofítica da Reserva de Jurubatiba. **Perspectivas online: Biológicas & Saúde**, 21(6): 6-12, 2016.

FREIRE, M.G.M.F., MUSSI-DIAS, V., MATTOSO, T.C., HENK, D.A., MENDES, A., MACEDO, L.R, TURATTI, C., MACHADO, S.W., SAMUELS, R.I. Survey of endophytic *Alternaria* species isolated from plants in the Brazilian restinga biome. **Journal of Pharmacy and Biological Sciences**, v 12 n. 2, p. 84-94, 2017.

HANIN, N.A., FITRIASARI, P.D. Identification of endophytic fungi from fruits and seeds of jambolana (*Syzygium cumini* L.) Skeels. **IOP Conf. Series: Earth and Environmental Science**, v. 276:012060, 2019.

HEPPERLE, D. **DNA Dragon 1.4.1 - DNA Sequence Contig Assembler Software**. 2011.

JACOBS, K., BERGDAHL, D.R., WINGFIELD, M.J., HALIK, S., SEIFERT, K. A, BRIGHT, D.E., WINGFIELD, B.D. *Leptographium wingfieldii* introduced into North America and found associated with exotic *Tomicus piniperda* and native bark beetles. **Mycological research**, v. 108, p. 411–418, 2004.

KUMAR, S., STECHER, G., TAMURA, K. Mega7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. **Molecular Biology and Evolution** v. 33, p.1870–1874. 2016.

LETUNIC, I., BORK, P. Interactive Tree Of Life (iTOL) v4: recent updates and new developments. **Nucleic Acids Research**, v. 47, 2019. https://doi.org/10.1093/nar/gkz239.

LIU, J., SUI, Y., WISNIEWSKI, M., XIE, Z., LIU, Y.,YOU,Y., ZHANG,X., SUN, Z., LI, W., LI, Y., WANG, Q.. The impact of the postharvest environment on the viability and virulence of decay fungi. **Critical Reviews in Food Science and Nutrition**, v. 58, n. 10, p. 1681-1687, 2018.

LOPES, U.P., ZAMBOLIM, L., PINHO, D.B., BARROS, A.V., COSTA, H., PEREIRA, O.L. Postharvest rot and mummification of strawberry fruits caused by *Neofusicoccum parvum* and *N. kwambonambiense* in Brazil. **Tropical Plant Pathology**, v. 39, n. 2, p. 178-183, 2014.

MACHADO, A.R., PINHO, D.B., PEREIRA, O.L. Phylogeny, identification and pathogenicity of the Botryosphaeriaceae associated with collar and root rot of the biofuel plant *Jatropha curcas* in Brazil, with a description of new species of *Lasiodiplodia*. **Fungal Diversity**, v. 67, p. 231–247, 2014.

MADDISON, W.P., MADDISON, D.R. Mesquite: a modular system for evolutionary analysis (2018) . Disponível em: http://mesquiteproject.org/mesquiteArchives/mesquite1.02/mesquite/download/MesquiteManual.pdf. Acesso em: 28/4/2020.

MORAL, J., AGUSTÍ-BRISACH, C., PÉREZ-RODRÍGUEZ, M., et al. Identification of fungal species associated with branch dieback of olive and resistance of table cultivars to *Neofusicoccum mediterraneum* and *Botryosphaeria dothidea*. **Plant Dis**. v. 101, n.2, p. 306-316, 2017.

MUSSI-DIAS, V., FERNANDES, D.C., FREIRE, M.G.M. Criação do banco de dados da micoteca do Laboratório de Química e Biomoléculas (LAQUIBIO) do ISECENSA. **Perspectivas Online: Biológicas & Saúde**, v.8, n.28, p. 92-102, 2018.

MUSSI-DIAS, V., FREIRE, M.G.M. Evaluation of postharvest stem-end rot on coconut fruits. **Journal of Agriculture and Environmental Sciences**. v. 5, n. 2, p. 25-35, 2016.

PALOU, L., MONTESINOS-HERRERO, C., BESADA, C, TABERNER, V. Postharvest fruit rot of persimmon (*Diospyros kaki*) in Spain caused by *Lasiodiplodia theobromae* and *Neofusicoccum* spp. **J Phytopathol,** v. 161, p. 625-631, 2013.

PALOU, L., TABERNER, V., GUARDADO, A., MONTESINOS-HERRERO, C. First report of *Alternaria alternata* causing postharvest black spot of persimmon in Spain. **Australas Plant Dis Notes**, v. 7, p. 41–42, 2012.

PENNYCOOK, S.R., SAMUELS, G.J. *Botryosphaeria* and *Fusicoccum* species associated with ripe fruit rot of *Actinidia deliciosa* (kiwifruit) in New Zealand. **Mycotaxon**, v. 24 p. 445–458, 1985.

PERES, A.P., SILVA-MANN, R., VIEIRA, M.G.G.C., MACHADO, J.C. Variabilidade morfocultural e genética de fungos associados à podridão peduncular do mamão. **Ciência Agrotécnica,** v. 27, n. 5, p. 1053-1062, 2003.

PILLAY, K., SLIPPERS, B., WINGFIELD, M.J., GRYZENHOUT, M. Diversity and distribution of co-infecting Botryosphaeriaceae from *Eucalyptus grandis* and *Syzygium cordatumin* South Africa. **South African Journal of Botany**, v. 84, p. 38–43. 2013.

POSADA, D., BUCKLEY, T.R. Model selection and model averaging in phylogenetics: advantages of akaike information criterion and bayesian approaches over likelihood ratio tests. **Systematic biology,** 53, p. 793–808. 2004.

RAMBAUT, A., DRUMMOND, A.J., XIE, D., BAELE, G., SUCHARD, M.A., 2018. Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7. Systematic biology. https://doi.org/10.1093/sysbio/syy032.

RONQUIST, F., HUELSENBECK, J.P. MrBayes 3: Bayesian phylogenetic inference under mixed models. **Bioinformatics** v. 19, p. 1572–1574, 2003.

SAKALIDIS, M.L, SLIPPERS, B., WINGFIELD, B.D., HARDY, G.E.ST.J., BURGESS, T.I. The challenge of understanding the origin, pathways and extent of fungal invasions: global populations of the *Neofusicoccum parvum–N. ribis* species complex. **Diversity and Distributions**, v. 19, p. 873–883, 2013.

SAKALIDIS, M.L., HARDY, G.E.S.J., BURGESS, T. Use of the Genealogical Sorting Index (GSI) to delineate species boundaries in the *Neofusicoccum parvum-Neofusicoccum ribis* species complex. **Molecular Phylogenetics and Evolution**, v. 60, p. 333-344, 2011.

SANTOS, P.H.D. DOS, OLIVEIRA, C.R.R. DE, GONÇALVES, V.M.L., AREDES, F.A.S., SILVEIRA, S.F. Phylogeographic distribution of *Asperisporium caricae* isolates in brazilian southeastern and northeastern coastal regions. **Bioscience Journal**, v., 35, p.859–868, 2019.

SANTOS, P.H.D., CARVALHO, B.M., AGUIAR, K.P., AREDES, F.A.S., POLTRONIERI, T.P.S., VIVAS, J.M.S., MUSSI-DIAS, V., BEZERRA, G.A., PINHO, D.B., PEREIRA, M.G., SILVEIRA, S.F. Phylogeography and population structure analysis reveals diversity by mutations in *Lasiodiplodia theobromae* with distinct sources of selection. **Genetics and Molecular Research**, v. 16, p.1–14, 2017.

SANTOS, P.H.D., CARVALHO, B.M., MUSSI DIAS, V., FREIRE, M.G.M. Isolamento do fungo endofítico *Phomopsis phyllanthicola* proveniente de restinga do norte fluminense. **VIII Congresso Brasileiro de Micologia**. Florianópolis - SC, p. 107, 2016.

SERGEEVA, V., ALVES, A., PHILLIPS, A.J.L. *Neofusicoccum luteum* associated with leaf necrosis and fruit rot of olives in New South Wales, Australia. **Phytopathol Mediterr**, v. 48, p. 294–298, 2009.

SILVA, L.M., BARBOSA, M.G., FERNANDES, M.B., RIBEIRO, R.C.F., MIZOBUTSI, E.H. Progresso temporal e controle da antracnose em banana no semiárido norte mineiro. **Rev. Bras. Frutic**., v.38, n.1, p.081-091. 2016.

SLIPPERS, B., WINGFIELD, M.J. Botryosphaeriaceae as endophytes and latent pathogens of woody plants: diversity, ecology and impact. **Fungal Biology Reviews**, v. 21, p. 90-106, 2007.

WHITE, T.J., BRUNS, T., LEE, S., TAYLOR, J. Amplification and direct sequencing of fungal ribosomal rna genes for phylogenetics, In: **PCR protocols**. p. 315–322. 1990.

WHITE, J.F., KINGSLEY, K.L., ZHANG, Q., et al. Review: endophytic microbes and their potential applications in crop management. **Pest Manag Sci**., v.75, n.10, p. 2558-2565, 2019.007.

YAN, L., ZHU, J., ZHAO, X., SHI, J., JIANG, C., SHAO, D. Beneficial effects of endophytic fungi colonization on plants. **Appl Microbiol Biotechnol**. v. 103, n.8, p. 3327-3340, 2019.

ZHAI, L., ZHANG, M. First report of *Neofusicoccum parvum* causing fruit rot on *Eriobotrya japonica* in China. Pant Disease, 2019. DOI: https://doi.org/10.1094/PDIS-09-18-1511-PDN.