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Endophytic fungi from *Vitis labrusca* L. ('Niagara Rosada') and its potential for the biological control of *Fusarium oxysporum*

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ABSTRACT. We investigated the diversity of endophytic fungi found on grape (*Vitis labrusca* cv. Niagara Rosada) leaves collected from Salesópolis, SP, Brazil. The fungi were isolated and characterized by amplified ribosomal DNA restriction analysis, followed by sequencing of the ITS1-5.8S-ITS2 rDNA. In addition, the ability of these endophytic fungi to inhibit the grapevine pathogen *Fusarium oxysporum* f. sp *herbemontis* was determined *in vitro*. We also observed that the climatic factors, such as temperature and rainfall, have no effect on the frequency of infection by endophytic fungi. The endophytic fungal community that was identified included *Aporospora terricola*, *Aureobasidium pullulans*, *Bjerkandera adusta*, *Colletotrichum boninense*, *C. gloeosporioides*, *Diaporthe helianthi*,

D. phaseolorum, *Epicoccum nigrum*, *Flavodon flavus*, *Fusarium subglutinans*, *F. sacchari*, *Guignardia mangiferae*, *Lenzites elegans*, *Paraphaeosphaeria pilleata*, *Phanerochaete sordida*, *Phyllosticta* sp, *Pleurotus nebrodensis*, *Preussia africana*, *Tinctoporellus epiniltinus*, and *Xylaria berteri*. Among these isolates, two, *C. gloeosporioides* and *F. flavus*, showed potential antagonistic activity against *F. oxysporum* f. sp *herbemontis*. We suggest the involvement of the fungal endophyte community of *V. labrusca* in protecting the host plant against pathogenic *Fusarium* species. Possibly, some endophytic isolates could be selected for the development of biological control agents for grape fungal disease; alternatively, management strategies could be tailored to increase these beneficial fungi.

Key words: Diversity; Grape; Endophytes; Fungal inhibition; ARDRA; Antagonism

INTRODUCTION

The plant phyllosphere, which may be divided into the endosphere and exosphere (Hardoin et al., 2008), represents an important habitat for a highly diverse fungal community. Although the nature of the interactions between endophytic fungi, which colonize the endosphere, and their hosts is not yet fully understood, this community is known to play an important role in plant growth, health, and stress maintenance (Araújo et al., 2001; Azevedo and Araújo, 2006).

The endophytic community structure is influenced by and varies depending on the environment to which the plant is adapted, and the fluctuations in abiotic factors such as site, temperature, and rainfall may have a role in the settling and establishment of some microbial species associated with the host plant (Arnold et al., 2003; Lana et al., 2011). These factors may interfere with the life strategy of endophytes in relation to the host plant. According to Hardoin et al. (2008), the endophytic community can be classified into three groups: 1) facultative endophytes, which can live inside the host plant but may be found in other habitats; 2) obligate endophytes, which are found strictly inside the host plant and cannot colonize other habitats, and 3) passenger endophytes, which randomly enter into the plant in the absence of selective factors such as plant protection and competition.

The majority of studies that have isolated endophytic fungi from grapevine have been performed in Europe and Africa and involve European grapevines (*Vitis vinifera*). These studies are usually concerned with the characterization of the endophytic communities (Mostert et al., 2000; Medina et al., 2005; Martini et al., 2009). Characterization of the endophytic fungal community of *Vitis labrusca* has been conducted by Brum (2006) and Lima (2010). Several studies have been performed using endophytic fungi as biocontrol agents of plant pathogens (Rubini et al., 2005; Sibounnavong et al., 2008; Küçük and Kivanç, 2008). As biocontrol agents, endophytic fungi are strategically advantageous against phytopathogens because they colonize the same ecological niches occupied by pathogenic microorganisms, ensuring competition for nutrients and space. Additionally, the endophytes may induce useful defense responses against pathogens in host plants. The colonization of grapevines by endophytes has

been related to the role of endophytic fungi in the biological control of pathogens, mainly *Plasmopora viticola* (Mostert et al., 2000; Musetti et al., 2006; Compant et al., 2008; Martini et al., 2009).

The genus *Fusarium* causes some of the most economically damaging plant diseases, affecting viticulture as well as other plant species throughout the world (Del Ponte et al., 2009; Matos et al., 2009). In grapes, *Fusarium*, mainly the species *Fusarium oxysporum* f. sp *herbemontis*, penetrates into root tissues, which can lead to plant death (Omer et al., 1999). The virulence of disease increases when plants are attacked by insects such as phylloxera, which produce wounds that facilitate fungal infection (Lotter et al., 1999; Edwards et al., 2007). The use of *Vitis* species and hybrids that are more resistant to the fungus may decrease the incidence of disease, but the degree of resistance is low in *V. labrusca* and *V. vinifera* (Botton et al., 2008). According to Fravel et al. (2003), the use of non-pathogenic *Fusarium* isolates is effective in the biocontrol of pathogenic isolates of this fungus. However, several species of *Fusarium* produce toxins, thereby invalidating their use for biocontrol owing to risks of food contamination (Mikusova et al., 2010). Therefore, the use of other fungal endophytic species for control becomes an important alternative; in fact, mycorrhizal inoculation and induction of enzymes such as chitinases has been shown to increase resistance to *Fusarium* in grapevine rootstocks (Dalla Costa et al., 2010).

The aim of the present study was to isolate endophytic fungi from *V. labrusca* cv. 'Niagara Rosada' and evaluate its *in vitro* potential for inhibiting the pathogenic fungus *F. oxysporum* f. sp *herbemontis*. In addition, the influence of abiotic factors (temperature and rainfall) on the frequency of endophytic fungi colonization was analyzed.

MATERIAL AND METHODS

Plant material and fungal isolation

Healthy leaves from six 4-year-old grape plants (*V. labrusca* cv. 'Niagara Rosada'), cultivated in Salesópolis, São Paulo, Brazil (23° 32' S and 45° 51' W), were collected approximately 1.0 m from the main stem and 1.5 m above the ground. Thirty leaves per plant were sampled in July (C1), October (C2), and December (C3) of 2004 as well as in March (C4) and August (C5) of 2005. The leaves were washed in tap water, immersed in ethanol (70%) for 30 s, surface disinfected in sodium hypochlorite (2% available Cl) for 3 min, and rinsed twice in sterile distilled water. As a control, the last water used to rinse the leaves was plated onto potato dextrose agar (PDA; Oxoid).

After surface disinfection, each leaf was cut, and six fragments (5 x 5 mm) per leaf were placed onto PDA containing 100 mg/mL tetracycline to inhibit bacterial growth and incubated at 28°C for 7-14 days, resulting in 1080 fragments per sampling time. The number of leaf fragments showing fungal growth was counted, and the tips of morphologically distinct hyphae were collected and subcultured on PDA plates for further identification. The infection frequency (IF) was calculated after each isolation as the ratio between the number of fragments that showed fungal growth and the total number of plant fragments analyzed. The fungal isolates were purified and stored submerged in mineral oil or water. In both methods, the isolates were kept at room temperature. The difference in IF was analyzed with a Tukey test at a 5% significance level.

DNA extraction and polymerase chain reaction (PCR)

The endophytic fungi were grown in PDB (potato dextrose broth) for 15 days, and the total DNA was extracted according to the method of Raeder and Broda (1985). The ITS1-5.8S-ITS2 ribosomal DNA (rDNA) sequence was amplified in a 50-µL final volume containing 2 µL 20 ng DNA, 0.2 mM of each primer [ITS1 (5'-TCCGATGGTGAACCTGC GG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3')], 0.2 mM of each deoxyribonucleotide triphosphate, 3.7 $MgCl_2$, and 0.05 U *Taq* DNA polymerase (Invitrogen, Brazil) in 20 mM Tris-HCl, pH 8.4, with 50 mM KCl. PCR assays without DNA were included as negative controls in all of the experiments. The reaction conditions were as follows: an initial denaturation of 94°C for 2 min and 35 cycles of denaturation, primer annealing, and primer extension at 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min, respectively. The reaction products were separated through electrophoresis on a 1.0% agarose gel and stained with ethidium bromide.

Amplified ribosomal DNA restriction analysis and sequencing analysis

The digestion mixture had a final volume of 11 µL consisting of 7 µL of the amplification product of ITS1-5.8S rDNA-ITS4 and 3 U restriction enzyme *Hin*fI. The reaction was incubated at 37°C for 3.0 h, further electrophoresed on 2.4% agarose gel, stained with ethidium bromide, and visualized on an ultraviolet transilluminator. The restriction fragments generated were analyzed and grouped into haplotypes according to the banding profile.

For endophytic fungal identification, the PCR products of isolates from each haplotype were purified using a GFX PCR DNA and gel band purification kit (GE Helthcare, NJ, USA) and sequenced using the ITS1 primer and a Big Dye Terminator System (Applied Biosystems). The sequences obtained were evaluated against GenBank sequences using the Basic Local Alignment Search Tool [http://www.ncbi.nlm.nih.gov (accessed January 26, 2012)]. The sequences were aligned and edited, and distance matrices and phylogenetic trees (Saitou and Nei, 1987) were calculated based on neighbor-joining algorithms using Molecular Evolutionary Genetics Analysis version 4. The nucleotide sequences obtained in this study were submitted to GenBank.

Endophytic antimicrobial activity against *Fusarium*

The antimicrobial activity of endophytic fungi from *V. labrusca* against *F. oxysporum* f. sp *herbemontis* was evaluated. *F. oxysporum* f. sp *herbemontis*, isolated from grape plants, was obtained from the culture collection of Embrapa Uva e Vinho (Bento Gonçalves, RS, Brazil). The *in vitro* selection of antagonists against the pathogen was conducted on PDA medium. For this method, mycelial disks (5 mm in diameter) of the phytopathogen were inoculated on PDA medium, and grape endophytic fungi were inoculated on the same plate 40 mm away from the *Fusarium* colony. The cultures were incubated at 28°C (with a photoperiod of 12 h) for 2-7 days, and the antagonism was detected by the formation of an inhibition halo. Control plates were prepared by inoculating *F. oxysporum* disks without endophytic fungi. The experiment was performed three times with triplicate plates in each experiment.

RESULTS

Isolation and identification of culturable fungi

No fungal growth was observed on control plates, indicating that the epiphytic community was effectively eliminated. Using the described methodology, the IF ranged from 0.79 to 1.0 over time, resulting in approximately 5184 fungal colonies, but this variation was unrelated to temperature and rainfall (Table 1). From these fungal colonies, 550 isolates were randomly selected and stored for further identification.

Table 1. Infection frequency (IF) of 1080 leaf fragments per isolation time, according to environmental conditions.

Isolation time	IF*	Temperature (°C)	Rainfall (mm^3)
C1	0.92[a]	16.0	2.3
C2	0.96[a]	17.5	0.0
C3	0.79[a]	20.2	3.6
C4	1.00[a]	22.0	9.3
C5	0.92[a]	22.4	1.5

*Values followed by the same letter were not significantly different at the Tukey test ($P < 0.05$).

The endophytic fungal community isolated from *V. labrusca* leaves included *Aporospora terricola*, *Aureobasidium pullulans*, *Bjerkandera adusta*, *Colletotrichum boninense*, *C. gloeosporioides*, *Diaporthe helianthi*, *D. phaseolorum*, *Epicoccum nigrum*, *Flavodon flavus*, *Fusarium subglutinans*, *F. sacchari*, *Guignardia mangiferae*, *Lenzites elegans*, *Paraphaeosphaeria pilleata*, *Phanerochaete sordida*, *Phyllosticta* sp, *Pleurotus nebrodensis*, *Preussia africana*, *Tinctoporellus epiniltinus*, and *Xylaria berteri* (Table 2).

Table 2. Identification of endophytic fungi from *Vitis labrusca* based on rRNA sequencing.

Isolate	Haplotype	Identification	GenBank No.	Maximal identity	*Fusarium* (grapevine phytopathogen)
4F6	H12	*Aporospora terricola*	EU272526.1	93%	++
1F2	H2	*Aureobasidium pullulans*	EU272526.1	97%	-
3F9	H5	*Bjerkandera adusta*	FJ810147.1	90%	++
2F3	H5	*Colletotrichum boninense*	FJ968603.1	100%	+
3F19	H5	*Colletotrichum boninense*	HM044134.1	100%	-
4F5	H5	*Colletotrichum boninense*	FJ441663.1	97%	-
2F1	H5	*Colletotrichum boninense*	EU822802.1	96%	+
5F17	H2	*Colletotrichum gloeosporioides*	GU222369.1	96%	+++
2F10	H3	*Diaporthe helianthi*	FJ441611.1	94%	++
3F2	H3	*Diaporthe helianthi*	EU888929.1	94%	+
2F8	H3	*Diaporthe phaseolorum*	EU272538.1	95%	++
2F9	H3	*Diaporthe phaseolorum*	EU622854.1	88%	+
1F4	H10	*Epicoccum nigrum*	FJ904828.1	99%	-
2F26	H11	*Flavodon flavus*	FJ010207.1	95%	+++
4F19	H3	*Fusarium subglutinans*	FJ158133.1	98%	-
4F21	H3	*Fusarium sacchari*	EF453121.1	98%	-
3F6	H8	*Guignardia mangiferae*	AB119119.1	91%	-
5F9	H6	*Lenzites elegans*	FJ711054.1	95%	-
2F7	H5	*Paraphaeosphaeria pilleata*	AF250821.1	94%	-
1F11	H13	*Phanerochaete sordida*	AB210078.1	99%	-
5F6	H8	*Phyllosticta* sp	FJ538346.1	95%	+
1F9	H8	*Phyllosticta* sp	AB119119.1	95%	+
1F10	H4	*Pleurotus nebrodensis*	FJ572269.1	98%	-
3F18	H10	*Preussia africana*	AY510420.1	95%	-
3F24	H10	*Preussia africana*	AYS10420.1	95%	-
3F17	H7	*Tinctoporellus epiniltinus*	GU731575.1	89%	+
3F16	H3	*Xylaria berteri*	GU324749.1	97%	++

(-) = no inhibition; (+) = inhibition haloes, 0.1-0.5 cm; (++) = inhibition haloes, 0.6-1.0 cm; (+++) = inhibition haloes, >1.0 cm.

Molecular characterization of endophytic fungi from *V. labrusca* leaves

From 550 selected isolates, 115 endophytic fungi from *V. labrusca* leaves were partially characterized with amplified ribosomal DNA restriction analysis (ARDRA) and rDNA (partial 18S, ITS1, 5.8S, ITS2, and partial 23S) sequencing. The ~650-bp fragments were cut with the *Hin*fI restriction enzyme, allowing the observation of 13 haplotypes (see Table 2). The identification of these haplotypes enabled fungi identification, which showed that the cultivable endophytic fungi associated with grape leaves belonged mainly to phylum Ascomycota, with the most frequent classes being Sordariomycetes (50% of cultivable endophytic fungi; Figure 1) and Dothideomycetes (27% of cultivable endophytic fungi; Figure 2). The phylum Basidiomycota was less frequently present (23% of cultivable endophytic fungi) and included isolates belonging to the class Agaricomycetes (Figure 3).

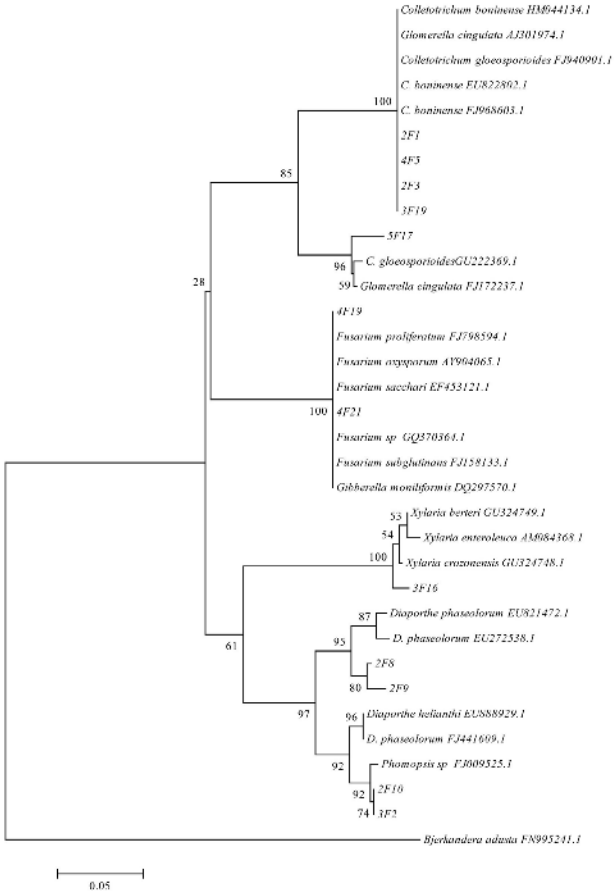

Figure 1. Phylogenetic relationships of endophytic fungi isolated from *Vitis labrusca* belonging to Ascomycota phylum; the isolates belonged to Class Sordariomycetes (Families: Diaporthaceae, Glomerellaceae, Xylariaceae, and Order Hypocreales (Family: Incertae sedis). The sequence analysis was based on partial ITS1-5.8S-ITS2 rRNA sequences obtained from endophytes and closely related sequences that are based on a distance analysis (neighbor-joining algorithm with the Jukes-Cantor model; 1000 bootstrap replicates performed).

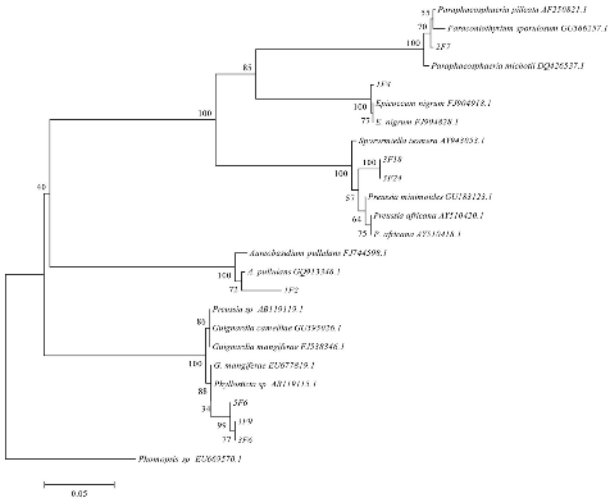

Figure 2. Phylogenetic relationships of endophytic fungi isolated from *Vitis labrusca* belonging to Ascomycota phylum; the isolates belonged to Class Dothideomycetes (Families: Botryosphaeriaceae, Dothioraceae, Montagnulaceae, Sporormiaceae and Genus *Epicoccum*, *Aporospora*, both Incertae sedis Family). The sequence analysis was based on partial ITS1-5.8S-ITS2 rRNA sequences obtained from endophytes and closely related sequences that are based on a distance analysis (neighbor-joining algorithm with the Jukes-Cantor model; 1000 bootstrap replicates performed).

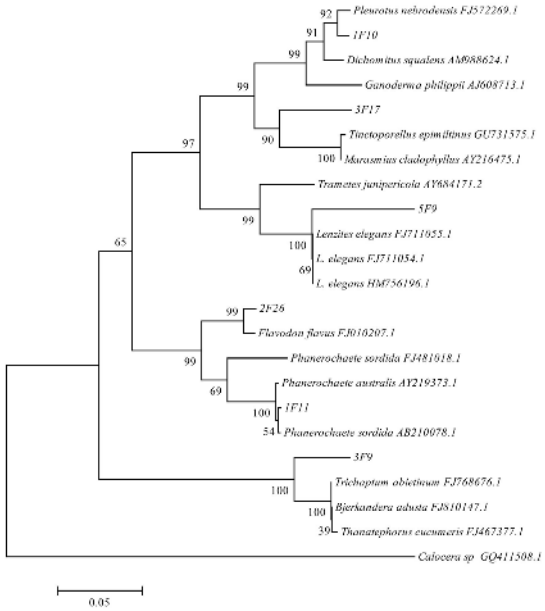

Figure 3. Phylogenetic relationships of endophytic fungi isolated from *Vitis labrusca* belonging to Basidiomycota phylum; the isolates belonged to Orders: Agaricales (Families: Schizophyllaceae and Pleurotaceae); Corticiales (Families: Corticiaceae); Polyporales (Family: Coriolaceae and Genus *Flavodon*, Incertae sedis Family); Russulales (Family: Meruliaceae). The sequence analysis was based on partial ITS1-5.8S-ITS2 rRNA sequences obtained from endophytes and closely related sequences that are based on a distance analysis (neighbor-joining algorithm with the Jukes-Cantor model; 1000 bootstrap replicates performed).

Haplotypes H3 and H5, identified as *Diaporthe* spp and *Colletotrichum* spp (Figure 4), respectively, were the most frequent groups in the *V. labrusca* endophytic community. The H3 isolates were obtained mainly in samples from C2, C3, and C4, whereas haplotype H5 was isolated from C2 and C3 (Figure 5).

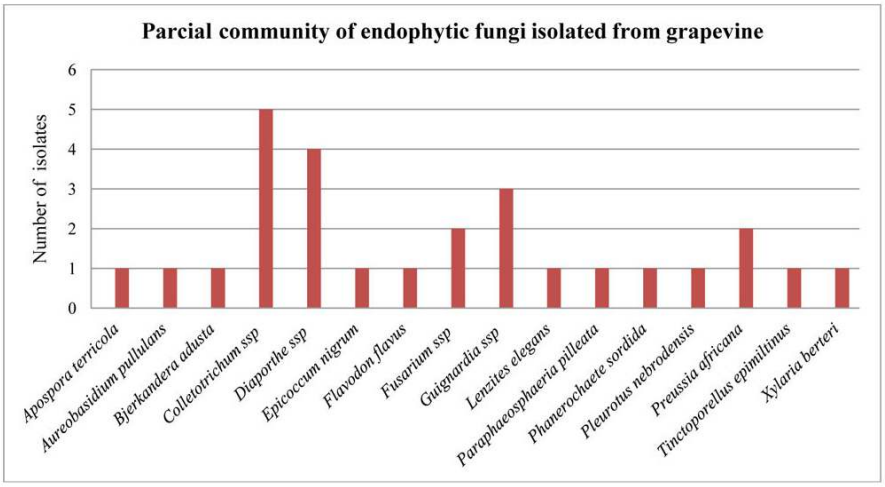

Figure 4. Partial community of endophytic fungi isolated from grapevine.

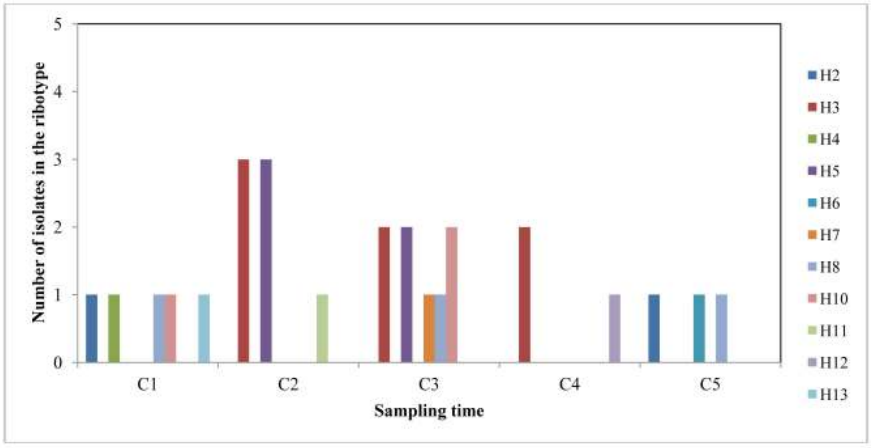

Figure 5. Distribution of endophytic fungi (haplotypes) in each sampling time (C1 to C5).

Antagonism of endophytic fungi from *V. labrusca* against *F. oxysporum* f. sp *herbemontis*

The activity of the endophytic fungi isolated from *V. labrusca* against *F. oxysporum* f. sp *herbemontis* was evaluated. We found that 52% of the endophytic fungi were capable of

inhibiting the growth of *F. oxysporum* f. sp *herbemontis*, but this frequency was unrelated to temperature, rainfall, or the ARDRA profile. Of the endophytic fungi tested, *A. terricola*, *B. adusta*, *D. phaseolorum*, *D. helianthi*, and *X. berteri* displayed moderate antagonism against *F. oxysporum* f. sp *herbemontis*. The endophytic *F. flavus* (2F26 strain) and *C. gloeosporioides* (5F17 strain) strongly inhibited *F. oxysporum* f. sp *herbemontis* (see Table 2).

DISCUSSION

The plant phyllosphere is a dynamic environment in which biotic and abiotic factors affect the structure and species composition of the fungal and bacterial communities that colonize roots, stems, branches, and leaves. The presence and composition of endophytic communities likely depend on the interaction with other endophytic or pathogenic microorganisms and may vary according to season (Araújo et al., 2001, 2002). These endophytic communities are ubiquitous and may improve plant fitness by reducing herbivory or phytopathogen settling (Benhamou et al., 2002) and promoting plant growth (Varma et al., 1999). The present study showed no correlation between the season and the community composition of endophytic isolates. Climatic variations in the regions studied may have been insufficient to cause changes in the frequency of isolation and composition of the endophytic fungal community, but additional investigations must be conducted to confirm this hypothesis.

The analysis of polymorphisms generated using the ARDRA technique is an important tool in the precise characterization of the diversity of endophytic communities and the organization of initial studies with large numbers of samples. The identification of 27 isolates, representing all haplotypes, obtained by sequencing the ITS1-5.8S-ITS2 and comparing it with sequences deposited in GenBank revealed that some haplotypes contained more than one genus. The endophytic fungal community of *V. labrusca* 'Niagara Rosada' showed great diversity, including species belonging to the phyla Ascomycota (77%) and Basidiomycota (23%). *Colletotrichum*, *Diaporthe*, *Epicoccum*, *Fusarium*, and *Guignardia* were the dominant genera in the endophytic community from Niagara Rosada leaves (see Table 2). These fungi have also been isolated from various plants from tropical and subtropical regions, including *Saccharum* sp (Mendes and Azevedo, 2007), *Theobroma cacao* (Rubini et al., 2005; Hamada, 2006), *Musa* (Cao et al., 2003), *Arachis* (Suryanarayanan and Murali, 2006), *Euterpe* (Rodrigues, 1994), and *Citrus* spp (Araújo et al., 2001; Durán et al., 2005). Several of the species we isolated have also been found as endophytes of *V. vinifera* (Medina et al., 2005; Martini et al., 2009) and *V. labrusca* (Lima, 2010). Other isolates, such as those belonging to the phylum Basidiomycota, including the species *B. adusta*, *F. flavus*, *P. sordida*, *P. nebrodensis*, and *T. epimiltinus*, were dominant and had not, to date, been isolated as endophytes from tropical plants. Similar results were observed for *P. africana* and *A. terricola* (Ascomycota).

Colletotrichum and *Diaporthe* species have also been cited in the literature as pathogenic to grapevines and other economically important crops. The evaluated plants from which these fungi were isolated neither displayed nor developed symptoms during the evaluation time (9 months), suggesting that although these isolates belong to genera associated with plant disease, they may be hypovirulent or have lost the ability to induce disease.

Fungi are well known to have antibacterial, antifungal, larvicidal, molluscicidal, and antioxidant effects (Keller et al., 2002). Among the classes of fungi, the ascomycetes are cited as active producers of antimicrobial compounds. In this study, 48.14% of endophytic fungi

isolated from *V. labrusca* belonged to the phylum Ascomycota, and most of these were capable of inhibiting *F. oxysporum* f. sp *herbemontis*. That several endophytes from Niagara Rosada inhibited the growth of *F. oxysporum* f. sp *herbemontis* and *F. oxysporum* suggests that these endophytic fungi may be involved in protecting the host plant against fungal diseases. Similar reports have been published describing grapevine endophytes protecting the host against a number of pathogenic plant fungi (Museti et al., 2006; Martini et al., 2009). The present study showed for the first time that a number of species from the endophytic community of *V. labrusca* can inhibit *in vitro F. oxysporum* f. sp *herbemontis* in a manner similar to that of mycorrhizal inoculation by the fungus *Glomus* in *Vitis* (Dalla Costa et al., 2010). Therefore, further studies should focus on the analysis and development of efficient strategies based on these endophytic fungi to control fungal grapevine diseases in plants. In addition, the identification of these metabolites could be part of a strategy for developing a sustainable product to control this plant disease.

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REFERENCES

Araújo WL, Maccheroni W Jr, Aguilar-Vildoso CI, Barroso PA, et al. (2001). Variability and interactions between endophytic bacteria and fungi isolated from leaf tissues of citrus rootstocks. *Can. J. Microbiol.* 47: 229-236.

Araújo WL, Marcon J, Maccheroni W Jr, Van Elsas JD, et al. (2002). Diversity of endophytic bacterial populations and their interaction with *Xylella fastidiosa* in citrus plants. *Appl. Environ. Microbiol.* 68: 4906-4914.

Arnold AE, Mejia LC, Kyllo D, Rojas EI, et al. (2003). Fungal endophytes limit pathogen damage in a tropical tree. *Proc. Natl. Acad. Sci. U. S. A.* 100: 15649-15654.

Azevedo JL and Araújo WL (2006). Fungi: Multifaceted Microbes. In: Diversity and Applications of Endophytic Fungi Isolated from Tropical Plants (Ganguli BN and Deshmukh SK, eds.). Anamaya Publishers, New Dehli, 189-207.

Benhamou N, Garand C and Goulet A (2002). Ability of nonpathogenic *Fusarium oxysporum* strain Fo47 to induce resistance against *Pythium ultimum* infection in cucumber. *Appl. Environ. Microbiol.* 68: 4044-4060.

Botton M, Soria SJ and Hickel ER (2008). Manejo de Pragas na Cultura da Videira. Available at [http://www.cnpuv.embrapa.br/]. Accessed October 19, 2010.

Brum MCP (2006). Microrganismos Endofiticos da Videira Niagara Rosada (*Vitis labrusca* L.) e o Controle Biológico de *Fusarium*. Master's thesis, Universidade de Mogi das Cruzes, Mogi das Cruzes.

Cao LX, You JL and Zhou SN (2003). Endophytic fungi from *Musa acuminate* leaves and roots in South China. *World J. Microbiol. Biotechnol.* 18: 169-171.

Compant S, Kaplan H, Sessitsch A, Nowak J, et al. (2008). Endophytic colonization of *Vitis vinifera* L. by *Burkholderia phytofirmans* strain PsJN: from the rhizosphere to inflorescence tissues. *FEMS Microbiol. Ecol.* 63: 84-93.

Dalla Costa M, Lovato PC and Sete PB (2010). Mycorrhizal inoculation and induction of chitinases and β-1,3-glucanases and fusarium resistance grapevine rootstock. *Pesq. Agrop. Bras.* 45: 375-383.

Del Ponte EM, Fernandes JMC and Pavan W (2009). A model-based assessment of vthe impacts of climate variability on *Fusarium* head blight seasonal risks in southern Brazil. *J. Phytopathol.* 157: 675-681.

Durán EL, Ploper LD, Ramalho JC and Picolo-Grandi R (2005). The foliar fungal endophytes of *Citrus limon* in Argentina. *Can. J. Bot.* 83: 350-355.

Edwards J, Norng S, Powell KS and Granett J (2007). Relationships between grape in *Phylloxera* abundance, fungal interactions and grapevine decline. *Acta Hortic.* 733: 151-157.

Fravel D, Olivain C and Alabouvette C (2003). *Fusarium oxysporum* and its biocontrole. *New Phytol.* 157: 493-502.

Hamada RE (2006). Fungos Endofíticos Visando o Controle Biológico da Podridão Parda dos Frutos do Cacaueiro e

Mecanismos de Ação do Antagonista. Doctoral thesis, Universidade Federal do Amazonas, Manaus.
Hardoin P, van Overbeek LS and van Elsas JD (2008). Properties of bacterial endophytes and their proposed role in plant growth. *Trends Microbiol.* 16: 463-471.
Keller C, Maillard M, Keller J and Hostettmann K (2002). Screening of European fungi for antibacterial, antifungal, larvicidal, molluscicidal, antioxidant and free-radical scavenging activities and subsequent isolation of bioactive compounds. *Pharmaceut. Biol.* 40: 518-525.
Küçük Ç and Kivanç M (2008). Mycoparasitism in the biological control of *Gibberella zeae* and *Aspergillus ustus* by *Trichoderma harzianum* strains. *J. Agric. Technol.* 4: 49-55.
Lana TG, Azevedo JL, Pomella AW, Monteiro RT, et al. (2011). Endophytic and pathogenic isolates of the cacao fungal pathogen *Moniliophthora perniciosa* (Tricholomataceae) are indistinguishable based on genetic and physiological analysis. *Genet. Mol. Res.* 10: 326-334.
Lima TEF (2010). Micobiota endofitica de *Vitis labrusca* L. CV Isabel no vale do Siriji, Pernambuco, Brasil. Masther's thesis, Departamento de Micologia, Universidade Federal de Pernambuco, Recife.
Lotter DW, Granett J and Omer AD (1999). Differences in grape Phyloxera-related grapevine root damage in organic and coinventionally managed vineyards in California. *Hortscience* 34: 1108-1111.
Martini M, Musetti R, Grisan S and Polizzotto R (2009). DNA-dependent detection of the grapevine fungal endophytes *Aureobasidium pullulans* and *Epicoccum nigrum*. *Plant Dis.* 93: 993-998.
Matos AP, Sanches NF, Teixeira FA and Simão AH (2009). Monitoramento da Fusariose em Plantios de Abacaxi 'Pérola' Conduzidos em Sistema de Produção Integrada no Estado do Tocantins. Doc. 184. Embrapa Mandioca e Fruticultura Tropical, Cruz das Almas.
Medina A, Mateo R, López-Ocaña L, Valle-Algarra FM, et al. (2005). Study of Spanish grape mycobiota and ochratoxin A production by Isolates of *Aspergillus tubingensis* and other members of *Aspergillus* section Nigri. *Appl. Environ. Microbiol.* 71: 4696-4702.
Mendes R and Azevedo JL (2007). Micologia: Avanços no Conhecimento. In: Valor Biotecnológico de Fungos Isolados de Plantas de Interesse Econômico (Maia LC, Malosso E and Yano-Melo AM, eds.). Editora Universitária da Universidade Federal de Pernambuco, Recife, 129-140.
Mikusova P, Ritieni A, Santini A and Juhasova G (2010). Contamiantion by moulds of grape berries in Slovakia. Part A: Chemical, analysis, control, exposure and risks assessment. *Food Addit. Contam.* 27: 738-747.
Mostert L, Crous PW and Petrini O (2000). Endophytic fungi associated with shoots and leaves of *Vitis vinifera*, with specific reference to the *Phomopsis viticola* complex. *Sydowia* 52: 46-58.
Musetti R, Polizzotto R, Vecchione A and Borselli Z (2006). Antifungal activity of diketopiperazines extracted from *Alternaria alternata* against *Plasmopara viticola*: An ultrastructural study. *Micron* 38: 643-650.
Omer AD, Granett J and Wakerman RJ (1999). Pathogenicity of *Fusarium oxysporum* on different *Vitis* rootstocks. *J. Phytopathol.* 147: 433-436.
Raeder U and Broda P (1985). Rapid preparation of DNA from filamentous fungi. *Lett. Appl. Microbiol.* 1: 17-20.
Rodrigues KF (1994). The foliar fungal endophytes of the Amazonian palm *Euterpe oleracea*. *Mycologia* 86: 376-385.
Rubini MR, Silva-Ribeiro RT, Pomella AW, Maki CS, et al. (2005). Diversity of endophytic fungal community of cacao (*Theobroma cacao* L.) and biological control of *Crinipellis perniciosa*, causal agent of Witches' Broom Disease. *Int. J. Biol. Sci.* 1: 24-33.
Saitou N and Nei M (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4: 406-425.
Sibounnavong P, Cynthia CD, Kanokmedhakul S and Soytong K (2008). The new antagonistic fungus, *Emericella nidulans* strain EN against *Fusarium* wilt of tomato. *J. Agr. Technol.* 4: 89-99.
Suryanarayanan TS and Murali TS (2006). Incidence of *Leptosphaerulina crassiasca* in symptomless leaves of peanut in southern India. *J. Basic Microbiol.* 46: 305-309.
Varma A, Savita V, Sudha, Sahay N, et al. (1999). *Piriformospora indica*, a cultivable plant-growth-promoting root endophyte. *Appl. Environ. Microbiol.* 65: 2741-2744.