

## ***Neofusicoccum eucalyptorum*, a *Eucalyptus* pathogen, on native Myrtaceae in Uruguay**

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*Neofusicoccum eucalyptorum* is a canker-associated fungus apparently highly specialized on *Eucalyptus*. However, in surveys of the microbial population inhabiting native Myrtaceae in Uruguay, fungal cultures resembling *N. eucalyptorum* were isolated. The possible occurrence of *N. eucalyptorum* on hosts other than *Eucalyptus* prompted further investigation. Several surveys were conducted throughout Uruguay to obtain samples from native forests, focusing primarily on species in the Myrtaceae. Fungal identification was based on morphology and confirmed using comparison sequences for the internal transcribed spacer (ITS) of the rDNA operon. Pathogenicity was evaluated by inoculating plants of a *Eucalyptus grandis* clone. Morphological and molecular identification confirmed the occurrence of *N. eucalyptorum* on *Blepharocalyx salicifolius*, *Myrceugenia glaucescens* and *Myrrhinium atropurpureum* var. *octandrum*. This is the first report of *N. eucalyptorum* occurring in hosts other than *Eucalyptus*. Pathogenicity tests confirmed the ability of this species to produce cankers on *E. grandis*. This study provides information that will assist breeding programmes in attempts to obtain disease-resistant *Eucalyptus* plantations and it also suggests that a *Eucalyptus* pathogen could have moved to native trees in Uruguay.

**Keywords:** botryosphaeria canker, *Botryosphaeria eucalyptorum*, host-jump events

### **Introduction**

*Neofusicoccum eucalyptorum* (teleomorph *Botryosphaeria eucalyptorum*) was first described by Smith *et al.* (2001) in South Africa as a canker pathogen of *Eucalyptus* trees. It was later found in eastern Australia as the dominant species of Botryosphaeriaceae isolated from cankers on native and planted *Eucalyptus* (Slippers *et al.*, 2004a), and as an endophyte in *Eucalyptus globulus* (Burgess *et al.*, 2006). Several species of Botryosphaeriaceae are common endophytes that cause disease after the onset of stress, with drought being the most commonly encountered predisposing factor (Old *et al.*, 1990; Pusey, 1989; Wene & Schoeneweiss, 1980; Slippers & Wingfield, 2007). Although the impact of opportunistic endophytes is difficult to assess, Smith *et al.* (2001) considered the pathogenicity of several isolates of *N. eucalyptorum*, and concluded that the species was pathogenic to eucalypts, even though isolates of *N. eucalyptorum* were less aggressive than those of the better-known *Botryosphaeria dothidea*.

Host specialization is observed for *N. eucalyptorum*, which has been reported only from *Eucalyptus* spp. (Smith *et al.*, 2001; Slippers *et al.*, 2004a; Burgess *et al.*, 2006). Its abundance and wide distribution in eastern Australia suggests that this pathogen probably originated in Australia and was introduced with planting stock or seeds into other countries where *Eucalyptus* spp. were planted (Slippers *et al.*, 2004a). In Uruguay, *N. eucalyptorum* appears to be common in *Eucalyptus* plantations and has been found as an endophyte infecting *Eucalyptus* and also sporulating on woody debris of *Eucalyptus maidenii* after pruning (Alonso, 2004; Pérez, 2008).

The area planted to *Eucalyptus* in Uruguay nearly tripled between 1995 and 2005, from 175 000 ha to c. 500 000 ha (MGAP, 2005), and this explosive increase was also associated with increased disease problems. Nevertheless, limited work has been carried out on *Eucalyptus* pathogens in the country and very little is known regarding the biology and epidemiology of *N. eucalyptorum*. In addition, the biotic interaction between introduced *Eucalyptus* and native tree species in the Myrtaceae is also of great concern. Uruguay has a rich diversity of native Myrtaceae, with a total of 35 tree species reported by Brussa & Grela (2007). Thus, the exchange of pathogens between introduced and native trees could result in negative economic impact, as well as

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ecological disturbance or even catastrophic damage (Anderson *et al.*, 2004; Slippers *et al.*, 2005; Woolhouse *et al.*, 2005; Desprez-Loustau *et al.*, 2007; Pavlic *et al.*, 2007). For this reason, several surveys over the main forest regions in Uruguay were conducted to gain an improved understanding of the Botryosphaeriaceae occurring on introduced and native hosts in Uruguay (Pérez, 2008). During the course of these surveys, a group of isolates resembling the morphology of *N. eucalyptorum* were isolated from native Myrtaceae species. The possible occurrence of *N. eucalyptorum* on hosts other than *Eucalyptus* prompted further investigation. The objectives of this study were, therefore, to identify the isolates obtained from native myrtaceous species, and to compare these, based on genetic variation and pathogenicity, with isolates obtained from *Eucalyptus* species.

## Materials and methods

### Fungal isolates

Plant material with and without disease symptoms was collected between 2005 and 2008 from several native forests, with special attention being paid to those located close to *Eucalyptus* plantations (less than 500 m away), throughout the country. Myrtaceae species within the native forests were chosen for study because of their close phylogenetic relationship with the genus *Eucalyptus* (Wilson *et al.*, 2005). Samples were also collected from *Eucalyptus* plantations for comparison. Endophytic isolates were obtained from symptomless living tissue.

Leaf, petiole and twig sections were sequentially surface-sterilized in 70% ethyl alcohol for 1 min, immersed in 0.4% sodium hypochlorite for 2 min, then rinsed twice in sterile distilled water and blotted dry on sterile filter paper. Surface-sterilized plant tissue was placed on 2% malt extract agar (MEA) (2% malt extract, 1.5% agar; Oxoid). Plates were incubated at room temperature (~20°C) for 1 week. Colonies resembling species of Botryosphaeriaceae were selected and maintained in 2% MEA at 8°C. To verify the efficacy of the surface sterilization and to assure the growth of only endophytic microorganisms, imprints of sample surfaces after sterilization were made on MEA plates and observed for 1 week to confirm that fungi did not grow. One specimen (isolate UY336) was obtained from a stem canker. In this case the isolation was made from wood tissue at the advancing zone of the lesion, which was surface-sterilization in 70% ethyl alcohol for 30 s, rinsed twice in sterile distilled water and blotted dry on sterile filter paper. Disinfected tissue was placed on 2% MEA and incubated at room temperature (~20°C) for 1 week. Colonies resembling species of Botryosphaeriaceae were subcultured to fresh 2% MEA plates for further investigation.

### Morphological characterization

Isolates were grown on 1.5% water agar (WA) (Sigma Chemicals) with sterilized pine needles placed on the sur-

face of the medium to stimulate the production of fruiting structures (pycnidia) and conidia. Plates were incubated at 22°C under near UV-light until pycnidia were observed on the pine needles (approx. 3 weeks after plating). Monosporic cultures were generated by plating a spore suspension taken from two pycnidia suspended in 300 µL sterile water on WA. Germinating conidia were lifted from the agar plates and transferred to fresh 2% MEA.

Pycnidia and conidia produced on pine needles were mounted on microscope slides, examined under a Nikon Eclipse E600 light microscope and photographed with a Nikon Digital Camera DXM1200F (Nikon Inc.). Five isolates with structures resembling *N. eucalyptorum* obtained from native hosts were analysed using molecular techniques (described below) and those of *N. eucalyptorum* obtained from *Eucalyptus* hosts were included for reference.

### DNA extraction

The five isolates from native myrtaceous trees plus those obtained from *Eucalyptus* hosts were grown in 2% MEA plates at room temperature for 1 week. Mycelium was scraped from the surface of the agar and transferred to microfuge (1.5-mL) tubes with 3-mm glass beads and extraction buffer of the Qiagen Plant DNeasy Mini Kit (Qiagen Inc.). Subsequently, the tubes were vigorously shaken using a vortex mixer for 1 min and placed in a water bath at 60°C for 1 h. DNA was extracted using the Qiagen Plant DNeasy Mini Kit following the manufacturer's instructions.

### Internal transcribed spacer (ITS) analysis

The ITS region of the ribosomal DNA operon (ITS) was amplified using primers ITS1 (5'-TTCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White *et al.*, 1990). A 25-µL reaction mixture containing 1.0 µL of 0.05% casein, 12.5 µL Amplitaq Gold PCR Master-Mix (Applied Biosystems), 1.0 µL of 10 mM ITS1 primer, 1.0 µL of 10 mM ITS4 primer, 8.5 µL ddH<sub>2</sub>O and 1.0 µL DNA template was used for polymerase chain reactions (PCR). Amplifications were performed in a MJ Research PTC 200 DNA Engine Thermal Cycler PCR with the following parameters: 5 min at 94°C; 1 min at 94°C; 1 min at 50°C; 1 min at 72°C; cycle to step 2, 35 times; 5 min at 72°C; hold at 10°C.

PCR products were visualized on 1.5% agarose gels, purified and prepared for sequencing using an ExoSAP-IT PCR clean-up kit (USB Corp.) following the manufacturer's instructions. The same primers were used for sequencing reactions with the ABI Prism Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) and an ABI Prism 377 automated DNA sequencer. CHROMASPRO software version 1.33 (Technelysium Pty Ltd.) was used for the assemblage of the forward and reverse sequences. Sequences obtained in this study were deposited in GenBank (Table 1). All ITS sequences were

Culture ID <sup>a</sup>	Species	Host	Accession number
UY77	<i>Neofusicoccum eucalyptorum</i>	<i>Eucalyptus grandis</i>	EU860370
UY185	<i>N. eucalyptorum</i>	<i>E. maidenii</i>	EU860371
UY336	<i>N. eucalyptorum</i>	<i>Myrceugenia glaucescens</i>	EU860372
UY394	<i>N. eucalyptorum</i>	<i>E. dunnii</i>	EU080919
UY587	<i>N. eucalyptorum</i>	<i>E. tereticornis</i>	EU080921
UY966	<i>N. eucalyptorum</i>	<i>Blepharocalyx salicifolius</i>	EU860373
UY1070	<i>N. eucalyptorum</i>	<i>E. maidenii</i>	EU080929
UY1074	<i>N. eucalyptorum</i>	<i>E. grandis</i>	EU860374
UY1149	<i>N. eucalyptorum</i>	<i>E. dunnii</i>	EU860375
UY1177	<i>N. eucalyptorum</i>	<i>Blepharocalyx salicifolius</i>	EU860376
UY1190	<i>N. eucalyptorum</i>	<i>E. globulus</i>	EU080930
UY1233	<i>N. eucalyptorum</i>	<i>E. viminalis</i>	EU080932
UY1298	<i>N. eucalyptorum</i>	<i>Myrrhinium atropurpureum</i> <i>var. octandrum</i>	EU080934
UY1314	<i>N. eucalyptorum</i>	<i>Myrrhinium atropurpureum</i> <i>var. octandrum</i>	EU860377
CMW10125	<i>N. eucalyptorum</i>	<i>E. grandis</i>	AF283686
CMW10126 <sup>T</sup>	<i>N. eucalyptorum</i>	<i>E. grandis</i>	AF283687
CBS115679	<i>N. eucalypticola</i>	<i>E. grandis</i>	AY615141
CBS115767	<i>N. eucalypticola</i>	<i>E. rossii</i>	AY615143
CBS118531	<i>N. mangiferae</i>	<i>Mangifera indica</i>	AY615185
CMW13998	<i>N. mangiferae</i>	<i>Syzygium cordatum</i>	DQ316081
CBS115476 <sup>T</sup>	<i>Botryosphaeria dothidea</i>	<i>Prunus</i> sp.	AY236949

<sup>a</sup>T, ex-type culture; UY, isolate obtained in this study; isolates in bold obtained from native Myrtaceae hosts.

subjected to BLAST searches in NCBI GenBank (<http://www.ncbi.nlm.nih.gov/blast/Blast.cgi>, verified 8 September 2008), and these sequences of the ex-type cultures of closest match species were downloaded from GenBank. Multiple sequence alignments were made online using the E-INS-i strategy in MAFFT version 6 (<http://align.bmr.kyushu-u.ac.jp/mafft/online/server/>, verified 8 September 2008) (Katoh *et al.*, 2005).

Phylogenetic analysis was performed using PAUP version 4.0b10 (Swofford, 2002). For neighbour-joining analysis the model TrN + I was selected using MODELTEST v. 3.7 (Posada & Crandall, 1998). Gaps were treated as missing data and all characters were treated as unordered and of equal weight. The heuristic search option with simple taxa additions and tree bisection and reconnection (TBR) as the branch-swapping algorithm was selected for maximum parsimony analysis. Support for the nodes of the shortest trees was determined by analysis of 1000 bootstrap replicates (Hillis & Bull, 1993) and tree length (TL), consistency index (CI), retention index (RI), and homoplasy index (HI) were calculated. The alignment was deposited in TreeBASE (SN3974).

### Pathogenicity tests

The pathogenicity of isolates obtained from native Myrtaceae and introduced *Eucalyptus* trees was tested following an adaptation of the method described by Simeto *et al.* (2007) using the mycelial-plug technique. The region of the stem to be wounded was surface-sterilized with 70% ethyl alcohol. A wound was made on the stems

of 4-month-old plants of a *Eucalyptus grandis* clone approximately 10 cm above the soil and between two nodes using a 5-mm-diameter cork borer to remove the bark and expose the cambium. Mycelial plugs from pure cultures grown for a week on 2% MEA at room temperature were taken using the same cork-borer size and placed into the wound with the mycelial surface facing the cambium. A piece of sterile cotton soaked in sterile water was attached to the inoculated wound with Ready Por no. 545 tape (Sagrin S.A.) to prevent desiccation of the plug. The five isolates obtained from native trees, along with eight isolates obtained from *Eucalyptus* (listed in Table 1), were inoculated in a randomized complete block design with three replicates. Plugs of sterile MEA were inoculated into stems of three trees as controls. Inoculated trees were maintained outside under a structure with a plastic roof and open sides, with temperature ranging from 15 to 25°C. Stem diameter at the site of the inoculation and lesion length were measured 1 and 3 weeks post-inoculation and photographed for records.

Data were subjected to analysis of variance (ANOVA) using the Generalized Linear Model procedure (PROC GLM) of SAS (release 9.1; SAS Institute, Inc.). When the F-test was significant ( $P < 0.05$ ), treatment means were compared using Tukey's studentized range (HSD) test at  $P = 0.05$ .

To complete Koch's postulates, one inoculated stem per isolate was randomly selected for re-isolation of the inoculated fungus. Thus, pieces of wood from the edges of the lesions were surface-sterilized in 70% ethyl alcohol for 1 min, immersed in 0.4% sodium hypochlorite for

**Table 1** List of *Neofusicoccum eucalyptorum* isolates and related species included in this study

2 min, then rinsed twice in sterile distilled water and blotted dry on sterile filter paper. Surface sterilized plant tissue was placed on 2% MEA and incubated at room temperature (~20°C) for 1 week. Fungal identification was based on colony and conidial morphology.

## Results

### Sampling and fungal isolates

A total of 216 trees representing 20 distinct species of the Myrtaceae were surveyed. A total of 130 isolates with colony and conidial morphology resembling species of Botryosphaeriaceae were obtained, of which 64 were isolated from native myrtaceous hosts and 66 were isolated from *Eucalyptus* spp. Five isolates resembling *N. eucalyptorum* were obtained from plant tissue from five different trees representing three different host species and at four different locations (Table 2). Four out of these five isolates were obtained from symptomless tissue as endophytes and the remaining isolate (UY336) was associated with a stem canker. A large number of isolates resembling *N. eucalyptorum* were obtained from *Eucalyptus* samples and a total of nine isolates were randomly selected for genetic and phenotypic comparisons, including at least one from each of the six *Eucalyptus* species considered (Table 1).

### Morphology and ITS sequence comparisons

The five isolates obtained from native hosts plus the nine from *Eucalyptus* spp. showed identical colony and conidial morphology and had morphological characteristics that were similar to those described by Smith *et al.* (2001) for *N. eucalyptorum*. Pycnidia were observed after 2 weeks of incubation on sterile pine needles in WA plates and they produced hyaline, granular, ovoid to slightly clavate conidia 18–25 µm long and 7–12 µm wide. Ascostromata and other teleomorph structures were not observed.

ITS sequence comparisons showed that the five isolates obtained from native myrtaceous trees and those from *Eucalyptus* spp. from Uruguay were identical in the 518 bp of the analysed ITS amplicon. When compared with the ex-type culture of *N. eucalyptorum* (CMW10126), the latter showed a mutation in position 25 of the alignment with a thiamine instead of a cytosine and a deletion in position 488 where the other isolates

had a cytosine. However, the isolates obtained in this study showed 100% similarity to isolate CMW10125, which was also included in the description of *N. eucalyptorum* by Smith *et al.* (2001) as BOT24 (Slippers *et al.*, 2004b).

The alignment contained 20 ingroup taxa, including five isolates obtained from native myrtaceous hosts, nine isolates obtained from *Eucalyptus* spp. in Uruguay and other Botryosphaeriaceae species closely related to *N. eucalyptorum* (Table 1). *Botryosphaeria dothidea* was the outgroup taxon. Of the 518 characters, 461 were constant, 36 variable characters were parsimony-uninformative and 21 were parsimony-informative. Heuristic search analysis of the data resulted in one most parsimonious tree (TL = 60 steps; CI = 0.983; RI = 0.971; HI = 0.017). Identical tree topology was obtained with the neighbour-joining analysis (Fig. 1).

### Pathogenicity tests

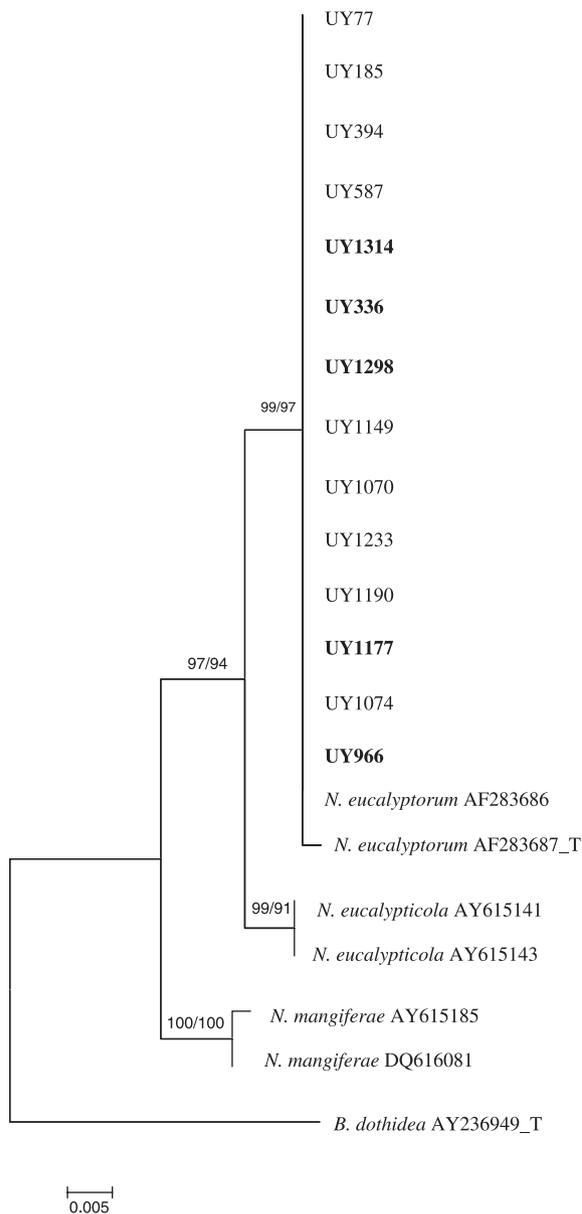
All of the tested isolates obtained from myrtaceous hosts were pathogenic on *E. grandis*, with lesion development and necrotic tissue advancing from the inoculated wound within a week of inoculation. No lesions were observed on seedlings inoculated with sterile MEA plugs that served as controls. In addition, statistical analyses indicated that no differences ( $P > 0.05$ ) were found among seedlings inoculated with different isolates for stem diameter, indicating that all the seedlings were of similar size at the inoculation time. However, lesion length measured 1 week after inoculation was different among inoculated isolates and also when evaluated 2 weeks later (i.e. 3 weeks after inoculation). With the exception of isolate UY1070 that was obtained from *E. maidenii*, all the isolates resulted in lesion lengths significantly different from the control treatment ( $P < 0.05$ ; Fig. 2). There were also significant differences ( $P > 0.05$ ) in the aggressiveness of the isolates. *Neofusicoccum eucalyptorum* was isolated from all the inoculated stems selected for re-isolations.

## Discussion

This study presents the first report of the *Eucalyptus* pathogen, *N. eucalyptorum*, infecting hosts outside of the genus *Eucalyptus*. These findings are contrary to previous assumptions that the pathogen has a narrow host range and is highly specialized to *Eucalyptus* (Slippers & Wing-

**Table 2** Isolates of *Neofusicoccum eucalyptorum* obtained from native Myrtaceae trees in Uruguay

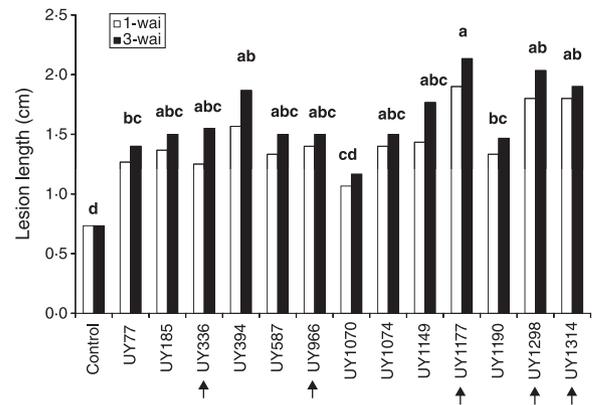
Isolate ID	Host	Isolated from	Location
UY336	<i>Myrceugenia glaucescens</i>	Stem canker	Rio Negro (32°53'S; 57°59'W)
UY966	<i>Blepharocalyx salicifolius</i>	Healthy leaf	Durazno (33°19'S; 56°17'W)
UY1177	<i>Blepharocalyx salicifolius</i>	Healthy twig	Lavalleja (34°11'S; 55°16'W)
UY1298	<i>Myrrhinium atropurpureum</i> var. <i>octandrum</i>	Healthy petiole	Maldonado (34°17'S; 54°41'W)
UY1314	<i>Myrrhinium atropurpureum</i> var. <i>octandrum</i>	Healthy petiole	Maldonado (34°20'S; 54°35'W)



**Figure 1** Distance tree based on neighbour-joining analysis of the ITS region using the TrN+I model confirming the identity of Uruguayan isolates as *Neofusicoccum eucalyptorum*. The alignment was based on a total of 518 characters, of which 461 were constant, 36 variable characters were parsimony-uninformative and 21 were parsimony-informative. Bootstrap values of 1000 replications for neighbour-joining and maximum parsimony analyses are shown, respectively, at the nodes. The tree was rooted with *Botryosphaeria dothidea*. Sequences obtained in this study are indicated with the prefix 'UY', isolates obtained from native Myrtaceae are in bold and ex-type cultures are labelled with a 'T' at the end. Scale bar indicates 0.005 substitutions per site.

field, 2007). Pathogenicity tests indicated that all isolates obtained from native Myrtaceae were able to infect and produce stem cankers on *E. grandis*.

The fact that *N. eucalyptorum* was found on three different species, namely *Blepharocalyx salicifolius*,



**Figure 2** Mean lesion length (cm) of three replicates for each *N. eucalyptorum* isolate and the control (sterile agar) inoculated onto *Eucalyptus grandis* cuttings 1 and 3 weeks after inoculation (WAI). Letters indicate mean separation based on Tukey's test ( $P<0.05$ ) shown only for lesion length at 3 WAI. Isolates obtained from native myrtaceous hosts are indicated with an arrow.

*Myrceugenia glaucescens* and *Myrrhinium atropurpureum* var. *octandrum*, provides evidence of a remarkably wider host range than previously believed (Slippers & Wingfield, 2007). *Neofusicoccum eucalyptorum* has previously been found occurring on several *Eucalyptus* species in Australia (Burgess *et al.*, 2006), Chile (Ahumada, 2003), South Africa (Smith *et al.*, 2001) and Uruguay (Alonso, 2004), but not on non-*Eucalyptus* hosts. These results strongly suggest that further investigations on the biology, ecology and epidemiology of this fungus are warranted and researchers should be alerted to the possibility of this pathogen affecting other non-*Eucalyptus* hosts in other countries.

Although *N. eucalyptorum* was found on three distinct host species, the small number of trees from which it was isolated suggests that it is still not extensively distributed in trees native to Uruguay. However, its occurrence on native trees in four different provinces of Uruguay is alarming and suggests that interactions among *Eucalyptus*, native trees and this pathogen are likely to be occurring countrywide. Continued investigations are needed to monitor the occurrence of this fungus and to obtain a better understanding of its relative importance.

Pathogenicity tests confirmed the ability of *N. eucalyptorum* to infect *E. grandis* and to cause stem cankers. Smith *et al.* (2001) reported pathogenicity of this fungus by inoculating five isolates obtained from *E. grandis* and *Eucalyptus nitens* onto a clone of *E. grandis* (ZG14), although they found no significant differences in lesion length among isolates. In contrast, the present study showed significant differences in pathogenicity among isolates, with those from native hosts producing the largest lesions. The pathogenicity observed on *E. grandis* for those isolates obtained from native trees indicates that this pathogen has the ability to move from one host species to another. Results obtained in this study also indicate the possibility of obtaining a precise phenotypic

characterization of isolate aggressiveness in just 1 week post-inoculation. Furthermore, the coefficient of variation observed 1 and 3 weeks post-inoculation was 14.1% and 13.5%, respectively (data not shown). Therefore, the use of clonal 4-month-old cuttings and the short period of time needed to give a consistent reaction (1 week) make this method appropriate for quick phenotypic characterization of *N. eucalyptorum* isolates.

The host specialization previously observed for *N. eucalyptorum* on only *Eucalyptus* and its abundance and wide distribution in eastern Australia (Smith *et al.*, 2001; Slippers *et al.*, 2004a; Burgess *et al.*, 2006) suggest that the pathogen is probably native to Australia. Its occurrence on *Eucalyptus* in other parts of the world appears to be the result of introductions that probably occurred via the large amounts of germplasm that have been traded. In Uruguay, it appears to have moved from *Eucalyptus* to native Myrtaceae. Historically, anthropogenic pathogen introduction has been considered the major driver of devastating host-jump experiences (Slippers *et al.*, 2005; Woolhouse *et al.*, 2005). Although inoculum pressure may not be a determinant for some species (Ficetola *et al.*, 2008), it is generally believed that high propagule pressure along with geographical proximity are mostly responsible for the appearance of new host–parasite combinations (Altizer *et al.*, 2003; Lockwood *et al.*, 2005). Therefore, species growing adjacent to infected plants are exposed to inoculum, which increases the probability of eventual infection. In Uruguay, *Eucalyptus* plantations are geographically located close to native Myrtaceae trees, and *N. eucalyptorum* has been very commonly found on *Eucalyptus* plantations throughout the main planted areas (Pérez, 2008). This suggests that both factors, high inoculum pressure and geographic proximity, may be responsible for the occurrence of *N. eucalyptorum* on native myrtaceous hosts.

Several studies have provided evidence that introduced *Eucalyptus* species and native myrtaceous trees can share pathogens (Coutinho *et al.*, 1998; Pavlic *et al.*, 2007). Additionally, Burgess *et al.* (2006) demonstrated that there was no restriction to the movement of *N. australe* between *E. globulus* plantations and native forest in Australia. In Uruguay, recent studies confirmed similar relationships between *Puccinia psidii* and *M. atropurpureum* var. *octandrum* (Pérez, 2008), and between *Quambalaria eucalypti* and *Myrcianthes pungens* (Pérez *et al.*, 2008). The present study adds *N. eucalyptorum* to the list and raises additional concerns regarding this pathogen, not only in Uruguay, but in other regions where *Eucalyptus* has been introduced. The negative impact of host-jump events in plant pathology is well documented and many examples have been repeatedly cited in the literature (Anderson *et al.*, 2004; Slippers *et al.*, 2005; Woolhouse *et al.*, 2005; Desprez-Loustau *et al.*, 2007). Host jumps have occurred in both directions, from native hosts to introduced plant species and vice versa (Milgroom *et al.*, 1996; Coutinho *et al.*, 1998) and biotic exchanges between both hosts are expected to increase as the

planted area and age of plantations increase (Strauss, 2001). Further investigation is needed to obtain a better understanding of the economic and ecological impact of *N. eucalyptorum* on both native and introduced species of Myrtaceae, since most isolates obtained from native trees were recovered from symptomless tissue.

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