# Chapter 3

## *BOTRYOSPHAERIA* SPECIES CAUSING DIEBACK ON *EUCALYPTUS* SPP. IN CHILE

## ABSTRACT

*Eucalytus* spp. represent the second largest forest plantation species in Chile with almost 360 000 ha already planted to these trees. The forestry industry has growing rapidly in this country, basing its *Eucalyptus* production mainly on *Eucalyptus globulus* and *E. nitens*. The increase in commercial plantations has resulted in a natural increase of new pests and diseases. Of these, Eucalyptus dieback associated with *Botryosphaeria* spp. in young and adult trees has been recognized as one of the diseases threatening *Eucalyptus* plantations in Chile. Very little research has been conducted on *Botryosphaeria* spp. on *Eucalyptus* and little is known regarding their identity or biology on this host. The aim of this chapter was, therefore, to identify *Botryosphaeria* spp. associated with dieback on commercially planted *Eucalyptus* trees in Chile. Symptomatic branches were collected from *E. globulus* and *E. nitens* plantations in Southern-central Chile, where dieback was severe. Species of *Botryosphaeria* were identified based on morphological characteristics and sequence data from the Internal Transcribed Spacer (ITS) region of the ribosomal RNA operon and the β-tubulin gene. Results showed that three *Botryosphaeria* species are present on *Eucalyptus* in the surveyed area. These species include *B. parva*, *B. eucalyptorum* and an undescribed species of *Botryosphaeria*. All three species represent new records for Chile. Pathogenicity trials conducted in greenhouses showed a significant difference in virulence between the three species with *B. parva* as the most virulent.

## INTRODUCTION

*Eucalyptus* L' Heritier is one of the largest genera in the Myrtaceae containing approximately 700 species (Potts & Pederick 2000). These species are native to Australia, Papua New Guinea, Indonesia and surrounding islands (Poynton 1979). *Eucalyptus* spp. are well recognized for the excellent quality and versatility of their wood, and the variety of other purposes for which they can be utilised. They have thus become one of the world's most widely planted forestry crops (Poynton 1979, Turnbull 1991). Approximately 10 million ha of *Eucalyptus* spp. had been planted by the year 2000, for commercial timber production, with a yield of more than 30 million tons per year (Turnbull 1991).

*Eucalyptus* spp. were introduced into Chile in the early 1800's, and since the 1980's when the Chilean government implemented a subsidy on tree planting, they have become the second most commonly planted trees, after *Pinus radiata* D. Don. (Jayawickrama, Schlatter & Escobar 1993; Kliejunas *et al.* 2001). Approximately 360 000 ha of commercially planted *Eucalyptus* trees have been established in Chile (INFOR 2001). The most widely planted species are *E. globulus* Labill. and *E. nitens* (Deane & Maid.) Maid, however, species such as *E. camaldulensis* Dehnhardt, *E. cladocolyx* F. Mueller and *E. siderexylon* A. Cunningham, *E. regnans* F. Muell., *E. fastigata* Deane & Maiden, *E. delegatensis* R.T. Barker and *E. viminalis* Labill. have also been planted in some areas (Jayawickrama *et al.* 1993, INFOR 1997).

Disease problems have not been serious on *Eucalyptus* in Chile in the past, but they are beginning to appear quite rapidly. The most important disease on *Eucalyptus* in the country is Mycosphaerella leaf disease (MLD), caused by a variety of *Mycosphaerella* spp. This disease is particularly important on juvenile leaves of *E. globulus* (see Chapter II). Various less important diseases have been reported in *Eucalyptus* plantations in Chile, but very little research has been conducted to determine the identity of the pathogens or to assess the damage that they cause (Wingfield 2001, unpublished report). In a recent survey conducted by the Bioforest S.A.- Arauco companies, symptoms of dieback on *E. globulus* and *E. nitens* plantations were noted as a common problem. Preliminary studies showed that the symptoms were consistently associated with *Botryosphaeria* spp.

The genus *Botryosphaeria* Ces. & De Not. (Pleosporales, Loculoascomycetes, *Botryosphaeriaceae*) includes 143 species with anamorph states such as *Fusicoccum* Corda in Sturm., *Diplodia* Fr. in Mont. and *Lasiodiplodia* Ellis & Everh. (Von Arx 1987, Jacobs & Rehner 1998, Denman *et al.* 2000). These species are mainly latent pathogens that colonise plant tissue after stress or senescence. Occasionally, they cause extensive disease in different woody hosts and this is usually associated with high levels of susceptibility and stress (Schoeneweiss 1979, Zhonghua & Michailides 2001). *Botryosphaeria* spp. are well known for their cosmopolitan distribution and wide host range, producing stem cankers, tip dieback, kino exudation and various other symptoms on *Eucalyptus* spp. (Von Arx & Müller 1954, Von Arx 1987, Denman *et al.* 2000).

Disease associated with *Botryosphaeria* spp. is spread from one plantation to another by conidia or ascospores, which are dispersed via rain splash and wind (Cresswell & Milholland 1988, Pusey 1989). *Botryosphaeria* spp. penetrate trees through wounds or natural openings, even though the infection is usually latent (Caroll 1988). Wounds, serve as primary infection sites, therefore, insects, animals, hail, windstorms and cultural practices can increase the opportunity for the infection to develop in trees (Brown & Hendrix 1981, Swart, Wingfield & Knox-Davis 1987, Cresswell & Milholland 1988). Symptoms of the disease are normally expressed when the host is stressed. Some authors, however, have been able to isolate *Botryosphaeria* spp. from asymptomatic plant tissue, confirming that the pathogen is commonly present in healthy host tissue (Luttrell 1950, Fisher, Petrini & Sutton 1993, Roux 1998). The early view that these fungi are typical wound infecting opportunities appears not to be sound and that infection of healthy tissue and disease manifestation after stress seems more common.

*Botryosphaeria* spp. have been recognised as important pathogens of *Eucalyptus* spp. in various parts of the world (Barnard *et al.* 1987, Shearer, Tippett & Bartle 1987, Smith, Wingfield & Petrini 1996, Roux *et al.* 2000, Roux *et al.* 2001). Of these *B. dothidea* (Moug.: Fr.) Ces. & De Not. causes stem canker (Barnard *et al.* 1987, Smith, Kemp & Wingfield 1994) and the more recently described *B. eucalyptorum* Crous, H. Smith et M. J. Wingf. causes dieback (Smith *et al.* 2001). To the best of our knowledge, the only *Botryosphaeria* spp. recorded on *Eucalyptus* in Chile is *B. dothidea* (Gonzalez 1997, Munoz 1999), which was found on leaves of *E. camaldulensis* Dehn. The only case of severe dieback has been observed on *E. nitens* trees in the Southern-central area (Valdivia

– Lanco – Los Lagos areas) of Chile during May of 2000, where approximately 30 % of the trees showed tip death. However, most trees recovered their foliage during the following growing season (Brito, pers. comm).

The taxonomy of *Botryosphaeria* spp. is confusing and teleomorph structures are rarely observed in nature or produced in culture. Therefore, identification of these fungi has generally been based on morphological characteristics of the anamorph states, although this approach is also complicated by the similarities between conidia in various species and changes in conidial morphology with age of these spores (Jacobs & Rehner 1998, Smith & Stanosz 2001, Zhou & Stanosz 2001). Recent research has combined morphological characteristics and various molecular techniques such as RAPD (Smith & Stanosz 2001) and ISSR markers (Powell *et al.* 1996, Zhou & Stanosz 2001), RFLP's (Powell *et al.* 1996) and analysis of sequence data from the ITS regions of the ribosomal RNA operon (Jacobs & Rehner 1998, Denman *et al.* 2000), the β-tubulin genes and translation elongation factor 1-α (EF1-α) gene (Smith *et al.* 2001, Slippers *et al.* 2002). These contemporary techniques and particularly DNA sequence data have given rise to an entirely new perspective on the taxonomy of *Botryosphaeria* spp.

The aim of this study was to identify the *Botryosphaeria* species associated with dieback of *Eucalyptus* in Chilean plantations. This was achieved by collecting symptomatic tissue from trees in plantations in various parts of Chile, and subsequently studying specimens and cultures linked to these collections. Identifications were made based on morphological characteristics of the fungi as well as analysis of DNA sequence data.

## MATERIALS AND METHODS

### *Collection of Samples*

Surveys were conducted in *Eucalyptus* plantations in Chile during April and October 2001. Small branches and stem pieces displaying symptoms of dieback were collected from trees in Southern-central Chile. Samples were collected from symptomatic tissue on *E. globulus* and *E. nitens* trees, located in eight commercial plantations (Figure 1). Ten samples were collected from each tree and five trees were sampled from each plantation. Samples were

maintained at 4 °C in brown paper envelopes. They were then transported to the laboratory for further study.

***Isolations and preparation of cultures***

Five branches from each tree were selected for the isolation of *Botryosphaeria* spp. Using a sterile scalpel, the surface of the bark was removed to expose the spore masses of pseudothecia or pycnidia. Spore masses were then lifted from the structures using a sterile needle and transferred to 60 mm Petri dishes containing 2 % malt extract agar (MEA) and incubated at 25 °C, under cool white light in an incubator for seven to ten days.

For the production of anamorph structures, mycelium was transferred to 2 % water agar (WA) with sterilised pine needles placed on the surface of the medium and incubated at 25 °C with 12 hour cycles of near ultra-violet (nuv, 250 nm) light and dark (Sutton 1980, Johnson 1992, Crous & Palm 1999).

***Morphological characteristics***

Pycnidia developed on pine needles within approximately ten days of incubation. Conidia were taken from these structures and used for morphological characterization as described by Johnson (1992) and, Crous & Palm (1999). Conidia were spread on the surface of WA medium and after 12 to 24 hours of incubation, single germinating conidia were transferred to malt extract agar (MEA). Pycnidia that formed on pine needles were broken with a scalpel to liberate the conidia, which were mounted in lactophenol on glass microscope slides. Conidia were examined under a Zeiss light microscope. The average length of 20 conidia was measured for each isolate studied. Between five and ten cultures representing three groups based on conidial morphology were then chosen for further identification using DNA sequencing (Table 1).

***DNA extraction and PCR amplification***

Mycelium from actively growing MEA cultures was scraped from the surface of the agar plates using a sterile scalpel and lyophilized for 24 hours. The dried mycelium was ground to a fine powder, using liquid nitrogen. DNA was extracted using the method described by Raeder and Broda (1985), with minor modifications. Thus 800 µl of extraction buffer

(200mM Tris-HCl pH 8.3, 150mM NaCl, 25mM EDTA pH8.0, SDS 0.5%) was added to the ground mycelium, and mixed. Phenol and chloroform (v/v 5:3) was added to all samples, vortexed and centrifuged three times. Extracted nucleic acids were precipitated from the aqueous layer using two volumes of absolute ethanol. Nucleic acids were pelleted by centrifugation and washed with 70% ethanol. The dry nucleic acid pellets were then re-suspended in water and 4 µl RNaseA (10 µg/µl) (Sigma, USA) was added to digest any residual RNA. The resulting DNA was quantified by UV light visualization after electrophoresis in a 1% agarose gel stained with ethidium bromide. A Beckman DU Series 60 Spectrophotometer (Beckman, Germany) was used to calculate the DNA concentration of all DNA samples.

The Internal Transcribed Spacer (ITS) region of the rRNA operon, were amplified using primers ITS1 (5' TCC GTA GGT GAA CCT GCG G 3'), and ITS4 (5' TCC TCC GCT TAT TGA TAT GC 3') (White *et al.* 1990). Part of the ß-tubulin 2-gene region was amplified for selected isolates using primers Bt 2a (5' GGT AAC CAA ATC GGT GCT GCT TTC 3') and Bt 2b (5' ACC CTC AGT GTA GTG ACC CTT GGC 3') (Glass & Donaldson 1995).

The same amplification and sequencing protocol was used to amplify both the ITS and ß-tubulin regions. The PCR reaction mixture contained 1 X PCR buffer and $MgCl_2$ (10 mM Tris-HCl, pH 8.0, 1.5 mM $MgCl_2$, 50 mM KCl, pH 8.3) (Roche Diagnostic, South Africa), 0.2 mM of each dNTP (Roche Diagnostics, SA), 0.15 µM of primers (MWG Biotech, Germany), 0.5U Expand™ High Fidelity Taq DNA polymerase (Roche Molecular Biochemicals, Almeda, CA). Sterile deionized water was used to make up the total volume of the reaction to 50 µl.

The PCR reaction consisted of an initial denaturation step at 96 °C for 1 min, followed by 35 cycles of template denaturation at 94 °C for 30 s, primer annealing at 56 °C for 30 s, for ITS and ß-tubulin, followed by extension at 72 °C for 90 sec. A step up of 5 sec. elongation was added with each cycle after the first 25 cycles. A final elongation of 10 min at 72 °C completed the program. PCR products were purified using the High Pure PCR product purification Kit (Roche Diagnostics, Germany). PCR products were visualised on 2% agarose gels in a TAE buffer electrophoresis system, stained with ethidium bromide and

visualized with UV light. A 100 bp (base pair) molecular marker (Roche Diagnostic, SA) was used to determine the sizes of PCR products.

***DNA sequencing and phylogenetic analysis***

PCR products were sequenced in forward and reverse directions using the primers ITS1, ITS4, Bt 2a and Bt 2b. Sequencing reactions were performed using the ABI PRISM™ Big DYE Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer Applied Biosystems), following the protocols of the manufacturer. One forward primer (ITS1) and one reverse primer (ITS4) was used for the ITS region and a forward primer (Bt 2a) and one reverse primer (Bt 2b), was used for the ß-tubulin gene region (White *et al.* 1990). Sequence reactions were run using Polyacrylamide Gel Electrophoresis (PAGE) on an ABI PRISM™ 377 and capillary electrophoresis on an ABI PRISM™ 3100 Autosequencer (Perkin-Elmer Applied Biosystems).

Sequences were analysed using Sequence Navigator version 1.0.1 (Perkin-Elmer Applied Biosystems, USA). Sequences were aligned manually and gaps were inserted wherever necessary. A partition homogeneity test was used to test the congruence and combinability of the two data sets (Huelsenbeck, Bull & Cunningham 1996). The data sets were congruent and thus, ITS and ß-tubulin sequence data sets were combined. Phylogenetic analysis of the aligned sequences were determined using Phylogenetic Analysis Using Parsimony (PAUP version 4.0b1) (Swofford 1998). All characters in the analysis were given equal weight and the MULPAR option was effective. The heuristic search function based on parsimony with tree bisection reconnection (TBR) was used to generate a phylogram. Branch supports were calculated by performing a Bootstrap analysis on 1000 replications of the aligned sequences.

To determine the phylogenetic relationships and the identities of the *Botryosphaeria* spp., sequences of known *Botryosphaeria* spp. were obtained from GenBank as well as from the study of Slippers *et al.* (2002), and included in the alignment for comparative purposes. All resulting trees were rooted to *Botryosphaeria rhodina* (Cooke) Arx, which has been shown (Slippers, pers. comm.) to be a more effective outgroup taxon for this group than *Guignardia philoprina* (Ellis) Viala & Ravaz used in various previous studies (Jacobs 2002, Slippers *et al.* 2002).

***Greenhouse Pathogenicity test***

A pathogenicity test was conducted using two isolates for each of the three *Botryosphaeria* species identified, based on morphology and sequence data. The isolates representing these three species were randomly selected from the larger group of isolates (Table 2). All six isolates that were used in the inoculations were grown on MEA at 25 °C for one week prior to inoculation. Preservation and maintenance of the cultures was the same as that used for the identification studies.

Inoculations were conducted on a *E. grandis* Hill ex Maid. clone (ZG14) known to be susceptible to Botryosphaeria canker. A total of 70 trees were acclimatised in a phytotron at approximately 22 - 25 °C for three weeks prior to inoculation.

Ten trees were inoculated with each of the six isolates selected for testing and ten trees were inoculated as controls. Stems of trees to be inoculated were surface disinfested with 70% EtOH prior to inoculation. Bark disks were removed between two nodes, using a cork borer (4 mm diam.). Mycelium plugs from the edges of actively growing cultures or sterile MEA plates (controls) were then placed into the wounds made with the cork borer. Wounds were covered with Parafilm (Pechiney plastic packing, Chicago, USA) to reduce desiccation and to avoid contamination.

Seven weeks after inoculation, lesion lengths (mm) were recorded. Small pieces of wood from the lesion were plated onto MEA to re-isolate the inoculated fungus. Analysis of data was conducted for all isolates separately and also by grouping the isolates by species, using the linear model of analysis of variance (ANOVA) and means were separated based on LSD (Least Significant Difference) using the STATISTICA software for Windows (StatSoft, Inc., 1995).

## RESULTS

***Isolations and preparation of cultures***

All samples used in this study originated from eight plantations, and included six *E. globulus* and two *E. nitens* trees. Symptoms were very similar, showing a death of young

trees or dead tips in adult trees (Figure 2). Newly developing symptoms were not common. In most cases, the material was collected from trees that were damaged one or two years before the surveys were conducted.

A total of 60 cultures were obtained from the various isolations made and 20 of these isolates were selected to identify the *Botryosphaeria* spp. present in the sampled area. All isolates sequenced in this study have been deposited in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa (Table 1).

***Morphological characteristics***

All primary isolations were made from bark pieces bearing teleomorph structures of *Botryosphaeria* spp. (Figure 3). Purified cultures were obtained from these primary isolations and they appeared to display very little difference in cultural morphology on MEA (Figure 3). All 20 isolates produced pycnidia on sterile pine needle (Figure 3). Single conidial cultures produced from these structures were used for subsequent sequencing and pathogenicity tests.

All conidia observed under the microscope were hyaline and characteristic of *Fusicoccum* spp. The average length of these conidia ranged from 19.3 to 26.0 µm, while the widths range from 6.7 to 7.8 µm (Figure 4). Three groups could be defined based on conidial size.

***DNA sequencing and phylogenetic analysis***

A total of 19 taxa were used for the sequencing analysis. A fragment of approximately 520 bp was amplified for the ITS region for all isolates. The amplicon produced for the ß-tubulin amplification was 440 bp. A partition homogeneity test to determine whether it is possible to combine both datasets showed that they are combinable ($p = 0.46$). The combined data set comprised 985 characters after alignment. A heuristic search was done on the combined data set. Of the 985 characters, 770 were constant, 30 of the variable characters were parsimony-uninformative and 185 were parsimony-informative. A single most parsimonious tree was generated with a Consistency index (CI = 0.888), retention

index (RI = 0.935) and homoplasy index (HI = 0.112). A bootstrap analysis of 1000 replications was done.

From the sequence analysis, seven clades emerged. Clade I includes isolates of *B. ribis* Grossenb. & Dugg. Two Chilean isolates (CMW 10532 & CMW 10549) were grouped in clade II, representing *B. parva* Pennycook & Samuels. Clade III contained *B. lutea*, while clade IV contained two Chilean isolates (CMW 10541 & CMW 10542) representing *B. eucalyptorum*. Clade V included two Chilean isolates (CMW 10530 & CMW 10531), as well as two isolates from Australia representing an undescribed *Botryosphaeria* sp. Clade VI and VII contained *B. dothidea* and *B. obtusa* (Schwein.) Shoem., respectively (Figure 5).

***Greenhouse Pathogenicity test***

All isolates tested for pathogenicity produced lesions on the outer bark, but more clearly in the xylem of the inoculated trees. No symptoms developed on trees inoculated as controls (Figure 6). The lesions observed in the xylem of the inoculated trees varied greatly in size and were not homogeneous. Thus, the variability between them was very high in some cases.

Mean inner lesion lengths on the trees inoculated with *Botryosphaeria* spp. showed a range of 21.5 mm for isolate CMW 10542 to 42.2 mm for isolate CMW 10532 (Table 2). Significant differences ($p < 0.001$) were found between control inoculations and trees inoculated (Table 2, Figure 7). The analysis showed three distinct groups with different lesion lengths. The first group of isolates (CMW 10547, CMW 10533, CMW 10532 and CMW 10539) gave rise to lesions ranging for 33.2 to 42.2 mm in length. The second group of isolates (CMW 10542 & CMW 10537) had lesions ranging in average length between 21.5 to 24.8 mm and the control gave rise to lesions and average of 9.1 mm including the size of the wound made by the cork borer (Table 2, Figure 7).

## DISCUSSION

In this study, we have considered, for the first time, *Botryosphaeria* spp. associated with dieback on *Eucalyptus* in Chile. The results have clearly shown that three species of *Botryosphaeria* are associated with these symptoms. They included *B. eucalyptorum*, *B. parva* and an undescribed *Botryosphaeria* sp. We have also been able to show that these fungi differ in their pathogenicity and probably contribute, in varying degrees, to the symptoms observed in the field.

*Botryosphaeria eucalyptorum* and *B. parva* are well known worldwide from *Eucalyptus* spp.(Smith *et al.* 2001, Jacobs 2002), but they have not previously been recorded in Chile. The third species identified in this study represents an undescribed species that has significant sequence identity with other isolates from Australia and South Africa (Slippers *et al.* 2002). This fungus now requires formal description. Prior reports of *B. dothidea* from *Eucalyptus* in Chile (Gonzalez 1997, Munoz 1999) could not be confirmed and we doubt that this species is present.

*Botryosphaeria eucalyptorum* (anamorph *Fusicoccum eucalyptorum* Smith and M.J. Wingf.) has recently been reported causing cankers on the main stem in *E. grandis* and *E. nitens* plantations in South Africa (Smith *et al.* 2001). Smith *et al.* (2001), were also able to demonstrate that the pathogenicity of *B. eucalyptorum* appears to be much lower than that of other species such as *B. dothidea*. The results reported in the study conducted by Smith *et al.* (2001) are in accordance with those obtained in this thesis where *B. eucalyptorum* produced smaller lesion sizes that the other species tested.

*Botryosphaeria parva* was first described in 1985 from Kiwifruit in New Zealand (Pennycook & Samuels 1985). It is now well known worldwide as a pathogen of woody plants and fruit trees such as Mango trees in South Africa (Von Arx 1987, Jacobs 2002). Controversy with respect to the identity of this species has been clarified by Slippers *et al.* (2002) using combined data from the ITS region with ß-tubulin and/or Elongation factor sequence data. From this thesis, it is clear that *B. parva* is completely different to *B. ribis* and this is supported by the combined analysis of the ITS region and ß-tubulin gene sequences. *Botryosphaeria parva* was the species with the highest average lesion lengths in the inoculation trials. These results were also similar with those recently obtained by

Nakabonge (pers. comm.) on Ugandan *Eucalyptus* trees where *B. parva* was most pathogenic.

From morphological studies we have observed few cultural differences between *B. eucalyptorum*, *B. parva* and the unidentified *Botryosphaeria* sp. Morphological characteristics of conidia based on size and shape, gave us an orientation with respect to the identity of the species mentioned. Slippers *et al.* (2002) observed that not all conidia share similar characteristics and that it is generally necessary to combine both morphological characteristics and sequence data to obtain reliable identifications. Our results support this view.

This study has shown that Botryosphaeria dieback is probably not a particularly important disease in Chile. It does, however, occur in isolated situations and it will be important to monitor its presence in plantations. The fact that species such as *B. eucalyptorum* that cause serious disease in countries such as South Africa, is of concern. Certainly, if conditions become conducive for disease development, this fungus is likely to cause serious damage (Shearer *et al.* 1987, Fisher *et al.* 1993, Smith *et al.* 1996, Smith *et al.* 2001, Roux *et al.* 2001). Although this study has shown that the three species of *Botryosphaeria* are pathogenic to a single *E. grandis* clone, it will be important to assess pathogenicity on *E. nitens* and *E. globulus* grown in Chile.

*Botryosphaeria* spp. are well known opportunistic pathogens of *Eucalyptus* spp., especially when the trees are under stress (Smith *et al.* 1996, Smith *et al.* 2001). Strategies to reduce the stress and selection of resistant clones and use of hybrids would provide opportunities to avoid disease development in the future. In Chile, it will be necessary to select planting sites not conducive to disease, especially when less resistant material is planted. Future activities should also include more extensive surveys to achieve a better understanding of the presence and distribution of *Botryosphaeria* spp. and their potential to negatively impact on Chilean forest production.

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**Table 1**. *Botryosphaeria* isolates from Chile used in this study for identification porposes.

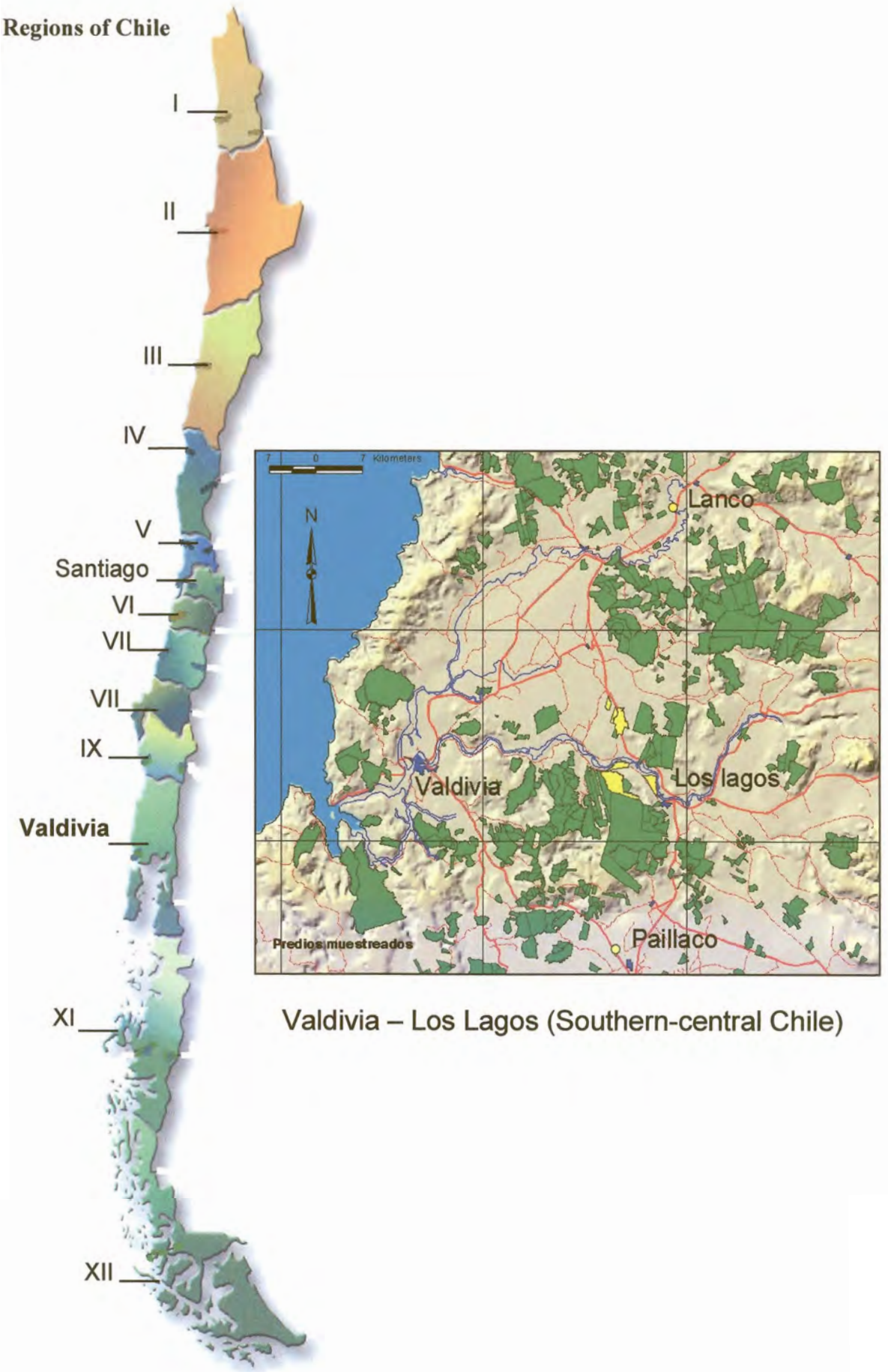
| Culture No | Identity | Host | Collection Area | Collector | Date of Isolation |
|---|---|---|---|---|---|
| CMW 10530 | *Botryosphaeria* sp. | *E. globulus* | Valdivia | R. Ahumada | July 2002 |
| CMW 10531 | *B.* sp. | *E. globulus* | Valdivia | R. Ahumada | July 2002 |
| CMW 10532 | *B. parva* | *E. globulus* | Valdivia | R. Ahumada | July 2002 |
| CMW 10533 | *B.* sp. | *E. globulus* | Valdivia | M.J. Wingfield | July 2002 |
| CMW 10534 | *B. eucalyptorum* | *E. globulus* | Valdivia | R. Ahumada | July 2002 |
| CMW 10535 | *B.* sp. | *E. globulus* | Valdivia | R. Ahumada | July 2002 |
| CMW 10536 | *B.* sp. | *E. globulus* | Valdivia | R. Ahumada | July 2002 |
| CMW 10537 | *B.* sp. | *E. globulus* | Valdivia | R. Ahumada | July 2002 |
| CMW 10538 | *B. parva* | *E. globulus* | Concepción | M.J. Wingfield | July 2002 |
| CMW 10539 | *B. parva* | *E. globulus* | Concepción | M.J. Wingfield | July 2002 |
| CMW 10540 | *B. eucalyptorum* | *E. globulus* | Los Lagos | R. Ahumada | October 2002 |
| CMW 10541 | *B. eucalyptorum* | *E. globulus* | Los Lagos | M.J. Wingfield | October 2002 |
| CMW 10542 | *B. eucalyptorum* | *E. globulus* | Los Lagos | M.J. Wingfield | October 2002 |
| CMW 10543 | Not identified | *E. globulus* | Los Lagos | R. Ahumada | October 2002 |
| CMW 10544 | Not identified | *E. globulus* | Los Lagos | R. Ahumada | October 2002 |
| CMW 10545 | *B.* sp. | *E. globulus* | Valdivia | M.J. Wingfield | October 2002 |
| CMW 10546 | *B. eucalyptorum* | *E. globulus* | Valdivia | M.J. Wingfield | October 2002 |
| CMW 10547 | *B. eucalyptorum* | *E. nitens* | Valdivia | R. Ahumada | October 2002 |
| CMW 10548 | *B.* sp. | *E. nitens* | Valdivia | R. Ahumada | October 2002 |
| CMW 10549 | *B. parva* | *E. nitens* | Valdivia | M.J. Wingfield | October 2002 |

**Table 2.** Lesion lengths on trees of a *E. grandis* clone (ZG14), resulting from inoculation with three *Botryosphaeria* spp. from Chile.

| Isolates | Identity | Lesion Lengths (mm) Min | Max | Means[1] | |
|---|---|---|---|---|---|
| Control | Pure MEA | 8 | 11 | 9.1 | a |
| CMW 10547 | *B. eucalyptorum* | 22 | 52 | 36.1 | cd |
| CMW 10542 | *B. eucalyptorum* | 12 | 46 | 21.5 | b |
| CMW 10537 | *B.* sp. | 13 | 49 | 24.8 | b |
| CMW 10533 | *B.* sp. | 21 | 65 | 38.7 | cd |
| CMW 10532 | *B. parva* | 11 | 66 | 42.2 | d |
| CMW 10539 | *B. parva* | 8 | 66 | 33.2 | c |

[1] Lesion lengths represent the mean of ten replications per isolates. Means with different letters are significantly different (LSD, $p < 0.005$).

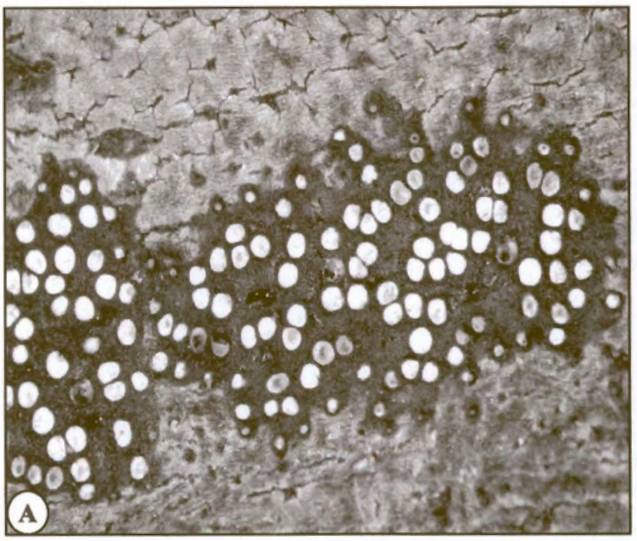
**Figure 1**. Geographic distribution of commercial *Eucalyptus* plantations in Chile, showing sampling areas.



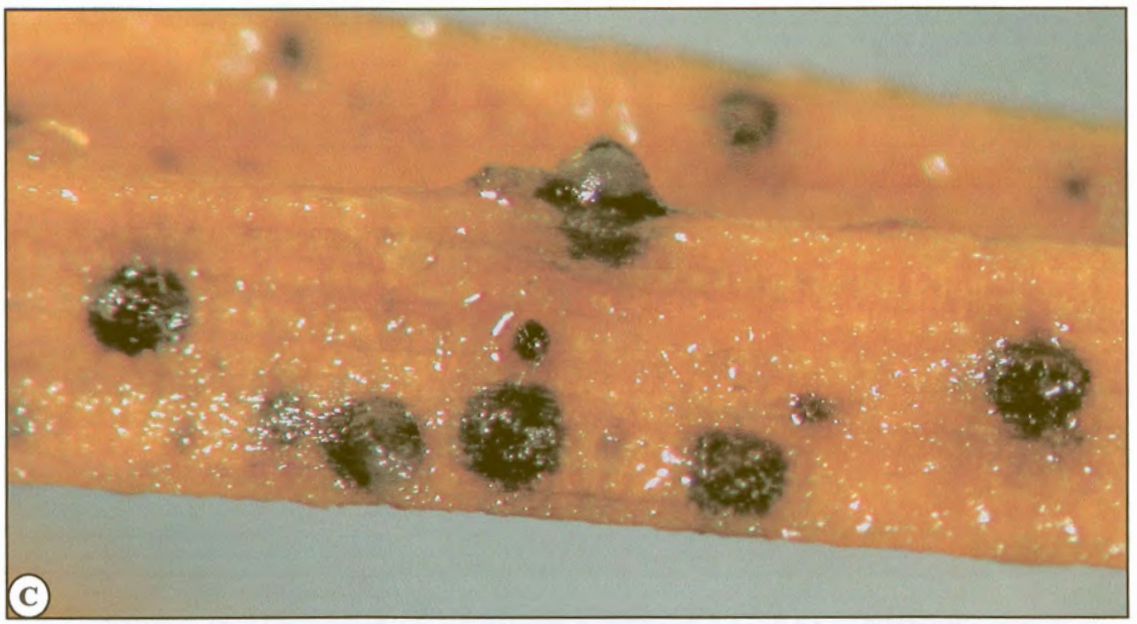

Valdivia – Los Lagos (Southern-central Chile)

**Figure 2**. Commercial plantation of *Eucalyptus* spp. affected by *Botryosphaeria* spp. in Southern-central area of Chile (Valdivia, Los Lagos).

**Figure 3**. Morphology of *Botryosphaeria* spp. found in Chile. **(A)** Perithecia on *Eucalyptus* bark from which isolations were made. **(B)** Asci and ascospores. **(C)** Pycnidia on sterile pine needles. **(D)** Examples of differences in culture morphology from the single conidia.







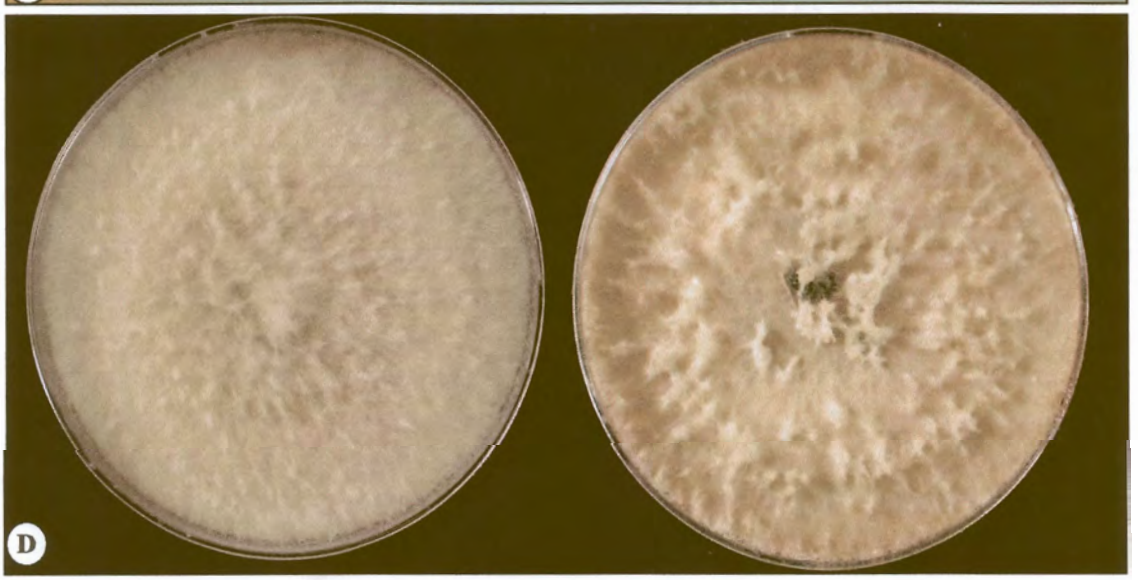
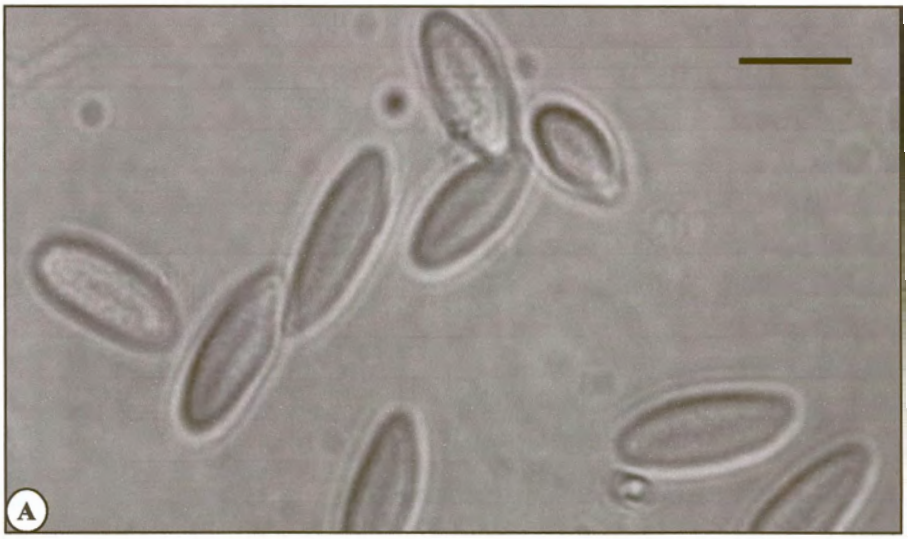
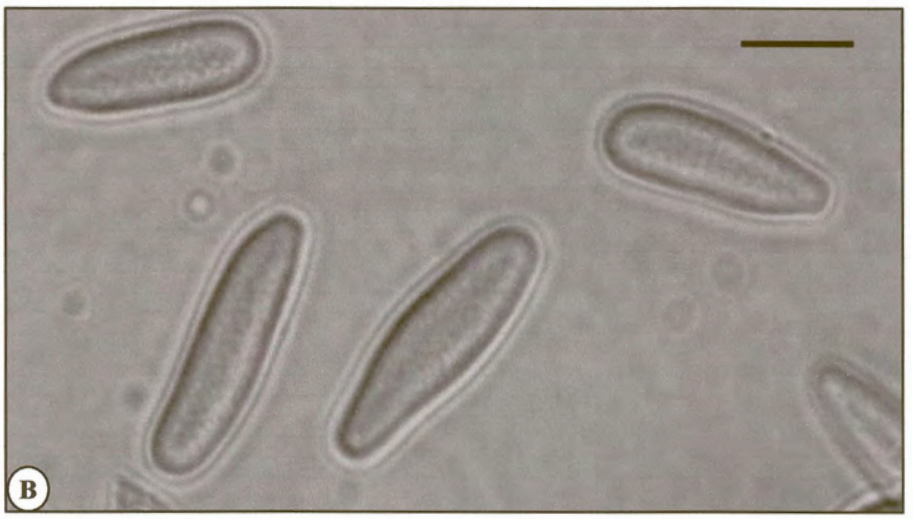



**Figure 4**. Conidia of the three species identified from *Eucalyptus* plantations in Chile, namely **(A)** *B. parva*. **(B)** *B. eucalyptorum* and **(C)** *Botryophaeria* sp. (Bars = 10 µm).



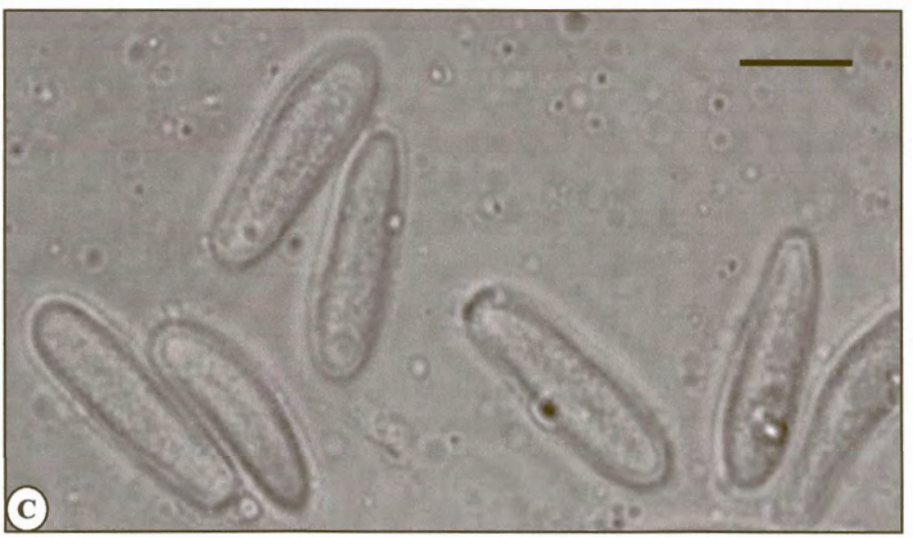



**Figure 5**. Phylogram generated from combined ITS and β-tubulin sequences from the rRNA operon of seven *Botryosphaeria* spp. (p = 0.46, CI = 0.888, RI = 0.935, HI = 0.112) inferred using heuristic search and branch swapping options of PAUP version 4.0b1. Bootstrap support from 1000 replications is given above the branches and branches length below the branches. The tree is rooted to the outgroup, *B. rhodina*.

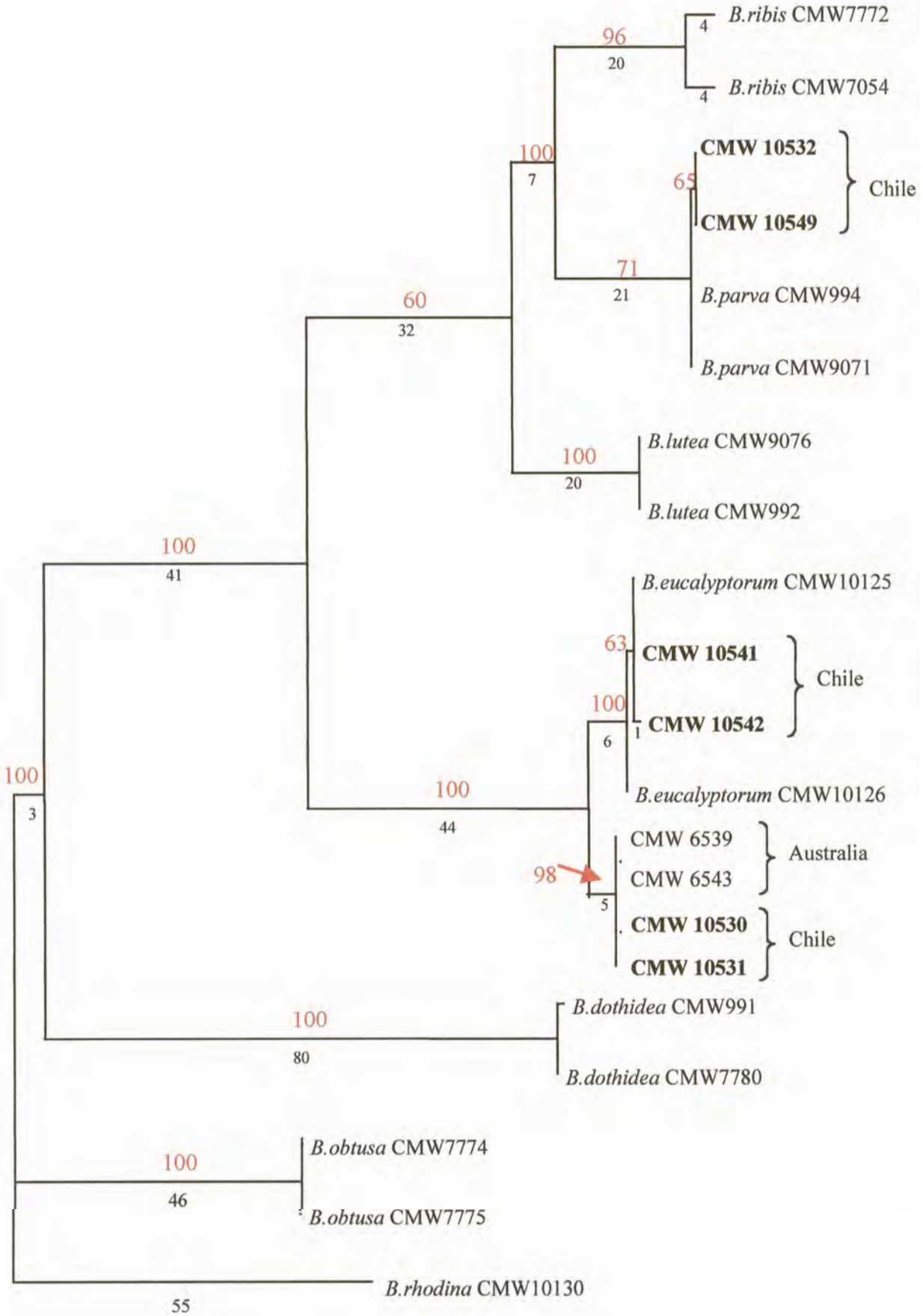

B.ribis CMW7772
B.ribis CMW7054
CMW 10532
CMW 10549
Chile
B.parva CMW994
B.parva CMW9071
B.lutea CMW9076
B.lutea CMW992
B.eucalyptorum CMW10125
CMW 10541
CMW 10542
Chile
B.eucalyptorum CMW10126
CMW 6539
CMW 6543
Australia
CMW 10530
CMW 10531
Chile
B.dothidea CMW991
B.dothidea CMW7780
B.obtusa CMW7774
B.obtusa CMW7775
B.rhodina CMW10130
96
20
4
4
100
7
65
71
21
60
32
100
20
100
41
63
100
6
1
100
3
100
44
98
5
100
80
100
46
55

**Figure 6**. Lesions observed in the xylem of an *E. grandis* clone, seven weeks after inoculation with three *Botryosphaeria* spp. and a control.

Control CMW 10547 CMW 10542 CMW 10537 CMW 10533 CMW 10532 CMW 10539

**Figure 7**. Average lesion lengths (mm) after inoculation of six isolates, representing the three *Botryosphaeria* spp. identified from Chile, and a control on an *E. grandis* clone.

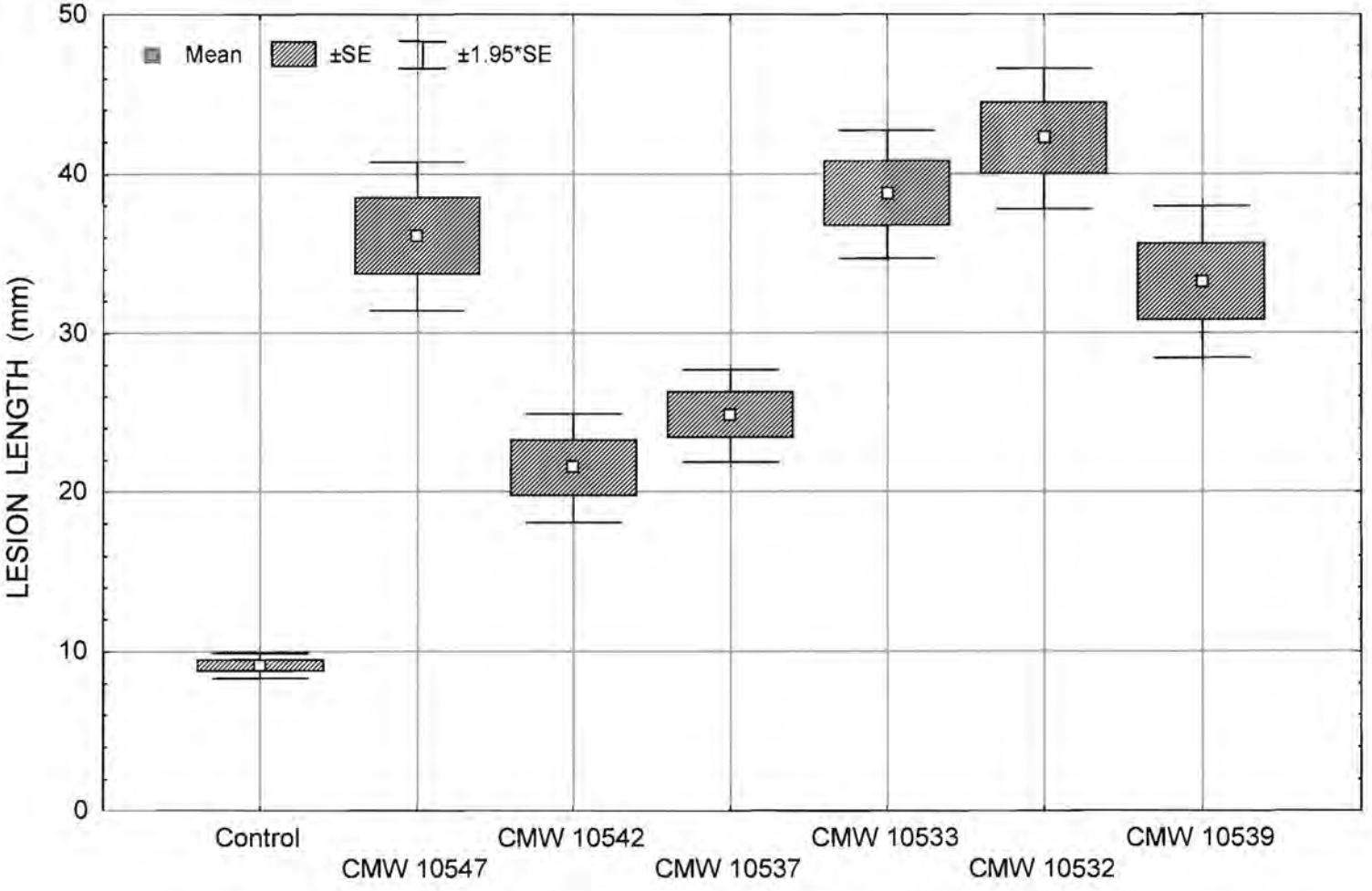


**Figure 8**. Combined ITS and β-tubulin sequences of *Botryosphaeria* species, consisted of 985 characteres of equal weight, with 771 constant characters of which 185 were parsimony-informative. Seuqence data set contains sequences of *Botryosphaeria* spp. isolated from *Eucalyptus* spp. from Chile.

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                                  10         20         30         40
B.ribis CMW7772                   GGAAGGATCA TTACCGAGTT GATTCGAGCT CCGGCTCGAC
B.ribis CMW7054                   GGAAGGATCA TTACCGAGTT GATTCGAGCT CCGGCTCGAC
CMW 10532                         GGAAGGATCA TTACCGAGTT GATTCGAGCT CCGGCTCGAC
CMW 10549                         GGAAGGATCA TTACCGAGTT GATTCGAGCT CCGGCTCGAC
B.parva CMW994                    GGAAGGATCA TTACCGAGTT GATTCGAGCT CCGGCTCGAC
B.parva CMW9071                   GGAAGGATCA TTACCGAGTT GATTCGAGCT CCGGCTCGAC
B.eucalyptorum CMW10125           GGAAGGATCA TTACCGAGTT GACTCGAGCT CCGGCTCGAC
B.eucalyptorum CMW10126           GGAAGGATCA TTACCGAGTT GACTCGAGCT CCGGCTCGAC
CMW 10541                         GGAAGGATCA TTACCGAGTT GACTCGAGCT CCGGCTCGAC
CMW 10542                         GGAAGGATCA TTACCGAGTT GACTCGAGCT CCGGCTCGAC
CMW 10530                         GGAAGGATCA TTACCGAGTT GACTCGAGCT CCGGCTCGAC
CMW 10531                         GGAAGGATCA TTACCGAGTT GACTCGAGCT CCGGCTCGAC
B.lutea CMW9076                   GGAAGGATCA TTACCGAGTT GATTCGAGCT CCGGCTCGAC
B.lutea CMW992                    GGAAGGATCA TTACCGAGTT GATTCGAGCT CCGGCTCGAC
B.dothidea CMW991                 GGAAGGATCA TTACCGAGTT GATTCGGGCT CCGGCCCGA-
B.dothidea CMW7780                GGAAGGATCA TTACCGAGTT GATTCGGGCT CCGGCCCGA-
B.obtusa CMW7774                  GGAAGNATCA TTACCGAGTT -CT-CGGGCT TCGGCTCGAA
B.obtusa CMW7775                  GGAAGGATCA TTACCGAGTT -CT-CGGGCT TCGGCTCGAA
B.rhodina CMW10130                GGAAGGATCA TTACCGAGTT --TTCGGGCT TCGGCTCGAC

                                  50         60         70         80
B.ribis CMW7772                   TC-TCCCACC CAATGTGTAC CTACCTCTGT TGCTTTGGCG
B.ribis CMW7054                   TC-TCCCACC CAATGTGTAC CTACCTCTGT TGCTTTGGCG
CMW 10532                         TC-TCCCACC CAATGTGTAC CTACCTCTGT TGCTTTGGCG
CMW 10549                         TC-TCCCACC CAATGTGTAC CTACCTCTGT TGCTTTGGCG
B.parva CMW994                    TC-TCCCACC CAATGTGTAC CTACCTCTGT TGCTTTGGCG
B.parva CMW9071                   TC-TCCCACC CAATGTGTAC CTACCTCTGT TGCTTTGGCG
B.eucalyptorum CMW10125           TC-TCCCACC CTATGTGTAC CTACCTCTGT TGCTTTGGCG
B.eucalyptorum CMW10126           TC-TCCCACC CTATGTGTAC CTACCTCTGT TGCTTTGGCG
CMW 10541                         TC-TCCCACC CTATGTGTAC CTACCTCTGT TGCTTTGGCG
CMW 10542                         TC-TCCCACC CTATGTGTAC CTACCTCTGT TGCTTTGGCG
CMW 10530                         TC-TCCCACC CTATGTGTAC CTACCTCTGT TGCTTTGGCG
CMW 10531                         TC-TCCCACC CTATGTGTAC CTACCTCTGT TGCTTTGGCG
B.lutea CMW9076                   TC-TCCCACC CCATGTGTAC CTACCTCTGT TGCTTTGGCG
B.lutea CMW992                    TC-TCCCACC CCATGTGTAC CTACCTCTGT TGCTTTGGCG
B.dothidea CMW991                 TCCTCCCACC CTTTGTGTAC CTACCTCTGT TGCTTTGGCG
B.dothidea CMW7780                TCCTCCCACC CTTTGTGTAC CTACCTCTGT TGCTTTGGCG
B.obtusa CMW7774                  TC-TCCCACC CTTTGTGAAC ATACCTCTGT TGCTTTGGCG
B.obtusa CMW7775                  TC-TCCCACC CTTTGTGAAC ATACCTCTGT TGCTTTGGCG
B.rhodina CMW10130                TC-TNCCACC CTTTGTGAAC GTACCTCTGT TGCTTTGGCG
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                                  90         100        110        120
B.ribis CMW7772                   GGCCGCGGTC CT--CCGC-A CCGG-CGCCC -TT--CG-GG
B.ribis CMW7054                   GGCCGCGGTC CT--CCGC-A CCGG-CGCCC -TT--CG-GG
CMW 10532                         GGCCGCGGTC CT--CCGC-A CCGG-CGCCC -TT--CG-GG
CMW 10549                         GGCCGCGGTC CT--CCGC-A CCGG-CGCCC -TT--CG-GG
B.parva CMW994                    GGCCGCGGTC CT--CCGC-A CCGG-CGCCC -TT--CG-GG
B.parva CMW9071                   GGCCGCGGTC CT--CCGC-A CCGG-CGCCC -TT--CG-GG
B.eucalyptorum CMW10125           GGCCGCGGTC CT--CCGC-A CCGG-CTCCC TTT---G-GG
B.eucalyptorum CMW10126           GGCCGCGGTC CT--CCGC-A CCGG-CTCCC TTT---G-GG
CMW 10541                         GGCCGCGGTC CT--CCGC-A CCGG-CTCCC TTT---G-GG
CMW 10542                         GGCCGCGGTC CT--CCGC-A CCGG-CTCCC TTT---G-GG
CMW 10530                         GGCCGCGGTC CT--CCGC-A CCGG-CCCCC TTT---G-GG
CMW 10531                         GGCCGCGGTC CT--CCGC-A CCGG-CCCCC TTT---G-GG
B.lutea CMW9076                   GGCCGCGGTC CT--CCGC-A CCGACCCCCG -TT--CG-GG
B.lutea CMW992                    GGCCGCGGTC CT--CCGC-A CCGACCCCCG -TT--CG-GG
B.dothidea CMW991                 GGCCGCGGTC CT--CCGCGG CCGGCCCCCC -TCCCCG-GG
B.dothidea CMW7780                GGCCGCGGTC CT--CCGCGG CCGGCCCCCC -TCCCCG-GG
B.obtusa CMW7774                  G-C-----TC TTTGCCGCGA --GGAGGCCC -T---CGCGG
B.obtusa CMW7775                  G-C-----TC TTTGCCGCGA --GGAGGCCC -T---CGCGG
B.rhodina CMW10130                G-C-----TC -------CG- ---------- ----------

                                  130        140        150        160
B.ribis CMW7772                   GGGGCTGGCC A--GCGC--- CCGCCAGAGG ACCAT-AAAA
B.ribis CMW7054                   GGGGCTGGCC A--GCGC--- CCGCCAGAGG ACCAT-AAAA
CMW 10532                         GGG-CTGGCC A--GCGC--- CCGCCAGAGG ACCAT-AAAA
CMW 10549                         GGG-CTGGCC A--GCGC--- CCGCCAGAGG ACCAT-AAAA
B.parva CMW994                    GGG-CTGGCC A--GCGC--- CCGCCAGAGG ACCAT-AAAA
B.parva CMW9071                   GGG-CTGGCC A--GCGC--- CCGCCAGAGG ACCAT-AAAA
B.eucalyptorum CMW10125           GG--CTGGCC A--GCGT--- CCGCCAGAGG ACCA-CAAAA
B.eucalyptorum CMW10126           GG--CTGGCC A--GCGT--- CCGCCAGAGG ACCA-CAAAA
CMW 10541                         GG--CTGGCC A--GCGT--- CCGCCAGAGG ACCA-CAAAA
CMW 10542                         GG--CTGGCC A--GCGT--- CCGCCAGAGG ACCA-CAAAA
CMW 10530                         GG--CTGGCC A--GCGC--- CCGCCAGAGG ACCA-CAAAA
CMW 10531                         GG--CTGGCC A--GCGC--- CCGCCAGAGG ACCA-CAAAA
B.lutea CMW9076                   GGG-CCGGCC A--GCGC--- CCGCCAGAGG ACCA-CAAAA
B.lutea CMW992                    GGG-CCGGCC A--GCGC--- CCGCCAGAGG ACCA-CAAAA
B.dothidea CMW991                 GGG--TGGCC A--GCGC--- CCGCCAGAGG ACCATCAAA-
B.dothidea CMW7780                GGG--TGGCC A--GCGC--- CCGCCAGAGG ACCATCAAA-
B.obtusa CMW7774                  GCC--CCCCC -GCGCGCTTT CCGCCAGAGG ACCTTCAAA-
B.obtusa CMW7775                  GCC--CCCCC -GCGCGCTTT CCGCCAGAGG ACCTTCAAA-
B.rhodina CMW10130                ---------- ---------- CCGCCAAAGG ACCTTCAAA-
```

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                                170        180        190        200
B.ribis CMW7772                 CTCCAGTCAG TGAAC-TTCG CAGTCTGAAA AACAAGTTAA
B.ribis CMW7054                 CTCCAGTCAG TGAAC-TTCG CAGTCTGAAA AACAAGTTAA
CMW 10532                       CTCCAGTCAG TGAAC-TTCG CAGTCTGAAA AACAAGTTAA
CMW 10549                       CTCCAGTCAG TGAAC-TTCG CAGTCTGAAA AACAAGTTAA
B.parva CMW994                  CTCCAGTCAG TGAAC-TTCG CAGTCTGAAA AACAAGTTAA
B.parva CMW9071                 CTCCAGTCAG TGAAC-TTCG CAGTCTGAAA AACAAGTTAA
B.eucalyptorum CMW10125         CTCCAGTCAG TAAACGTT-G CAGTCTGAAA AACAAGTTAA
B.eucalyptorum CMW10126         CTCCAGTCAG TAAACGTT-G CAGTCTGAAA AACAAGTTAA
CMW 10541                       CTCCAGTCAG TAAACGTT-G CAGTCTGAAA AACAAGTTAA
CMW 10542                       CTCCAGTCAG TAAACGTT-G CAGTCTGAAA AACAAGTTAA
CMW 10530                       CTCCAGTCAG TAAACGTT-G CAGTCTGAAA ACCAAGTTAA
CMW 10531                       CTCCAGTCAG TAAACGTT-G CAGTCTGAAA ACCAAGTTAA
B.lutea CMW9076                 CTCCAGTCAG TAAACG-TCG CAGTCTGAGA AACAAGTTAA
B.lutea CMW992                  CTCCACTCAC TAAACG-TCC CAGTCTCAGA AACAAGTTAA
B.dothidea CMW991               CTCCAGTCAG TAAACGAT-G CAGTCTGAAA AACAT-TTAA
B.dothidea CMW7780              CTCCAGTCAG TAAACGAT-G CAGTCTGAAA AACAT-TTAA
B.obtusa CMW7774                CTCCAGTCAG TAAACG-TCG ACGTCTGATA AACAAGTTAA
B.obtusa CMW7775                CTCCAGTCAG TAAACG-TCG ACGTCTGATA AACAAGTTAA
B.rhodina CMW10130              CTCCAGTCAG TAAACGCA-G ACGTCTGATA AACAAGTTAA

                                210        220        230        240
B.ribis CMW7772                 TAAACTAAAA CTTTCAACAA CGGATCTCTT GGTTCTGGCA
B.ribis CMW7054                 TAAACTAAAA CTTTCAACAA CGGATCTCTT GGTTCTGGCA
CMW 10532                       TAAACTAAAA CTTTCAACAA CGGATCTCTT GGTTCTGGCA
CMW 10549                       TAAACTAAAA CTTTCAACAA CGGATCTCTT GGTTCTGGCA
B.parva CMW994                  TAAACTAAAA CTTTCAACAA CGGATCTCTT GGTTCTGGCA
B.parva CMW9071                 TAAACTAAAA CTTTCAACAA CGGATCTCTT GGTTCTGGCA
B.eucalyptorum CMW10125         TAAACTAAAA CTTTCAACAA CGGATCTCTT GGTTCTGGCA
B.eucalyptorum CMW10126         TAAACTAAAA CTTTCAACAA CGGATCTCTT GGTTCTGGCA
CMW 10541                       TAAACTAAAA CTTTCAACAA CGGATCTCTT GGTTCTGGCA
CMW 10542                       TAAACTAAAA CTTTCAACAA CGGATCTCTT GGTTCTGGCA
CMW 10530                       TAAACTAAAA CTTTCAACAA CGGATCTCTT GGTTCTGGCA
CMW 10531                       TAAACTAAAA CTTTCAACAA CGGATCTCTT GGTTCTGGCA
B.lutea CMW9076                 TAAACTAAAA CTTTCAACAA CGGATCTCTT GGTTCTGGCA
B.lutea CMW992                  TAAACTAAAA CTTTCAACAA CGGATCTCTT GGTTCTGGCA
B.dothidea CMW991               TAAACTAAAA CTTTCAACAA CGGATCTCTT GGTTCTGGCA
B.dothidea CMW7780              TAAACTAAAA CTTTCAACAA CGGATCTCTT GGTTCTGGCA
B.obtusa CMW7774                TAAACTAAAA CTTTCAACAA CGGATCTCTT GGTTCTGGCA
B.obtusa CMW7775                TAAACTAAAA CTTTCAACAA CGGATCTCTT GGTTCTGGCA
B.rhodina CMW10130              TAAACTAAAA CTTTCAACAA CGGATCTCTT GGTTCTGGCA
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                                  250         260         270         280
B.ribis CMW7772                   TCGATGAAGA ACGCAGCGAA ATGCGATAAG TAATGTGAAT
B.ribis CMW7054                   TCGATGAAGA ACGCAGCGAA ATGCGATAAG TAATGTGAAT
CMW 10532                         TCGATGAAGA ACGCAGCGAA ATGCGATAAG TAATGTGAAT
CMW 10549                         TCGATGAAGA ACGCAGCGAA ATGCGATAAG TAATGTGAAT
B.parva CMW994                    TCGATGAAGA ACGCAGCGAA ATGCGATAAG TAATGTGAAT
B.parva CMW9071                   TCGATGAAGA ACGCAGCGAA ATGCGATAAG TAATGTGAAT
B.eucalyptorum CMW10125           TCGATGAAGA ACGCAGCGAA ATGCGATAAG TAATGTGAAT
B.eucalyptorum CMW10126           TCGATGAAGA ACGCAGCGAA ATGCGATAAG TAATGTGAAT
CMW 10541                         TCGATGAAGA ACGCAGCGAA ATGCGATAAG TAATGTGAAT
CMW 10542                         TCGATGAAGA ACGCAGCGAA ATGCGATAAG TAATGTGAAT
CMW 10530                         TCGATGAAGA ACGCAGCGAA ATGCGATAAG TAATGTGAAT
CMW 10531                         TCGATGAAGA ACGCAGCGAA ATGCGATAAG TAATGTGAAT
B.lutea CMW9076                   TCGATGAAGA ACGCAGCGAA ATGCGATAAG TAATGTGAAT
B.lutea CMW992                    TCGATGAAGA ACGCAGCGAA ATGCGATAAG TAATGTGAAT
B.dothidea CMW991                 TCGATGAAGA ACGCAGCGAA ATGCGATAAG TAATGTGAAT
B.dothidea CMW7780                TCGATGAAGA ACGCAGCGAA ATGCGATAAG TAATGTGAAT
B.obtusa CMW7774                  TCGATGAAGA ACGCAGCGAA ATGCGATAAG TAATGTGAAT
B.obtusa CMW7775                  TCGATGAAGA ACGCAGCGAA ATGCGATAAG TAATGTGAAT
B.rhodina CMW10130                TCGATGAAGA ACGCAGCGAA ATGCGATAAG TAATGTGAAT

                                  290         300         310         320
B.ribis CMW7772                   TGCAGAATTC AGTGAATCAT CGAATCTTTG AACGCACATT
B.ribis CMW7054                   TGCAGAATTC AGTGAATCAT CGAATCTTTG AACGCACATT
CMW 10532                         TGCAGAATTC AGTGAATCAT CGAATCTTTG AACGCACATT
CMW 10549                         TGCAGAATTC AGTGAATCAT CGAATCTTTG AACGCACATT
B.parva CMW994                    TGCAGAATTC AGTGAATCAT CGAATCTTTG AACGCACATT
B.parva CMW9071                   TGCAGAATTC AGTGAATCAT CGAATCTTTG AACGCACATT
B.eucalyptorum CMW10125           TGCAGAATTC AGTGAATCAT CGAATCTTTG AACGCACATT
B.eucalyptorum CMW10126           TGCAGAATTC AGTGAATCAT CGAATCTTTG AACGCACATT
CMW 10541                         TGCAGAATTC AGTGAATCAT CGAATCTTTG AACGCACATT
CMW 10542                         TGCAGAATTC AGTGAATCAT CGAATCTTTG AACGCACATT
CMW 10530                         TGCAGAATTC AGTGAATCAT CGAATCTTTG AACGCACATT
CMW 10531                         TGCAGAATTC AGTGAATCAT CGAATCTTTG AACGCACATT
B.lutea CMW9076                   TGCAGAATTC AGTGAATCAT CGAATCTTTG AACGCACATT
B.lutea CMW992                    TGCAGAATTC AGTGAATCAT CGAATCTTTG AACGCACATT
B.dothidea CMW991                 TGCAGAATTC AGTGAATCAT CGAATCTTTG AACGCACATT
B.dothidea CMW7780                TGCAGAATTC AGTGAATCAT CGAATCTTTG AACGCACATT
B.obtusa CMW7774                  TGCAGAATTC AGTGAATCAT CGAATCTTTG AACGCACATT
B.obtusa CMW7775                  TGCAGAATTC AGTGAATCAT CGAATCTTTG AACGCACATT
B.rhodina CMW10130                TGCAGAATTC AGTGAATCAT CGAATCTTTG AACGCACATT
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| | 330 | 340 | 350 | 360 |
|---|---|---|---|---|
| *B.ribis* CMW7772 | GCGCCCCTTG | GTATTCCGAG | GGGCATGCCT | GTTCGAGCGT |
| *B.ribis* CMW7054 | GCGCCCCTTG | GTATTCCGAG | GGGCATGCCT | GTTCGAGCGT |
| **CMW 10532** | GCGCCCCTTG | GTATTCCGAG | GGGCATGCCT | GTTCGAGCGT |
| **CMW 10549** | GCGCCCCTTG | GTATTCCGAG | GGGCATGCCT | GTTCGAGCGT |
| *B.parva* CMW994 | GCGCCCCTTG | GTATTCCGAG | GGGCATGCCT | GTTCGAGCGT |
| *B.parva* CMW9071 | GCGCCCCTTG | GTATTCCGAG | GGGCATGCCT | GTTCGAGCGT |
| *B.eucalyptorum* CMW10125 | GCGCCCCTTG | GTATTCCGAG | GGGCATGCCT | GTTCGAGCGT |
| *B.eucalyptorum* CMW10126 | GCGCCCCTTG | GTATTCCGAG | GGGCATGCCT | GTTCGAGCGT |
| **CMW 10541** | GCGCCCCTTG | GTATTCCGAG | GGGCATGCCT | GTTCGAGCGT |
| **CMW 10542** | GCGCCCCTTG | GTATTCCGAG | GGGCATGCCT | GTTCGAGCGT |
| **CMW 10530** | GCGCCCCTTG | GTATTCCGAG | GGGCATGCCT | GTTCGAGCGT |
| **CMW 10531** | GCGCCCCTTG | GTATTCCGAG | GGGCATGCCT | GTTCGAGCGT |
| *B.lutea* CMW9076 | GCGCCCCTTG | GTATTCCGAG | GGGCATGCCT | GTTCGAGCGT |
| *B.lutea* CMW992 | GCGCCCCTTG | GTATTCCGAG | GGGCATGCCT | GTTCGAGCGT |
| *B.dothidea* CMW991 | GCGCCCTTTG | GTATTCCGAA | GGGCATGCCT | GTTCGAGCGT |
| *B.dothidea* CMW7780 | GCGCCCTTTG | GTATTCCGAA | GGGCATGCCT | GTTCGAGCGT |
| *B.obtusa* CMW7774 | GCGCCCCCTG | GCATTCCGGG | GGGCATGCCT | GTTCGAGCGT |
| *B.obtusa* CMW7775 | GCGCCCCCTG | GCATTCCGGG | GGGCATGCCT | GTTCGAGCGT |
| *B.rhodina* CMW10130 | GCGCCCCTTG | GTATTCCGGG | GGGCATGCCT | GTTCGAGCGT |

| | 370 | 380 | 390 | 400 |
|---|---|---|---|---|
| *B.ribis* CMW7772 | CATTTCAACC | CTCAAGCT-C | TGCTTGGTAT | TGGGCTCCGT |
| *B.ribis* CMW7054 | CATTTCAACC | CTCAAGCT-C | TGCTTGGTAT | TGGGCTCCGT |
| **CMW 10532** | CATTTCAACC | CTCAAGCT-C | TGCTTGGTAT | TGGGCCTCGT |
| **CMW 10549** | CATTTCAACC | CTCAAGCT-C | TGCTTGGTAT | TGGGCCTCGT |
| *B.parva* CMW994 | CATTTCAACC | CTCAAGCT-C | TGCTTGGTAT | TGGGCCCCGT |
| *B.parva* CMW9071 | CATTTCAACC | CTCAAGCT-C | TGCTTGGTAT | TGGGCCCCGT |
| *B.eucalyptorum* CMW10125 | CATTTCAACC | CTCAAGCTT- | TGCTTGGTAT | TGGGCCCCGT |
| *B.eucalyptorum* CMW10126 | CATTTCAACC | CTCAAGCTT- | TGCTTGGTAT | TGGGCCCCGT |
| **CMW 10541** | CATTTCAACC | CTCAAGCTT- | TGCTTGGTAT | TGGGCCCCGT |
| **CMW 10542** | CATTTCAACC | CTCAAGCTT- | TGCTTGGTAT | TGGGCCCCGT |
| **CMW 10530** | CATTTCAACC | CTCAAGCT-C | TGCTTGGTAT | TGGGCTCCGT |
| **CMW 10531** | CATTTCAACC | CTCAAGCT-C | TGCTTGGTAT | TGGGCTCCGT |
| *B.lutea* CMW9076 | CATTTCAACC | CTCAAGCT-C | TGCTTGGTAT | TGGGCTCCGT |
| *B.lutea* CMW992 | CATTTCAACC | CTCAAGCT-C | TGCTTGGTAT | TGGGCTCCGT |
| *B.dothidea* CMW991 | CATTACAACC | CTCAAGCT-C | TGCTTGGTAT | TGGGCACCGT |
| *B.dothidea* CMW7780 | CATTACAACC | CTCAAGCT-C | TGCTTGGTAT | TGGGCACCGT |
| *B.obtusa* CMW7774 | CATTACAACC | CTCAAGCT-C | TGCTTGGTAT | TGGGCGCCGT |
| *B.obtusa* CMW7775 | CATTACAACC | CTCAAGCT-C | TGCTTGGTAT | TGGGCGCCGT |
| *B.rhodina* CMW10130 | CATTACAACC | CTCAAGCT-C | TGCTTGGAAT | TGGGCACCGT |

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                                410        420        430        440
B.ribis CMW7772                 CCTCCA---C GGACGCGCCT TAAAGACCTC GGCGGTGGC-
B.ribis CMW7054                 CCTCCA---C GGACGCGCCT TAAAGACCTC GGCGGTGGC-
CMW 10532                       CCTCCA---C GGACGCGCCT TAAAGACCTC GGCGGTGGC-
CMW 10549                       CCTCCA---C GGACGCGCCT TAAAGACCTC GGCGGTGGC-
B.parva CMW994                  CCTCCA---C GGACGCGCCT TAAAGACCTC GGCGGTGGC-
B.parva CMW9071                 CCTCCA---C GGACGCGCCT TAAAGACCTC GGCGGTGGC-
B.eucalyptorum CMW10125         CCT-CT--GT GGACGCGCCT CAAAGACCTC GGCGGTGGC-
B.eucalyptorum CMW10126         CCT-CT--GT GGACGCGCCT CAAAGACCTC GGCGGTGGC-
CMW 10541                       CCT-CT--GT GGACGCGCCT CAAAGACCTC GGCGGTGGC-
CMW 10542                       CCT-CT--GT GGACGCGCCT CAAA-ACCTC GGCGGTGGC-
CMW 10530                       CCT-CT--GT GGACGCGCCT CAAAGACCTC GGCGGTGGC-
CMW 10531                       CCT-CT--GT GGACGCGCCT CAAAGACCTC GGCGGTGGC-
B.lutea CMW9076                 CCT-CT--GT GGACGCGCCT CGAAGACCTC GGCGGTGGC-
B.lutea CMW992                  CCT-CT--GT GGACGCGCCT CGAAGACCTC GGCGGTGGC-
B.dothidea CMW991               CCT--T-TGC GGGCGCGCCT CAAAGACCTC GGCGGTGGC-
B.dothidea CMW7780              CCT--T-TGC GGGCGCGCCT CAAAGACCTC GGCGGTGGC-
B.obtusa CMW7774                CCT-CTCTGC GGACGCGCCT TAAAGACCTC GGCGGTGGCT
B.obtusa CMW7775                CCT-CTCTGC GGACGCGCCT TAAAGACCTC GGCGGTGGCT
B.rhodina CMW10130              CCT-CACTGC GGACGCGCCT CAAAGACCTC GGCGGTGGCT

                                450        460        470        480
B.ribis CMW7772                 GTCTTGCC-T CAAGCGTAGT AGAAAA--CA CCTCGCTTTG
B.ribis CMW7054                 GTCTTGCC-T CAAGCGTAGT AGAAAA--CA CCTCGCTTTG
CMW 10532                       GTCTTGCC-T CAAGCGTAGT AGAAAA--CA CCTCGCTTTG
CMW 10549                       GTCTTGCC-T CAAGCGTAGT AGAAAA--CA CCTCGCTTTG
B.parva CMW994                  GTCTTGCC-T CAAGCGTAGT AGAAAA--CA CCTCGCTTTG
B.parva CMW9071                 GTCTTGCC-T CAAGCGTAGT AGAAAA--CA CCTCGCTTTG
B.eucalyptorum CMW10125         GTCTTGCC-T CAAGCGTAGT AGAA-AT-CA CCTCGCTTTG
B.eucalyptorum CMW10126         GTCTTGCC-T CAAGCGTAGT AGAA-AT-CA CCTCGCTTTG
CMW 10541                       GTCTTGCC-T CAAGCGTAGT AGAA-AT-CA CCTCGCTTTG
CMW 10542                       GTCTTGCC-T CAAGCGTAGT AGAA-AT-CA CCTCGCTTTG
CMW 10530                       GTCTTGCC-T CAAGCGTAGT AGAA-AT-CA CCTCGCTTTG
CMW 10531                       GTCTTGCC-T CAAGCGTAGT AGAA-AT-CA CCTCGCTTTG
B.lutea CMW9076                 GTCTTGCC-T CAAGCGTAGT AGAAAA--CA CCTCGCTTTG
B.lutea CMW992                  GTCTTGCC-T CAAGCGTAGT AGAAAA--CA CCTCGCTTTG
B.dothidea CMW991               GTCTTGCC-T CAAGCGTAGT AGAACATACA TCTCGCTTCG
B.dothidea CMW7780              GTCTTGCC-T CAAGCGTAGT AGAACATACA TCTCGCTTCG
B.obtusa CMW7774                GTTCAGCCCT CAAGCGTAGT AGAA--TACA CCTCGCTTTG
B.obtusa CMW7775                GTTCAGCCCT CAAGCGTAGT AGAA--TACA CCTCGCTTTG
B.rhodina CMW10130              GTTCAGCCCT CAAGCGTAGT AGAA--TACA CCTCGCTTTG
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                                570        580        590        600

B.ribis CMW7772                 TCTGGTTTGT TGCCAAAACA CTCCCGCTCC CGCGCCCCC-
B.ribis CMW7054                 TCTGGTTTGT TGCCAAAACA CTCCCGCTCC CGCGCCCCC-
CMW 10532                       TCTGGTTTGT TGCCAAAACA CTCCCGCTCC CGCGCCCCC-
CMW 10549                       TCTGGTTTGT TGCCAAAACA CTCCCGCTCC CGCGCCCCC-
B.parva CMW994                  TCTGGTTTGT TGCCAAAACA CTCCCGCTCC CGCGCCCCC-
B.parva CMW9071                 TCTGGTTTGT TGCCAAAACA CTCCCGCTCC CGCGCCCCC-
B.eucalyptorum CMW10125         TCTGGTTTGT TGCCAAAACA CTCTCGCTCC TGCGCCCCC-
B.eucalyptorum CMW10126         TCTGGTTTGT TGCCAAAACA CTCTCGCTCC TGCGCCCCC-
CMW 10541                       TCTGGTTTGT TGCCAAAACA CTCTCGCTCC TGCGCCCCC-
CMW 10542                       TCTGGTTTGT TGCCAAAACA CTCTCGCTCC TGCGCCCCC-
CMW 10530                       TCTGGTTTGT TGCCAAAACA CTCTCGCTCC TGCGCCCCC-
CMW 10531                       TCTGGTTTGT TGCCAAAACA CTCTCGCTCC TGCGCCCCC-
B.lutea CMW9076                 TCTGGTTTGT TGCCAAAACA CTGCCGCTCC CGCGCCCCC-
B.lutea CMW992                  TCTGGTTTGT TGCCAAAACA CTGCCGCTCC CGCGCCCCC-
B.dothidea CMW991               TCTGGTTTGT TGCCAAAACA C-CC-GCTCC CGCGCCCCC-
B.dothidea CMW7780              TCTGGTTTGT TGCCAAAACA C-CC-GCTCC CGCGCCCCC-
B.obtusa CMW7774                TCTGGTTTGT TGCCAAAACA CTCCCGCTGC CGCGCCCCCC
B.obtusa CMW7775                TCTGGTTTGT TGCCAAAACA CTCCCGCTGC CGCGCCCCCC
B.rhodina CMW10130              TCTGGTTTGT TGCCAAAACA CTCCTGCTCC TGCGCCCCCC

                                610        620        630        640

B.ribis CMW7772                 GCTGACGCGA ATCGACACCA CAGGCAGACC ATTTCCGGCG
B.ribis CMW7054                 GCTGACGCGA ATCGACACCA CAGGCAGACC ATTTCCGGCG
CMW 10532                       GCTGACGCGA ATCGACACCA CAGGCAGACC ATTTCTGGCG
CMW 10549                       GCTGACGCGA ATCGACACCA CAGGCAGACC ATTTCTGGCG
B.parva CMW994                  GCTGACGCGA ATCGACACCA CAGGCAGACC ATTTCTGGCG
B.parva CMW9071                 GCTGACGCGA ATCGACACCA CAGGCAGACC ATTTCTGGCG
B.eucalyptorum CMW10125         GCTGACGCGA ATCGACACCA TAGGCAGACC ATTTCTGGTG
B.eucalyptorum CMW10126         GCTGACGCGA ATCGACACCA CAGGCAGACC ATTTCTGGTG
CMW 10541                       GCTGACGCGA ATCGACACCA TAGGCAGACC ATTTCTGGTG
CMW 10542                       GCTGACGCGA ATCGACACCA TAGGCAGACC ATTTCTGGTG
CMW 10530                       GCTGACGCGA ATCGACACCA CAGGCAGACC ATTTCTGGCG
CMW 10531                       GCTGACGCGA ATCGACACCA CAGGCAGACC ATTTCTGGCG
B.lutea CMW9076                 GCTGACGCGA ATCGACACCG CAGGCAGACC ATTTCTGGCG
B.lutea CMW992                  GCTGACGCGA ATCGACACCG CAGGCAGACC ATTTCTGGCG
B.dothidea CMW991               GCTAACGCGA ATCGACACCA CAGGCAGACC ATCTCCGGCG
B.dothidea CMW7780              GCTAACGCGA ATCGACACCA CAGGCAGACC ATCTCCGGCG
B.obtusa CMW7774                GCTGACGCCA ATCGACACCA CAGGCAGACT ATCTCTGGCG
B.obtusa CMW7775                GCTGACGCCA ATCGACACCA CAGGCAGACT ATCTCTGGCG
B.rhodina CMW10130              GCTGACGG-A AGCGACACCA TAGGCAGACC ATCTCCGGCG
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                                650        660        670        680

B.ribis CMW7772                 AGCACGGCCT GGACGGCTCT GGCGTGTGAG TCTGCGCCGT
B.ribis CMW7054                 AGCACGGCCT GGACGGCTCT GGCGTGTGAG TCTGCGCCGT
CMW 10532                       AGCACGGCCT GGACGGCTCT GGCGTGTAAG TCTGCGCCGT
CMW 10549                       AGCACGGCCT GGACGGCTCT GGCGTGTAAG TCTGCGCCGT
B.parva CMW994                  AGCACGGCCT GGACGGCTCT GGCGTGTAAG TCTGCGCCGT
B.parva CMW9071                 AGCACGGCCT GGACGGCTCT GGCGTGTAAG TCTGCGCCGT
B.eucalyptorum CMW10125         AACACGGCCT GGACGGCTCT GGCGTGTAAG TCTGCGCCGT
B.eucalyptorum CMW10126         AACACGGCCT GGACGGCTCT GGCGTGTAAG TCTGCGCCGT
CMW 10541                       AACACGGCCT GGACGGCTCT GGCGTGTAAG TCTGCGCCGT
CMW 10542                       AACACGGCCT GGACGGCTCT GGCGTGTAAG TCTGCGCCGT
CMW 10530                       AACACGGCCT GGACGGCTCT GGCGTGTAAG TCTGCGCCGT
CMW 10531                       AACACGGCCT GGACGGCTCT GGCGTGTAAG TCTGCGCCGT
B.lutea CMW9076                 AGCACGGCCT GGACGGCTCT GGCGTGTAAG TCTGCGCCGT
B.lutea CMW992                  AGCACGGCCT GGACGGCTCT GGCGTGTAAG TCTGCGCCGT
B.dothidea CMW991               AGCACGGCCT GGACGGCTCT GGCGTGTAAG TCTGCATC-A
B.dothidea CMW7780              AGCACGGCCT GGACGGCTCT GGCGTGTAAG TCTGCATC-A
B.obtusa CMW7774                AGCACGGCCT GGACGGCTCC GGCGTGTAAG TTTGCG-C-T
B.obtusa CMW7775                AGCACGGCCT GGACGGCTCC GGCGTGTAAG TTTGCG-C-T
B.rhodina CMW10130              AGCACGGCCT GGATGGCTCC GGTGTGTAAG TGTGCG-CC-

                                690        700        710        720

B.ribis CMW7772                 TTC----CCG CGC-----GA A--TGGCAAT GGCTGACCC-
B.ribis CMW7054                 TTC----CCG CGC-----GA A--TGGCAAT GGCTGACCC-
CMW 10532                       TTC----CCG CGC-----GA A--TGGCAAT GGCTGACCC-
CMW 10549                       TTC----CCG CGC-----GA A--TGGCAAT GGCTGACCC-
B.parva CMW994                  TTC----CCG CGC-----GA A--TGGCAAT GGCTGACCC-
B.parva CMW9071                 TTC----CCG CGC-----GA A--TGGCAAT GGCTGACCC-
B.eucalyptorum CMW10125         TTC----CCG CTC-----GA A--TGGCAAT GGCTGACCC-
B.eucalyptorum CMW10126         TTC----CCG CTC-----GA A--TGGCAAT GGCTGACCC-
CMW 10541                       TTC----CCG CTC-----GA A--TGGCAAT GGCTGACCC-
CMW 10542                       TTC----CCG CTC-----GA A--TGGCAAT GGCTGACCC-
CMW 10530                       TTC----CCG CTC-----GA A--TGGCAAT GGCTGACCC-
CMW 10531                       TTC----CCG CTC-----GA A--TGGCAAT GGCTGACCC-
B.lutea CMW9076                 TTCTT----G CGC-----GA A--TGGCAAT GGCTGACCC-
B.lutea CMW992                  TTCTT----G CGC-----GA A--TGGCAAT GGCTGACCC-
B.dothidea CMW991               TTCTCA---G CG-TGGGAGA ACAT--CAAT GACT-AAACT
B.dothidea CMW7780              TTCTCA---G CG-TGGGAGA ACAT--CAAT GACT-AAACT
B.obtusa CMW7774                GTCTTTGCCG CGCTC----- ---TG-CAAT CGCTGACCCT
B.obtusa CMW7775                GTCTTTGCCG CGCTC----- ---TG-CAAT CGCTGACCCT
B.rhodina CMW10130              TTCTCCGCCG CG-------- -CATGGCAAT CGCTGAC-CT
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                                730        740        750        760

B.ribis CMW7772                 GTAGCAGCTA CAATGGCACC TCCGACCTGC AGCTCGAGCG
B.ribis CMW7054                 GTAGCAGCTA CAATGGCACC TCCGACCTGC AGCTCGAGCG
CMW 10532                       GCAGCAGCTA CAATGGCACC TCCGACCTGC AGCTCGAGCG
CMW 10549                       GCAGCAGCTA CAATGGCACC TCCGACCTGC AGCTCGAGCG
B.parva CMW994                  GCAGCAGCTA CAATGGCACC TCCGACCTGC AGCTCGAGCG
B.parva CMW9071                 GCAGCAGCTA CAATGGCACC TCCGACCTGC AGCTCGAGCG
B.eucalyptorum CMW10125         GCAACAGCTA CAATGGCACC TCCGACCTCC AGCTCGAGCG
B.eucalyptorum CMW10126         GCAACAGCTA CAATGGCACC TCCGACCTCC AGCTCGAGCG
CMW 10541                       GCAACAGCTA CAATGGCACC TCCGACCTCC AGCTCGAGCG
CMW 10542                       GCAACAGCTA CAATGGCACC TCCGACCTCC AGCTCGAGCG
CMW 10530                       GCAACAGCTA CAATGGCACC TCCGACCTCC AGCTCGAGCG
CMW 10531                       GCAACAGCTA CAATGGCACC TCCGACCTCC AGCTCGAGCG
B.lutea CMW9076                 GCAGCAGCTA CAATGGCACC TCCGACCTCC AGCTCGAGCG
B.lutea CMW992                  GCAGCAGCTA CAATGGCACC TCCGACCTCC AGCTCGAGCG
B.dothidea CMW991               GTAGCAGCTA CAATGGCACC TCGGACCTTC AGCTCGAGCG
B.dothidea CMW7780              GTAGCAGCTA CAATGGCACC TCGGACCTTC AGCTCGAGCG
B.obtusa CMW7774                TG-GCAGCTA CAATGGCACC TCCGACCTCC AGCTGGAGCG
B.obtusa CMW7775                TG-GCAGCTA CAATGGCACC TCCGACCTCC AGCTGGAGCG
B.rhodina CMW10130              GTAGCAGCTA CAATGGCACT TCGGACCTCC AACTGGAGCG

                                770        780        790        800

B.ribis CMW7772                 CATGAACGTC TACTTCAACG AGGTACTCTC TCACTAATTG
B.ribis CMW7054                 CATGAACGTC TACTTCAACG AGGTACTCTC TCACTAATTG
CMW 10532                       CATGAACGTC TACTTCAACG AGGTACTCTC TCACTAATTG
CMW 10549                       CATGAACGTC TACTTCAACG AGGTACTCTC TCACTAATTG
B.parva CMW994                  CATGAACGTC TACTTCAACG AGGTACTCTC TCACTAATTG
B.parva CMW9071                 CATGAACGTC TACTTCAACG AGGTACTCTC TCACTAATTG
B.eucalyptorum CMW10125         CATGAACGTC TACTTCAACG AGGTACTCTC TCACTAATCA
B.eucalyptorum CMW10126         CATGAACGTC TACTTCAACG AGGTACTCTC TCACTAATCA
CMW 10541                       CATGAACGTC TACTTCAACG AGGTACTCTC TCACTAATCA
CMW 10542                       CATGAACGTC TACTTCAACG AGGTACTCTC TCACTAATCA
CMW 10530                       CATGAACGTC TACTTCAACG AGGTACTCTC TCACTAATCA
CMW 10531                       CATGAACGTC TACTTCAACG AGGTACTCTC TCACTAATCA
B.lutea CMW9076                 CATGAACGTC TACTTCAACG AGGTACTCTC TCACTAATCG
B.lutea CMW992                  CATGAACGTC TACTTCAACG AGGTACTCTC TCACTAATCG
B.dothidea CMW991               CATGAACGTC TATTTCAACG AGGTACTCTC TCACTAATTA
B.dothidea CMW7780              CATGAACGTC TATTTCAACG AGGTACTCTC TCACTAATTA
B.obtusa CMW7774                CATGAACGTC TACTTCAACG AGGTACTCTC T-ACTAGTTA
B.obtusa CMW7775                CATGAACGTC TACTTCAACG AGGTACTCTC T-ACTAGTTA
B.rhodina CMW10130              CATGAACGTC TACTTCAACG AGGTACTCTC TCCATAATTA
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                                  810        820        830        840
B.ribis CMW7772                   CACAAACACG TAAAGTATGG CAATCTTCTG AACGCGCAGC
B.ribis CMW7054                   CACAAACACG TAAAGTATGG CAATCTTCTG AACGCGCAGC
CMW 10532                         CACAAACACG TAAAGTATGG CAATCTTCTG AACGCGCAGC
CMW 10549                         CACAAACACG TAAAGTATGG CAATCTTCTG AACGCGCAGC
B.parva CMW994                    CACAAACACG TAAAGTATGG CAATCTTCTG AACGCGCAGC
B.parva CMW9071                   CACAAACACG TAAAGTATGG CAATCTTCTG AACGCGCAGC
B.eucalyptorum CMW10125           CACAAACACA TAAAGTATGG CAATCTTCTG AACGCGCAGC
B.eucalyptorum CMW10126           CACAAACACA TAAAGTATGG CAATCTTCTG AACGCGCAGC
CMW 10541                         CACAAACACA TAAAGTATGG CAATCTTCTG AACGCGCAGC
CMW 10542                         CACAAACACA TAAAGTATGG CAATCTTCTG AACGCGCAGC
CMW 10530                         CACAAACACG TAAAGTATGG CAATCTTCTG AACGCGCAGC
CMW 10531                         CACAAACACG TAAAGTATGG CAATCTTCTG AACGCGCAGC
B.lutea CMW9076                   CACGAACACG TAAAGTATGG CAATCTTCTG AACGCGCAGC
B.lutea CMW992                    CACGAACACG TAAAGTATGG CAATCTTCTG AACGCGCAGC
B.dothidea CMW991                 GACAAACACG TAAAGTATGG CAATCTTCTG AACGCGCAGC
B.dothidea CMW7780                GACAAACACG TAAAGTATGG CAATCTTCTG AACGCGCAGC
B.obtusa CMW7774                  GACAAACACG TAAAGTATGG CAATCTTCTG AACGCGCAGC
B.obtusa CMW7775                  GACAAACACG TAAAGTATGG CAATCTTCTG AACGCGCAGC
B.rhodina CMW10130                GACAAACACG TAAAGTATGG CAATCTTCTG AACGCGCAGC

                                  850        860        870        880
B.ribis CMW7772                   AGGCGTCCAA CAACAAGTAC GTTCCTCGTG CCGTCCTCGT
B.ribis CMW7054                   AGGCGTCCAA CAACAAGTAC GTTCCTCGTG CCGTCCTCGT
CMW 10532                         AGGCGTCCAA CAACAAGTAC GTTCCTCGTG CCGTCCTCGT
CMW 10549                         AGGCGTCCAA CAACAAGTAC GTTCCTCGTG CCGTCCTCGT
B.parva CMW994                    AGGCGTCCAA CAACAAGTAC GTTCCTCGTG CCGTCCTCGT
B.parva CMW9071                   AGGCGTCCAA CAACAAGTAC GTTCCTCGTG CCGTCCTCGT
B.eucalyptorum CMW10125           AGGCGTCCAA CAACAAGTAC GTTCCTCGTG CCGTCCTCGT
B.eucalyptorum CMW10126           AGGCGTCCAA CAACAAGTAC GTTCCTCGTG CCGTCCTCGT
CMW 10541                         AGGCGTCCAA CAACAAGTAC GTTCCTCGTG CCGTCCTCGT
CMW 10542                         AGGCGTCCAA CAACAAGTAC GTTCCTCGTG CCGTCCTCGT
CMW 10530                         AGGCGTCCAA CAACAAATAC GTTCCTCGTG CCGTCCTCGT
CMW 10531                         AGGCGTCCAA CAACAAATAC GTTCCTCGTG CCGTCCTCGT
B.lutea CMW9076                   AGGCGTCGAA CAACAAGTAC GTTCCTCGTG CCGTCCTCGT
B.lutea CMW992                    AGGCGTCGAA CAACAAGTAC GTTCCTCGTG CCGTCCTCGT
B.dothidea CMW991                 AGGCGTCCAA CAACAAGTAC GTTCCTCGTG CCGTCCTCGT
B.dothidea CMW7780                AGGCGTCCAA CAACAAGTAC GTTCCTCGTG CCGTCCTCGT
B.obtusa CMW7774                  AGGCATCCAA CAATAAGTAC GTTCCTCGTG CTGTCCTCGT
B.obtusa CMW7775                  AGGCATCCAA CAATAAGTAC GTTCCTCGTG CTGTCCTCGT
B.rhodina CMW10130                AGGCGTCCAA CAACAAGTAC GTTCCTCGTG CTGTCCTCGT
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                                890        900        910        920

B.ribis CMW7772                 CGACCTCGAG CCCGGCACCA TGGATGCCGT CCGCGCCGGC
B.ribis CMW7054                 CGACCTCGAG CCCGGCACCA TGGATGCCGT CCGCGCCGGC
CMW 10532                       CGACCTCGAG CCCGGCACCA TGGATGCCGT CCGCGCCGGC
CMW 10549                       CGACCTCGAG CCCGGCACCA TGGATGCCGT CCGCGCCGGC
B.parva CMW994                  CGACCTCGAG CCCGGCACCA TGGATGCCGT CCGCGCCGGC
B.parva CMW9071                 CGACCTCGAG CCCGGCACCA TGGATGCCGT CCGCGCCGGC
B.eucalyptorum CMW10125         TGACCTCGAG CCCGGCACCA TGGATGCCGT CCGCGCCGGC
B.eucalyptorum CMW10126         TGACCTCGAG CCCGGCACCA TGGATGCCGT CCGCGCCGGC
CMW 10541                       TGACCTCGAG CCCGGCACCA TGGATGCCGT CCGCGCCGGC
CMW 10542                       TGACCTCGAG CCCGGCACCA TGGATGCCGT CCGCGCCGGC
CMW 10530                       TGACCTCGAG CCTGGCACCA TGGATGCCGT CCGCGCCGGC
CMW 10531                       TGACCTCGAG CCTGGCACCA TGGATGCCGT CCGCGCCGGC
B.lutea CMW9076                 CGACCTCGAG CCCGGCACCA TGGATGCCGT CCGCGCCGGC
B.lutea CMW992                  CGACCTCGAG CCCGGCACCA TGGATGCCGT CCGCGCCGGC
B.dothidea CMW991               CGACCTCGAG CCCGGCACGA TGGATGCTGT CCGCGCCGGC
B.dothidea CMW7780              CGACCTCGAG CCCGGCACGA TGGATGCCGT CCGCGCCGGC
B.obtusa CMW7774                TGACCTCGAG CCCGGCACCA TGGATGCCGT CCGCGCCGGC
B.obtusa CMW7775                TGACCTCGAG CCCGGCACCA TGGATGCCGT CCGCGCCGGC
B.rhodina CMW10130              CGACCTCGAG CCCGGCACCA TGGATGCCGT CCGCGCCGGC

                                930        940        950        960

B.ribis CMW7772                 CCCTTCGGCC AGCTCTTCCG CCCTGACAAC TTCGTCTTCG
B.ribis CMW7054                 CCCTTCGGCC AGCTCTTCCG CCCTGACAAC TTCGTCTTCG
CMW 10532                       CCCTTCGGCC AGCTCTTCCG CCCTGACAAC TTCGTCTTCG
CMW 10549                       CCCTTCGGCC AGCTCTTCCG CCCTGACAAC TTCGTCTTCG
B.parva CMW994                  CCCTTCGGCC AGCTCTTCCG CCCTGACAAC TTCGTCTTCG
B.parva CMW9071                 CCCTTCGGCC AGCTCTTCCG CCCTGACAAC TTCGTCTTCG
B.eucalyptorum CMW10125         CCCTTCGGCC AGCTCTTCCG CCCCGACAAC TTTGTCTTCG
B.eucalyptorum CMW10126         CCCTTCGGCC AGCTCTTCCG CCCCGACAAC TTTGTCTTCG
CMW 10541                       CCCTTCGGCC AGCTCTTCCG CCCCGACAAC TTTGTCTTCG
CMW 10542                       CCCTTCGGCC AGCTCTTCCG CCCCGACAAC TTTGTCTTCG
CMW 10530                       CCCTTCGGCC AGCTCTTCCG CCCCGACAAC TTTGTCTTCG
CMW 10531                       CCCTTCGGCC AGCTCTTCCG CCCCGACAAC TTTGTCTTCG
B.lutea CMW9076                 CCCTTCGGCC AGCTCTTCCG CCCCGACAAC TTTGTCTTCG
B.lutea CMW992                  CCCTTCGGCC AGCTCTTCCG CCCCGACAAC TTTGTCTTCG
B.dothidea CMW991               CCCTTCGGCC AGCTTTTCCG CCCCGACAAC TTCGTCTTCG
B.dothidea CMW7780              CCCTTCGGCC AGCTTTTCCG CCCCGACAAC TTCGTCTTCG
B.obtusa CMW7774                CCCTTCGGCC AGCTCTTCCG TCCCGACAAC TTCGTTTTCG
B.obtusa CMW7775                CCCTTCGGCC AGCTCTTCCG TCCCGACAAC TTCGTTTTCG
B.rhodina CMW10130              CCCTTCGGCC AGCTCTTCCG CCCCGACAAC TTCGTCTTCG
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                                970       980    985
B.ribis CMW7772                 GTCAGTCTGG CGCCGGTAAC AACTG
B.ribis CMW7054                 GTCAGTCTGG CGCCGGTAAC AACTG
CMW 10532                       GTCAGTCTGG CGCCGGTAAC AACTG
CMW 10549                       GTCAGNNNNN NNNNNNNNNN NNNNN
B.parva CMW994                  GTCAGTCTGG CGCCGGTAAC AACTG
B.parva CMW9071                 GTCAGNNNNN NNNNNNNNNN NNNNN
B.eucalyptorum CMW10125         GCCAGTCTGG TGCCGGTAAC AATTG
B.eucalyptorum CMW10126         GCCAGTCTGG TGCCGGTAAC AATTG
CMW 10541                       GCCAGTCTGG TGCCGGTAAC AATTG
CMW 10542                       GCCAGTCTGG TGCCGGTAAC AATTG
CMW 10530                       GCCAGTCTGG TGCCGGTAAC AATTG
CMW 10531                       GCCAGTCTGG TGCCGGTAAC AATTG
B.lutea CMW9076                 GCCAGTCTGG TGCCGGTAAC AACTG
B.lutea CMW992                  GCCAGTCTGG TGCCGGTAAC AACTG
B.dothidea CMW991               GTCAGTCCGG TGCCGGTANN NNNNN
B.dothidea CMW7780              GTCAGTCCGG TGCCGGTAAC AACTG
B.obtusa CMW7774                GCCAGTCTGG TGCCGGTAAC AACTG
B.obtusa CMW7775                GCCAGTCTGG TGCCGGTAAC AACTG
B.rhodina CMW10130              GCCAGTCTGG TGCCGGTAAC AACTG
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# SUMMARY

*Eucalyptus* spp. represent the second largest commercial plantation species in Chile. Despite this fact, very little is known regarding diseases of these trees in the country. The research presented in this thesis is focused on two groups of pathogens. These include, *Mycosphaerella* spp. that are the cause of severe damage to leaves and *Botryosphaeria* spp. which infect shoots and stems of *Eucalyptus globulus* and *E. nitens* in Chile. The aim of the study has also been to expand the base of knowledge pertaining to *Eucalyptus* diseases and to utilise contemporary techniques to identify pathogens.

The first chapter of this thesis presents a general overview of *Pinus* and *Eucalyptus* plantation forestry in Chile, with special attention being given to the influence of pests and diseases. This review has shown that most of the existing research has been conducted on *P. radiata* plantations, where a relatively solid base of information exists. In contrast, it is clear that there is a lack of information regarding *Eucalyptus* diseases. Most of the taxonomy used to identify pathogens in Chile has been based on morphological characters. Phylogenetic analyses have generally not been utilised, prior to the studies presented in this thesis. Outcomes of this thesis also emphasise the need, not only to understand the importance of tree pathogens, but also the need to publish data pertaining to pests and diseases affecting commercial plantations in Chile. In this way, forestry research in the country will be more extensively exposed to the research groups elsewhere in the world and opportunities for collaboration will be increased.

In the second chapter of this thesis, it was possible to provide important information pertaining to the identification of *Mycosphaerella* spp. associated with leaf disease of *E. globulus* and *E. nitens* in Chile. Thus, six *Mycosphaerella* spp. were shown to be associated with *Eucalyptus* leaf disease in the plantations that were surveyed. These species include *M. africana, M. grandis, M. lateralis, M. molleriana, M. parkii* and *M. walkeri*, of which only *M. walkeri* has been reported in Chile previously. *Mycosphaerella grandis* appeared to be the most predominant species, which was present in northern and southern-central Chile, on *E. globulus* and *E. nitens* and also in juvenile and adult leaves. This species has also been reported as a cause of serious defoliation in Australia. *Mycosphaerella africana, M. lateralis, M. molleriana, M. parkii* and *M. walkeri* are

apparently not associated with the severe defoliation that has been observed during the last two years in Chile. However, to confirm this, it will be important to conduct further investigations and disease surveys. Previous suggestions that the important pathogens, *M. cryptica* and *M. nubilosa* are present in Chile have not been confirmed. This is encouranging, considering that these are two of the most pathogenic *Mycosphaerella* species on *Eucalyptus*. Knowledge resulting from this study will now be used to develop effective management strategies to reduce the impact caused by *Mycosphaerella* on *Eucalyptus* forestry in Chile.

The third chapter of this thesis deals with disease known as Botryosphaeria dieback. This disease appears to be increasing in importance in Chile and the species of *Botryosphaeria* associated with the disease have, as yet, not been identified. Results of this study using conventional morphological techniques and analysis of DNA sequences have shown that three *Botryosphaeria* species are present in the surveyed, which included *E. globulus* and *E. nitens* plantations in northern and southern-central Chile. These species include *B. parva*, *B. eucalyptorum* and an undescribed species (*B. sp.*). Previous reports based on morphology, identifying *B. dothidea* were probably incorrect. The three species identified in this study all represent new records for Chile. Pathogenicity trials conducted in a greenhouse showed that all of the species are pathogenic, but *B. parva* appeared to be the most virulent and more so than *B. eucalyptorum*. It will be important, in the future to re-evaluate the pathogenicity of those species on mature *E. globulus* and *E. nitens* in Chile. Even though, *Botryosphaeria* spp. are considered as opportunistic pathogens, it is important to carefully select plantation sites to reduce potential stress that may be placed on the trees. It will also be important in the future to select resistant planting stock that is able to withstand infections by *Botryospaheria* spp.

The knowledge generated from studies that make up this thesis will certainly be an important step in the process of developing a forest pathology program in Chile. New techniques for the identification of tree pathogens have been acquired and it is hoped that these can be further applied in the Chilean forestry situation in the future. Little attention was given to control of the pathogens studied in this thesis and this will be an important step in the future. In conclusion, it is hoped that the studies in this thesis will stimulate further research on *Eucalyptus* diseases in Chile and promote the field of forest pathology in the country.