




Chapter 1

A white outline map of Venezuela is centered on a grey background. The map shows the country's borders and internal state boundaries.

**The taxonomy and pathology of
Botryosphaeria spp., with special
reference to their relevance in *Eucalyptus*
plantations of Venezuela**

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INTRODUCTION

Fungi in the genus *Botryosphaeria* (Loculoascomycetes, Dothideales, Botryosphaeriaceae) (Barr, 1987; Hawksworth *et al.*, 2001) have a cosmopolitan distribution. They occur on a wide range of monocotyledonous, dicotyledonous and gymnospermous hosts, on woody twigs and branches, herbaceous leaves, stems of grasses, and in lichen thalli (Barr, 1987). *Botryosphaeria* spp. result in many different symptoms such as shoot blights, stem cankers, fruit rots, die-back and gummosis (Ciesla *et al.*, 1996; Old, 2000; Old & Davison, 2000).

Very little is known regarding the *Botryosphaeria* spp. in Venezuela. The anamorphs of *Botryosphaeria* spp. have been reported to include *Lasiodiplodia theobromae* (Pat.) Griffon & Maublanc., *Diplodia pinea* (Desm.) Kickx (= *Sphaeropsis sapinea* (Fr.) Dyko & Sutton), *D. mutila* Fr. apud Mont., and a species of *Dothiorella* Sacc. (Cedeño & Palacios-Pru, 1992; Cedeño *et al.*, 1994, 1995, 1996; Mohali, 1993, 1997; Mohali & Encinas, 2001; Mohali *et al.*, 2002; De Wet *et al.*, 2003). Identifications of these fungi, originating from disease symptoms on both agricultural crops and forest trees, have been based on conidial morphology. In recent years there have, however, been significant advances in the identification of *Botryosphaeria* spp. through combination of morphological and DNA-based techniques (Jacobs & Rehner,

1998; Denman *et al.*, 2000; Smith *et al.*, 2001a; Smith & Stanosz, 2001; Zhou & Stanosz, 2001a; Slippers *et al.* 2004b). These studies are given rise to a relatively robust taxonomy for *Botryosphaeria* and this is rapidly leading to a deeper understanding of host pathogen relationships and geographic distribution of species. This new knowledge has not been applied to *Botryosphaeria* in Venezuela.

Botryosphaeria species from Australian woody plants such as *Acacia* spp., *Eucalyptus* spp. and Proteaceae, have been relatively well studied because the plants have been used in commercial plantations and orchards world-wide (Ciesla *et al.*, 1996; Old, 2000; Old & Davison, 2000; Wingfield, *et al.*, 2001a, b; Denman *et al.*, 2003, Slippers *et al.* 2004c, d). These studies have shown that the establishment of such exotic plantations is followed by the introduction of pathogens such as *Botryosphaeria* spp. on infected planting stock and seeds (Ciesla *et al.*, 1996; Old, 2000; Old & Davison, 2000; Wingfield, *et al.*, 2001a, b; Denman *et al.*, 2003, Slippers *et al.* 2004c, d). These exotic plantations are also affected by pathogens that are native to the regions where these trees are planted. Both natural and introduced pathogens are thus a potential threat to the productivity of these plantations, as well as the native plants that surround them. This is very likely also true for Venezuela, but has not been studied to date.

Large-scale clonal plantations of *Eucalyptus* have been established in Venezuela for the production of wood and fiber. The *Eucalyptus* clones are selected for fast growth and desirable wood properties. The selection and need for uniformity in plantations results in lowered genetic variation. Such low genetic diversity in the host increases the risk of potential pathogens, such as *Botryosphaeria* spp., especially when the eucalypt trees are growing under stress.

Botryosphaeria spp. occur in Venezuela on various hosts on which they are thought to be important pathogens. Basic knowledge regarding many of these fungi is.

however, lacking in many cases, as illustrated above. This review, therefore, aims to provide a framework and foundation for the characterisation, risk assessment and attempts at control of *Botryosphaeria* spp. in Venezuela.

HIGHER CLASSIFICATION AND TELEOMORPH TAXONOMY

The genus of *Botryosphaeria* was established in 1863 by Cesati and De Notaris to accommodate bitunicate Ascomycetes with eight, aseptate, hyaline ascospores. Before this time, these taxa were placed under the group name *Sphaeria* (Fries 1823). Cesati and De Notaris (1863) identified 12 species at the time, including *B. dothidea*. More species were soon added, starting with four species, including *B. berengeriana* De Not added by De Notaris (1863). Today, more than 200 species have been described in *Botryosphaeria* (Index Fungorum; <http://www.indexfungorum.org>).

The type species for *Botryosphaeria* was not selected by Cesati and De Notaris (1863). Barr (1972) rejected propositions that either *B. quercuum* (Schwein.) Sacc. or *B. berengeriana* be designated as lectotype species for the genus because these species were not part of the original description. Therefore, Barr (1972) designated *B. dothidea* (Moug.:Fr.) Ces. & De Not. (= *Sphaeria dothidea* Moug.:Fr.), one of the original species included by Cesati and De Notaris (1863), as the lectotype of the genus.

The higher classification of *Botryosphaeria* and the related genus *Guignardia* has been controversial. Arx and Müller (1954) regarded both *Botryosphaeria* and *Guignardia* as genera of the family Botryosphaeriaceae. Petrak (1957) studied *Guignardia* and concluded that it should be merged with *Botryosphaeria*. Barr (1970), however, argued that *Botryosphaeria* and *Guignardia* are amerosporous representatives of the families Dothioraceae and Dothideaceae, respectively. Luttrell (1973) classified

Botryosphaeria in the order Pleosporales and *Guignardia* in the order Dothideales. Soon afterwards, however, Arx and Müller (1975) placed the two genera in the order Dothideales, family Botryosphaeriaceae. Sivanesan (1984) followed the same classification, but placed *Botryosphaeria* in the family Dothideaceae. Barr (1987) again placed *Botryosphaeria* in the order Pleosporales. The currently accepted classification, however, considers *Botryosphaeria* as member of the family Botryosphaeriaceae in the order Dothideales and *Guignardia* in the family Mycosphaerellaceae in the order Mycosphaerellales (Hawksworth *et al.*, 2001).

Despite the fact that the genus has been known for more than 140 years, the taxonomy of *Botryosphaeria* species is still problematic for several reasons. Teleomorphs are uncommonly encountered in nature and are difficult to induce in culture (Laundon, 1973; Jacobs & Rehner, 1998). Furthermore, there is often not sufficient diversity of teleomorph morphology to allow differentiation at the species level. There are also differences between young developing ascospores and older released ascospores, which often complicate comparisons between collections (Laundon, 1973). Despite recent clarifications of the taxonomy of key species, the majority of species in the genus are in need of revision using modern taxonomic methods.

ANAMORPH TAXONOMY

Botryosphaeria species have coelomycetous anamorphs (Sutton, 1980; Barr, 1987). Identification of *Botryosphaeria* spp. is often dependent on its anamorphs. These forms are more commonly encountered in nature and in culture and the morphological features are also more variable and useful for species identification (Sutton, 1980; Pennycook &

Samuels, 1985; Hanlin, 1990; Jacobs & Rehner, 1998). The anamorph characters most frequently used are conidial size, colour, septation, wall thickness and texture, as well as the presence of microconidia, and mode of conidiogenesis (Sutton, 1980; Sivanesan, 1984; Pennycook & Samuels, 1985; Denman *et al.*, 2000; Smith *et al.*, 2001b; Zhou and Stanosz, 2001a). The use of the conidial characters can also be problematic because the conidial size varies within species and on different hosts, or may overlap between species and change with age (Butin, 1993; Jacobs & Rehner, 1998; Sutton, 1980; Sivanesan, 1984; Pennycook & Samuels, 1985; Slippers *et al.*, 2004b).

Sutton (1980) used variations in the pycnidia and conidiogenesis to separate *Botryosphaeria* anamorph genera. Denman *et al.* (2000) studied the taxonomy of the most common *Botryosphaeria* anamorphs identified at the time, which amounted to 22 (even more have since been recognised). Of these, the most commonly used were *Fusicoccum* Corda, *Diplodia* Fr., *Macrophoma* (Sacc.) Berl & Vogl., *Dothiorella* Sacc., *Lasiodiplodia* Ellis & Everh., *Sphaeropsis* Sacc. and *Botryodiplodia* Sacc. Denman *et al.* (2000) separated these *Botryosphaeria* anamorphs into two groups based on conidial colour, shape and wall texture. One group has hyaline, fusoid and thin-walled conidia that might become translucent brown and septate prior to germination. These taxa are typical of *Fusicoccum*. The other group has hyaline to dark-conidia, most often ellipsoidal, 0-1 euseptate and opaque brown when mature. They can have prominent melanin deposits on the insides of the conidial walls giving the impression of striations, or the conidial walls can be smooth and thick (sometimes glassy). These taxa are considered representative of *Diplodia* (including *Lasiodiplodia*).

Zhou and Stanosz (2001a) studied the relationships of 52 *Botryosphaeria* isolates and their associated anamorphic fungi based on conidial morphology. They too identified two sections amongst the anamorphs. The first group, section *Hyala*, included

B. corticis, *B. dothidea*, *B. mamane*, *B. parva*, and *B. ribis* (all with known *Fusicoccum* anamorphs), and anamorph species *Fusicoccum luteum*. The conidia in this group were considered to usually be hyaline, but to become light brown when old or before germination. Widths of the conidia usually less than 10 μm . This section corresponds to the *Fusicoccum* group of Denman *et al.* (2000). The second group was named as section *Brunnea*, and included *B. obtusa*, *B. quercuum*, *B. rhodina*, *B. stevensii*, and *B. tsugae* (having *Diplodia*, *Lasiodiplodia* or *Sphaeropsis* anamorphs), *Diplodia pinea* f. sp. *cupressi*, and both the A and B groups of *Diplodia pinea*. The mature conidia were described as usually light to dark brown, but could be hyaline when young, and widths of conidia were usually greater than 10 μm . This section corresponds to the *Diplodia* group of Denman *et al.* (2000).

Alves *et al.* (2004) highlighted the fact that some *Botryosphaeria* species accommodated in *Diplodia*-anamorphs have mostly hyaline conidia such as: *B. corticola*, *B. stevensii* and *B. quercuum*. Therefore, they considered the distinction between the two sections developed by Zhou and Stanosz (2001a) on the basis of conidium coloration as tenuous. The name *Diplodia* should thus not be restricted to anamorphs with brown conidia, but also apply to some species with hyaline conidia, which also was reflected by Denman *et al.* (2000). Alves *et al.* (2004) pointed out that there was a clearer distinction between *Fusicoccum* and *Diplodia* in the widths of the conidia and cell wall thickness. *Fusicoccum* is generally <10 μm wide and thin walled, while *Diplodia* is generally >10 μm wide and thicker walled.

Today there are three genera validly recognized and used for anamorphs of *Botryosphaeria*. These are *Fusicoccum* with hyaline and narrow conidia; *Diplodia* and *Lasiodiplodia* with dark and broad conidia, becoming longitudinally striate in the latter

genus (Denman *et al.*, 2000; Zhou & Stanosz, 2001a; Phillips *et al.*, 2002; Alves, *et al.*, 2004).

***Fusicoccum* Corda.**

Petrak (1922) placed the type species of *Fusicoccum*, called *F. aesculi* Corda by Saccardo (1880, 1884, 1886), in the genus *Dothiorella*. Sutton (1980), however, considered the material described by Saccardo (*F. aesculi*) as best accommodated in the genus *Fusicoccum*, including the anamorphs of ascomycetes such as *B. dothidea* and *B. ribis*. Sutton (1980) described *Fusicoccum* as a Coelomycetes group that has hyaline, aseptate, straight conidia, with obtuse apices and truncate bases (fusiform). Pennycook and Samuels (1985) described two new *Fusicoccum* species, *F. parvum* and *F. luteum*, and they distinguished these two species from *F. aesculi* based on conidial size and pigmentation, as well as culture morphology. They thereby showed that *Fusicoccum* conidia can also be pigmented. Crous and Palm (1999) and Denman *et al.* (2000) supported the observations of Sutton (1980) and Pennycook and Samuels (1985). They, however, added other characteristics which could be used to identify the species in this genus such as the mode of proliferation of the conidiogenous cell for example, proliferation at the same level resulting in periclinal thickening, or percurrently resulting in annellations.

The 180 *Fusicoccum* species listed in Index fungorum might be misleading. Denman *et al.* (2000) and Slippers *et al.* (2005) suggested that many species previously described under *Dothiorella* are likely to be *Fusicoccum* species. There are more than 350 species of *Dothiorella* (Index fungorum: <http://www.indexfungorum.org>). Similarly, a number of species of *Phyllosticta* have recently been transferred to

Fusicoccum (Van der Aa & Vanev, 2002). Furthermore, recent studies have shown that some well recognized *Fusicoccum* species might contain cryptic species (Slippers *et al.*, 2004b, c, d). There are thus likely to be many more *Fusicoccum* spp. than those currently described and this genus, as well as the allied genera, are clearly in need of revision.

***Diplodia* Fr. (= *Sphaeropsis* Sacc.)**

According to Sutton (1980), the original generic description of *Diplodia* was compiled by Fries in 1834, and identified as *Diplodia mutila* Fr. The teleomorph of *D. mutila* was discovered by Stevens (1936) who cited it as *Physalospora mutila* (Fr.) N. E. Stevens. Shoemaker (1964) renamed *P. mutila* as *Botryosphaeria stevensii* Shoem. Sutton (1980) described the genus as having pycnidial, unilocular conidiomata, with a central ostiole; conidiophores are absent; proliferation is percurrent in conidiogenous cells; conidia are oblong to clavate, straight, aseptate (but developing eusepta prior to germination). The apexes obtuse and tapered to a truncate base.

Recent treatments have shown that the name *Diplodia* should preside over names such as *Sphaeropsis* (Denman *et al.*, 2000; De Wet *et al.*, 2003). Denman *et al.* (2000) and De Wet *et al.* (2003) considered the argument of percurrent proliferation and time of septation of *S. sapinea* as insufficient to separate it from the genus *Diplodia*. They suggested, therefore, that the older name *Diplodia pinea* be used for *Sphaeropsis sapinea* and also described a new cryptic species from isolates of this latter taxon as *D. scrobiculata* De Wet, Slippers & Wingfield (De Wet *et al.*, 2003).

Diplodia is the largest of the anamorph genera of *Botryosphaeria*, with more than 1200 species listed in Index Fungorum (<http://www.indexfungorum.org>). Many of

these species are, however, likely to be synonyms, while others will refer to species complexes. Like the other *Botryosphaeria* anamorph genera, *Diplodia* is in need of revision.

***Lasiodiplodia* Ellis & Everh.**

Lasiodiplodia theobromae, is a common plant pathogen in the tropics where it is often reported as *Botryodiplodia theobromae*. *Lasiodiplodia*, however, produces solitary pycnidia, which differentiates it from *Botryodiplodia*, which produces pycnidia in a valsoid stroma (Crous & Palm, 1999). The genus was first described by Ellis and Everhart in 1896 with *Lasiodiplodia tubericola* Ellis & Everh., as the type species (Taubenhaus, 1915). *Lasiodiplodia* spp. are characterized as having eustromatic, uni or multifocular conidiomata; conidiophores are absent; conidia are hyaline when young, later dark-brown and euseptate, and thick walled. Bases are truncate with longitudinal striations from apex to base (Sutton, 1980). Uduebo (1975) showed that the conidial wall ornamentation is made up by deposits of melanin on the inside of the wall, creating an illusion of striations on surfaces of conidia.

Lasiodiplodia theobromae (= *Botryodiplodia theobromae* Pat.) is the most commonly reported species (Punithalingam, 1976, 1980; Arx von, 1987). *Lasiodiplodia theobromae* has previously been reported as the anamorph of *Physalospora rhodina* Berk. & Curt. Apud Cooke (Punithalingam, 1980; Sutton, 1980), but now considered as the anamorph of *Botryosphaeria rhodina* (Cooke) Von Arx (Arx von, 1987).

There are 10 *Lasiodiplodia* species recorded in Index Fungorum (<http://www.indexfungorum.org/>). Some of these species are likely synonyms of *L. theobromae*. However, the recent description of a new species, *L. gonubiensis* Pavlic,

Slippers & M. J. Wingf. (Pavlic *et al.*, 2004), also shows that there might be more species to be identified on previously unstudied hosts and environments.

DNA-BASED CHARACTERIZATION

The identification of *Botryosphaeria* spp. and their anamorphs based on morphological characteristics requires an experienced researcher and large numbers of samples to compensate for variation within and between species. DNA based characters have been successful in resolving species level taxonomic questions in different groups of fungi (Berbee & Taylor, 1993). Many researchers have thus attempted to develop non-subjective molecular techniques to identify *Botryosphaeria* spp. RAPDs and isozymes have been used in *Botryosphaeria* taxonomy since the 1990's, especially with regard to *D. pinea* (= *S. sapinea*) morphotypes (Smith & Stanosz, 1995; Stanosz, *et al.*, 1999). Jacobs and Rehner (1998) were the first authors to combine DNA sequence data and morphological characters to consider interspecific relationships in *Botryosphaeria*. Since then, numerous other studies have combined morphological characters and different forms of DNA based data to characterize and study the phylogeny of *Botryosphaeria* (Jacobs & Rehner, 1998; Burgess *et al.* 2001b; Smith *et al.*, 2001a; Zhou & Stanosz, 2001a; Phillips *et al.*, 2002; Denman *et al.*, 2003; Alves *et al.*, 2004; Slippers *et al.*, 2004b and others).

Isozymes and Randomly Amplified Polymorphic DNA (RAPD) markers

Isozyme patterns and Randomly Amplified Polymorphic DNA (RAPD) markers have been useful tools to study the A and B morphotypes of *D. pinea* from different hosts and

localities (Smith & Stanosz, 1995; Stanosz *et al.*, 1996, 1999). With this technique the relationship between the *Botryosphaeria* spp. is determined using cluster analyses of presence or absence data for amplification fragments produced by RAPD markers and proteins extracted from different cultures (Smith & Stanosz, 1995; Stanosz *et al.*, 1999). The advantage of this technique is that it gives a genome-wide perspective.

Data from isozymes and RAPD's did not provide sufficient support to separate some closely related *Botryosphaeria* species, such as those in the *B. ribis*-*B. parva* and *B. lutea*-*B. australis* species complexes (Smith & Stanosz, 2001). In other cases these markers can also over-estimate diversity, such as in the four distinct groups in *D. pinea*, which were separated using RAPD's (Stanosz, *et al.*, 1996). The four groups were later shown to be only three *Botryosphaeria* species (*D. pinea*, *D. scrobiculata* and *B. obtusa*) (De Wet *et al.*, 2000).

Internal Simple Sequence Repeat (ISSR)

Zhou *et al.* (2001) worked with inter simple or short sequence repeats (ISSR) to differentiate *Botryosphaeria* species with very similar or identical ITS sequences and morphologies. The cluster analysis of the ISSR fingerprints among species of the light and narrow conidial-group (*Hyala*) indicated a close relationships between a group composed of *F. luteum*, *B. ribis* and *B. parva*; and another group composed of *B. mamane*, *B. corticis* and *B. dothidea*, respectively. However, fingerprint analysis also indicated that all these species are distinct. The results supported earlier work that separated *B. dothidea* and *B. ribis* (Zhou *et al.*, 2001), however, it also suggested the differentiation of *B. parva* and *B. ribis*, for which ITS sequences and RAPD marker analyses previously suggested were the same species (Zhou *et al.*, 2001). These results

have subsequently been confirmed with other techniques (see below). Thus, there is value in using ISSRs for species delimitation in *Botryosphaeria*, given that sufficient isolates are available for comparison.

Polymerase Chain Reaction - Restriction Fragment Length Polymorphism

(PCR-RFLPs) analysis

A method that has been commonly used in recent years to distinguish *Botryosphaeria* spp. is that based on RFLP profiles. This technique provides a quick and effective way to distinguish species and study their geographic distribution and population compositions (Slippers, 2003).

Jacobs (2002) developed an identification system for *Botryosphaeria* species from mango using sequence data of the ITS region of sequenced isolates. Slippers (2003) and Slippers *et al.* (2004c) also used RFLPs of the ITS region to distinguish *Botryosphaeria* spp. from fruit trees and *Eucalyptus* respectively. Pavlic (2004) used this technique to distinguish *Botryosphaeria* spp. from *Syzgium* trees in South Africa. The results of these studies allowed a quick and easy identification of some *Botryosphaeria* species, but could not distinguish between the cryptic species group's *B. parva*, *B. ribis* and *B. australis* (Jacobs, 2002; Slippers, 2003).

Slippers *et al.* (2004a) developed primers that amplified a microsatellite-containing region in both species *B. ribis* and *B. parva*. Restriction patterns for one of these unidentified DNA regions (Locus *BotF15*) have a unique restriction site for *B. ribis* and were thus used to distinguish *B. ribis* and *B. parva* (Slippers *et al.* 2004b). The isolates distinguished in this way are considered to represent *B. ribis sensu lato* and *B. parva sensu lato* (Slippers, 2003). This is because uncertainty remains as to whether the

variation within these groups represents speciation events or population variation within species as discussed by Slippers (2003).

Alves, *et al.* (2005) extended the ITS RFLP based techniques for distinguishing *Botryosphaeria* species, by also including the D1 and D2 regions of the LSU rDNA in the initial amplification. The method is termed amplification of the ribosomal DNA and restriction analysis (ARDRA), and is also used for taxonomic studies of other fungi (Guarro *et al.*, 1999). The technique was used to separate 35 strains into the respective 10 *Botryosphaeria* species. The results obtained demonstrated that the ARDRA technique is a useful tool for the identification of the major species in the genus *Botryosphaeria*, including some cryptic species such as *B. ribis* / *B. parva* and *B. sarmentorum* / *B. iberica* (Alves *et al.*, 2005; Phillips, *et al.*, 2005). The differentiation of these cryptic species was impossible by ARDRA analysis of the ITS region alone (Slippers *et al.*, 2004c).

Internal Transcribed Spacers (ITS) sequence data

Jacobs & Rehner (1998) considered nuclear rDNA ITS sequence analysis together with conidial characters and cultural studies to distinguish *Botryosphaeria* species. For 22 isolates, they found inconsistencies between the ITS clustering and traditional identifications. For example *B. dothidea* grouped in two clades, of which one was shared with *B. ribis*. This study showed the usefulness of DNA sequence data in identifying such problems and laid the foundation for future taxonomic studies.

Smith and Stanosz (2001) and Smith *et al.* (2001b) supported the separation of *B. ribis* from *B. dothidea* with data of ITS and 5.8 rDNA sequences. These studies indicated that the two species are distinct monophyletic groups, with *B. ribis* more

closely related to *B. parva* and *F. luteum*, and *B. dothidea* more closely related to *B. corticis* and *B. mamane*. This affirmation was supported with the addition of ITS sequences and conidial characterization of many more isolates of these taxa (Smith & Stanosz, 2001; Slippers *et al.*, 2004b), allowing clear separation of *B. ribis* and *B. dothidea*.

The use of internal transcribed spacers (ITS1 and ITS2) has been successfully employed to separate *Botryosphaeria* isolates on several hosts. Examples of different hosts include *Vitis* spp., *Eucalyptus* spp., *Quercus* sp., *Pinus* spp., *Pistacia vera* and fruit trees (Ogata *et al.*, 2000; Smith *et al.*, 2001a; Phillips *et al.*, 2002; Alves *et al.*, 2004). PCR primers have been developed based on ITS sequences for identification of *Fusicoccum* sp. from pistachio and other host plants in California (Ma & Michailides, 2002).

Despite the fact that ITS sequence data supported the separation of some *Botryosphaeria* species, this single DNA locus can not resolve all species uncertainties. Like RAPD markers, closely related species such as *B. ribis* and *B. parva* can not be separated with confidence based on ITS data alone (Smith & Stanosz, 2001; Zhou & Stanosz, 2001a; Slippers *et al.*, 2004b).

Mitochondrial Small Subunit Ribosomal RNA (mt SSU rDNA)

Zhou and Stanosz (2001b), worked with mitochondrial small subunit ribosomal RNA (mt SSU rDNA) to differentiate *Botryosphaeria* species with very similar or identical ITS sequences and morphologies. This phylogenetic analysis of mt SSU rDNA sequences did not support the separation of the genera *Fusicoccum* and *Diplodia*, as was

Botryosphaeria spp. can also be involved in causing a specific canker disease on a host. For example, the fungal gummosis or gummosis cankers, characterized by numerous gum deposits on the trunk, limbs, and twigs of peach (*Prunus persica* (L.) Batsch) trees (Reilly & Okie, 1982), is caused by three *Botryosphaeria* species, namely *B. obtusa*, *B. dothidea* and *B. rhodina*. These species are indistinguishable on the basis of symptoms. They, however, differ in frequency with *B. obtusa* isolated more frequently than *B. dothidea* and *B. rhodina* (Britton & Hendrix, 1982, 1986; Britton *et al.*, 1990).

Apart from *Prunus* mentioned above, *Botryosphaeria* spp. are important for causing canker diseases in various other agricultural crop trees or woody shrubs. *Botryosphaeria dothidea* and *B. obtusa* cause major diseases of apple in Georgia (Britton & Hendrix, 1986). Both *B. dothidea* and *B. obtusa* produce cankers on apple that can result in decline of entire trees. In Chile, cankers on the trunks of Red King Oregon apple trees are caused by *B. dothidea* (Latorre & Toledo, 1984). *Lasiodiplodia theobromae* is associated with tan lesions of the inner wood, copious gumming, and a consistent association with freeze-damage tissue, which was observed on young citrus trees (Davis *et al.*, 1987; Sangchote, 1991). *Botryosphaeria corticis* causes cankers on different varieties of blueberry (Clayton & Fox, 1963; Milholland & Galletta, 1969; Milholland, 1984).

Botryosphaeria spp. are important canker pathogens of forestry trees, such as *Eucalyptus*. The most common symptoms on *Eucalyptus* are the presence of cankers on stems and branches and resultant dieback (death of tree tops). The cankers are characterized by swelling of the stems or lateral branches. Usually the bark cracks around the lesions and copious amounts of black reddish resin (excretion of polyphenols) are produced (Ciesla, *et al.*, 1996; Old, 2000; Old & Davison, 2000).

Other symptoms

Apart from cankers, *Botryosphaeria* spp. also produces various other symptoms such as sapwood (blue stain), fruits, leaves, stems, twigs and roots of woody plants. Some of these are as follows:

Fruits - *Botryosphaeria obtusa* causes white and black rot of apple fruit, while *B. dothidea* causes ring rot on immature and mature fruits (Brown & Britton, 1986; Brown-Rytlewski & McManus, 2000; Ogata *et al.*, 2000). *Lasiodiplodia theobromae* is associated with stem end rot disease of mango (Davis *et al.*, 1987; Sangchote, 1991). Ripe fruit rot of *Actinidia deliciosa* (kiwifruit) is caused by *F. aesculi*, *F. parvum* and *F. luteum* (Pennycook & Samuels, 1985). *Dothiorella aromatica* (which possibly represents *F. aesculi* or *F. luteum*) is associated with symptoms of fruit rot on avocado (Darvas & Kotze, 1987).

Die-backs and other diseases on twigs, branches and stems - A complex of *B. ribis*, *F. luteum* and other fungi with *Fusicoccum* anamorphs cause panicle and shoot blight on California pistachio (Smith *et al.*, 2001b; Ntahimpera *et al.*, 2002; Ma & Michailides, 2002). Plants of high-bush blueberry with stem blight in North Carolina was reportedly caused by *B. dothidea* (Witcher & Clayton, 1963). *Botryosphaeria populi* (synonym of *B. dothidea*) was found on dead branches of *Populus nigra* L. (Phillips, 2000). Dieback on branches of macadamia (*Macadamia integrifolia* and *M. tetraphylla*) is induced by *B. ribis* (Herbert & Grech, 1985).

Leaves - *Botryosphaeria pipturi* causes leaf spot on the endemic understory species *Pipturus hawaiiensis* Levl. (Gardner & Hodges, 1998). Leaf blight on coconut palms in Brazil is attributed to *B. cocogena* (Subileau *et al.*, 1994). In South Africa, *B. ribis* occurs in lesions on *Eucalyptus camaldulensis*, *E. cladocalyx*, *E. grandis*, *E. globulus* and *E. nitens* leaves (Crous *et al.*, 1989). Likewise, *Botryosphaeria ribis* has been reported from leaf spots on eucalypts in Spain (Ruperez & Munoz, 1980).

Uncommonly reported diseases - *Botryosphaeria ribis* has been reported causing seed capsule abortion and twig dieback on *E. camaldulensis* in Florida (Barnard *et al.*, 1987; Webb, 1983). Root rot on *Eucalyptus* sp. is reportedly caused by *B. ribis* in Argentina (Frezzi, 1952). Uncommon branch contortions, swellings, witches-broom, and eventual death of tissue are associated with *B. mamane* on the leguminous forest species *Sophora chrysophylla* in Hawaii (Gardner, 1997).

BOTRYOSPHAERIA AS AN ENDOPHYTIC FUNGUS

Endophytic fungi are able to colonise healthy plant tissue without exhibiting virulence, thus not causing obvious damage at the time of infection (Carroll, 1990; McCutcheon *et al.*, 1993). Latent pathogens also share an endophytic relationship with their hosts, causing quiescent infections for long periods of time and symptoms appear only when the physiological or ecological conditions favour virulence (Tokuna & Ohira, 1973; Pusey, 1989; Bettucci & Saravay, 1993; Smith *et al.*, 1996a, b).

Species of *Botryosphaeria* can be endophytic in *Eucalyptus* tissue (Fisher *et al.*, 1993; Smith *et al.*, 1996a), as well as pathogenic in stressed trees (Pusey, 1989; Old *et al.*, 1990). In South Africa, potentially pathogenic *Botryosphaeria* spp. (e.g. *B. dothidea*

and *B. parva*) have been shown to occur as symptomless endophytic infections on leaves and in xylem of *Eucalyptus* (Smith *et al.*, 1996b). The presence of *Fusicoccum eucalypti* in healthy (endophytic fungus) and symptomatic tissue of twigs of *E. grandis* was reported from Uruguay (Bettucci & Alonso, 1997; Bettucci *et al.*, 1999).

OCCURRENCE OF *BOTRYOSPHAERIA* ON *EUCALYPTUS*

Various *Botryosphaeria* spp. have been associated with *Eucalyptus* cankers in Australia where these trees are native. In species selection trials of *E. radiata*, *B. ribis* was reported to cause dark brown discoloration of the phloem with extensive kino veins often formed ahead of phloem necrosis and light purplish brown discoloration of the xylem (Davison & Tay, 1983; Shearer *et al.*, 1987). *Botryosphaeria dothidea* and *B. ribis* were isolated from the bark of trees and stumps of *E. marginata*, causing basal canker and die-back in Australia (Davison & Tay, 1983). Slippers *et al.* (2004c) identified five *Botryosphaeria* spp. (*B. parva*, *B. dothidea*, *B. eucalyptorum*, *B. australis* and *B. eucalypticola*) from *Eucalyptus* in native forest and plantations in eastern Australia. *Botryosphaeria eucalyptorum* was the most abundant, and represented almost 50% of isolates in this last study.

Different *Botryosphaeria* spp. have been associated with *Eucalyptus* in the different countries where these trees have been introduced. A survey of the most important forestry areas of South Africa showed several eucalypt species and clones with die-back and canker associated with extreme environmental conditions, and where *B. dothidea* was consistently isolated from the trees (Smith *et al.*, 1994). *Botryosphaeria ribis* was also reported from leaves of eucalypts in South Africa (Crous *et al.*, 1989). Later studies by Smith *et al.* (2001a) and Slippers *et al.* (2004c), however,

showed that *B. parva*, *B. eucalyptorum* and *B. eucalypticola* are the dominant species on *Eucalyptus* in this country. In other regions of Africa, such as the Congo and Uganda, *Lasiodiplodia theobromae* (teleomorph = *B. rhodina*) has been reported as an important threat to the eucalypt plantations, causing stem cankers with copious exudation of resin and xylem discoloration (Roux *et al.*, 2000, 2001).

In South America several *Botryosphaeria* spp. are known to occur on *Eucalyptus*. For example, *B. ribis* is reported to cause symptoms such as stick rot and canker on *E. grandis* and *E. citriodora* in Brazil (de Arruda Silveira, 2001) and cankers on stems and branches in Argentina (Frezzi, 1952). In Colombia, both *B. ribis* and *B. dothidea* were found to cause *Eucalyptus* diseases (Rodas, 2003). In Chile, however, *B. parva*, *B. eucalyptorum* and *B. eucalypticola* are the most dominant species associated with *Botryosphaeria* canker and die-back diseases on *Eucalyptus* (Ahumada, 2003).

Despite the fact that *Botryosphaeria* spp. are commonly associated with disease of *Eucalyptus*, these fungi are considered to be weak pathogens of this host. They cause disease mostly on wounded or stressed plants, following drought, hot or cold winds, nutritional imbalance, water logging, hail wounds, insect damage and damage by other pathogens (Smith *et al.*, 1994, 1996b). In Australia, the pathogenicity (based on their ability to cause stem lesions) of several fungal species was tested on 12-month-old seedlings of *E. nitens* and *E. globulus* (Yuan & Mohammed, 1999). The *Botryosphaeria* isolates used in that study were shown to be intermediately or weakly pathogenic.

OCCURRENCE OF *BOTRYOSPHAERIA* ON *PINUS*

Botryosphaeria spp. cause damage on different *Pinus* species. The *Botryosphaeria* species most commonly reported on *Pinus* spp. is *Diplodia pinea* (= *Sphaeropsis*

supinea). This pathogen causes extensive losses in commercial plantation forestry, especially where susceptible *Pinus* spp. are intensively propagated (Zwolinski *et al.*, 1990).

In Brasil, *Diplodia pinea* has been reported on *Pinus radiata* and *P. pinaster* from 3-4 year-old trees causing die-back, blue-stain and mortality (Ferreira, 1989). In South Africa the fungus is considered the most important pathogen of pines, causing serious annual losses due to dieback after hail on *Pinus radiata* and *P. patula* (Swart *et al.*, 1985, 1987; Zwolinski *et al.*, 1990). In addition, *P. radiata* is susceptible to drought, and are vulnerable to damage by *D. pinea* in New Zealand (Thomson, 1969) and Australia (Marks & Minko, 1969; Davison *et al.*, 1991)

D. pinea has been shown to be present as latent endophytic infections in cones, shoots, needles and from stems of pines seedlings (Smith *et al.*, 1996a; Stanosz *et al.*, 1997; Burgess *et al.*, 2001a), and it has also been found on various *Pinus* spp. producing various disease symptoms, of which die-back, cankers, root disease, crown wilt and a saprophytic inhabitant of sapwood (blue stain) are most common (Mohali, 1997; Swart *et al.*, 1985, 1987; De Wet *et al.*, 2000).

Botryosphaeria dothidea has been reported causing wilt and death of *Pinus taeda* and *P. elliottii* var. *elliottii* in Hawaii (Hodges, 1983). *Lasiodiplodia theobromae* has been associated with discolouration from seeds of *Pinus elliottii* in South Africa, which reduced the germination (Carneiro, 1986; Rees, 1988; Fraedrich & Miller, 1989). Tip die-back of *Pinus taeda* and *P. elliottii* seedlings and blue stain of *P. massoniana* has been attributed to *L. theobromae* (Rowan, 1982; Fu *et al.*, 1988).

***BOTRYOSPHAERIA* IN VENEZUELA**

One of the most serious problems caused by a *Botryosphaeria* spp. in Venezuela is blue stain of Caribbean pine (*Pinus caribaea* var. *hondurensis*). There are approximately 400.000 ha of this pine species planted in the country. Blue stain of the Caribbean pine timber is a problem for sawmills, especially in the eastern part of Venezuela. The blue stain produces changes in the natural colour of the wood, making it aesthetically undesirable for the carpentry and paper production industry. This alteration in wood colour also results in a reduction in the wood price of up to 50%, representing large economical losses to the forestry industry (Mohali, 1993).

Blue stain of Caribbean pine is caused by different species of *Botryosphaeria*, of which *L. theobromae* (teleomorph *B. rhodina*) is the most important. Recently it has been shown that *D. mutila* also causes blue stain on Caribbean pine logs (Mohali & Encinas, 2001). Furthermore, Mohali (1997) reported *D. pinea* (= *S. sapinea*) as the causal agent of chlorosis and needle fall, as well as blue stain of the stems on Caribbean pine, in Yaracuy state, Venezuela.

Botryosphaeria anamorphs have been reported on agricultural hosts in Venezuela. Brown rot disease was observed on harvested peach fruits and is consistently associated with *Dothiorella dothidea* (an invalid name, which might refer to a number of different *Fusicoccum* species) (Cedeño *et al.*, 1994). *Lasiodiplodia theobromae* was reported as the causal agent of the dieback on passion fruit vines, which caused a significant reduction in the production (Cedeño *et al.*, 1995).

OCCURRENCE OF *BOTRYOSPHAERIA* ON *ACACIA*

Very severe cankers have been reported by Pongpanich (1997) on *Acacia auriculiformis* associated with infection by *Botryosphaeria* spp. in a trial in Western Thailand, with 80 % mortality in some seed. A trial the same provenances (*A. auriculiformis*) from Thailand planted in South Kalimantan, Indonesia, was severely affected by a canker disease caused by *Lasiodiplodia theobromae* (Hadi & Nuhamara, 1997).

A plantation of 2 ha of single seed source of *Acacia aulacocarpa* at Sakaerat in eastern Thailand suffered about 40% mortality through combined attack by borers and infection by *Botryosphaeria* spp. (Pongpanich, 1997). In southern India, severe basal cankers in a small planting of *Acacia crassicarpa* in two locations were associated with infection by *L. theobromae* (Sharma & Florence, 1997).

During a 2-year period, a survey of diseases, pathogenicity tests on *Acacia mearnsii* were conducted in South Africa (Roux & Wingfield, 1997). *Diplodia* and *Botryosphaeria* species were frequently isolated from diseased tissue, besides producing noticeable lesions in inoculation tests (Roux & Wingfield, 1997).

Botryosphaeria rhodina and *B. dothidea* have both been reported from native Australian *Acacia* spp., where these trees are planted as exotics in South Africa (Roux, 1998). In contrast, isolations from the same species as natives Australia have yielded *Botryosphaeria australis*, as new specie which appears to be native to the Southern Hemisphere (Slippers *et al.*, 2004).

CONCLUSIONS

Botryosphaeria spp. are known as important pathogens of various crops in Venezuela. Identifications thus far have, however, only been based on morphological data. Those identifications are outdated with respect to modern taxonomic treatments of the group. The correct identification of such *Botryosphaeria* spp., including previously unrecognized species and cryptic species, must be the first step for future studies and eventual efforts to control them. Based on such information, questions regarding the role of different pathogens, distribution and movement of pathogens and host resistance to key pathogens, can be addressed. This is especially true for *Botryosphaeria* spp. that occur on *Eucalyptus*. The growing importance of this crop in Venezuela and the dangers of common pathogens such as *Botryosphaeria* to clonal forestry makes this important and urgent.

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