14 Cankers and Other Diseases Caused by the *Botryosphaeriaceae*

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14.1 Pathogens, Significance and Distribution

Species of the ascomycete family *Botryosphaeriaceae* are important pathogens of trees and other woody plants. They have been reported from more than 1000 plant hosts. These include commercially important trees utilized in agriculture and forestry as well as those in native forests. Various disease symptoms have been linked to infection by species in the family, including cankers, dieback and blight of individual plant parts or entire trees (Slippers and Wingfield, 2007) (Plate 18). Several publications have predicted the increasing importance of the *Botryosphaeriaceae* in the light of global climate change (Desprez-Loustau *et al*., 2007; Slippers and Wingfield, 2007; Thompson *et al*., 2010). Furthermore, recent research has linked species in the family to outbreaks of new diseases (Alvarez-Loayza *et al*., 2008; Dakin *et al*., 2010; Piškur *et al*., 2011) or to the re-emergence of known diseases (Giraud, 2009).

A cryptic and more recently recognized aspect of the life cycle of the *Botryosphaeriaceae* is their ability to infect host plants and to persist endophytically as latent pathogens. Before this discovery, it was thought that these fungi were systemic pathogens (Webb, 1983; Shearer *et al*., 1987). Since they were first recognized as tree pathogens, the *Botryosphaeriaceae* have been treated as wound-infecting opportunists, although it was also known that they were capable of infecting via natural openings (stomata, lenticels and others). The first evidence of endophytism in these fungi emerged when Petrini and Fisher (1988) isolated *Diplodia pinea* (Desm.) J. Kickx f. (syn. *Sphaeropsis sapinea* (Fr.) Dyko & B. Sutton) from healthy stems of Scots pine (*Pinus sylvestris* L.). Later, Fisher *et al*. (1993) isolated *Botryosphaeria dothidea* (Moug.) Ces. & De Not. from leaves, twig bark and asymptomatic xylem of shining gum (*Eucalyptus nitens* (H. Deane & Maiden) Maiden). Subsequent studies have confirmed that virtually every species in the family occupies a plant host endophytically for at least part of its life cycle (Slippers and Wingfield, 2007).

Several reviews addressing various aspects of the *Botryosphaeriaceae* have appeared in the past decade (Crous *et al*., 2006; Slippers and Wingfield, 2007; Phillips *et al*., 2008; Slippers *et al*., 2009). These have underscored the importance of the *Botryosphaeriaceae* as plant pathogens. Some of them have addressed the taxonomy of the family (Crous *et al*., 2006; Phillips *et al*., 2008) by clarifying the identity or resolving the taxonomy of individual species

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(de Wet *et al*., 2003; Slippers *et al*., 2004a; Phillips *et al*., 2006), while others have clarified host associations (Slippers *et al*., 2004b, 2007, 2009). However, the ecological role of most of these fungi remains poorly understood (Slippers and Wingfield, 2007).

At least 20 genera are classified in the family *Botryosphaeriaceae*. Molecular tools have enabled the systematic grouping of these genera within the last decade, and clarified many complex taxonomic questions. In many cases, the sexual state linked to an asexual genus remains undiscovered (e.g. *Endomelanconiopsis*, *Lasiodiplodia*, *Pseudofusicoccum*), while in other cases asexual states have been linked as synanamorphs or to sexual morphs (e.g. *Botryosphaeria* with *Fusicoccum*, *Phaeobotryosphaeria* with *Sphaeropsis* and *B. dothidea*, *Neofusicoccum* and *Neoscytalidium* with *Dichomera*) (Phillips, 2002; Slippers *et al*., 2004a; Phillips *et al*., 2005, 2008; Crous *et al*., 2006; Pavlic *et al*., 2008). Regardless of the taxonomic issues, most of these fungi are known pathogens or have been isolated from diseased plants.

14.2 Diagnosis

Numerous symptoms accompany infection by the *Botryosphaeriaceae*, and these typically become obvious once plants are subjected to stress. Disease symptoms have been comprehensively listed by Slippers and Wingfield (2007) and include: cankers and dieback of twigs, branches and the stem (Plate 18); root rot and cankers of the roots; blue-stain; blossom blights, fruit rots and seed capsule abortion; seedling damping-off and blight; and tree death. The diversity of *Botryosphaeriaceae* associated with most of these disease symptoms necessitates laboratory isolation and subsequent DNA-based diagnostics.

The association of one or more species of the *Botryosphaeriaceae* with disease symptoms typically commences with the collection of diseased material. For comparative purposes, asymptomatic or seemingly healthy material should also be collected. Material is then stored at 4°C and can be processed immediately after collection or left for a week or more. During processing, material can be sectioned, surface disinfected to eliminate epiphytic fungi and placed on to any generalist media (e.g. 2% malt extract agar, potato dextrose agar) for isolation. Media can be supplemented with antibiotics to inhibit the growth of potentially competitive bacteria. As a general guide, isolates with a 'fluffy' mycelium, either white in colour to creamy or pigmented 'greenish brown' or grey to grey black potentially represent the *Botryosphaeriaceae* and can be grouped according to culture morphology (Slippers and Wingfield, 2007).

Isolates can be induced to sporulate by transferring a mycelial plug to water agar overlaid with sterile pine needles (Pavlic *et al*., 2004) or sterile host material, either branch sections (Mehl *et al*., 2011) or leaves (Inderbitzin *et al*., 2010). Plates can then be placed under continual near-ultraviolet light (Pavlic *et al*., 2004), in the dark, or under artificial laboratory bench light (Inderbitzin *et al*., 2010). The resulting pycnidia are useful for studying conidial morphology and making single spore cultures for DNA-based studies.

The sequencing of species of the *Botryosphaeriaceae* was initially confined to the internal transcribed spacer (ITS) ribosomal DNA (rDNA) locus (consisting of ITS1, 5.8S and ITS2) (Jacobs and Rehner, 1998; Zhou and Stanosz, 2001). Virtually every subsequent study has used several loci, including ITS, because combined sequence data from multiple loci results in improved resolution among taxa (de Wet *et al*., 2003; Inderbitzin *et al*., 2010; Mehl *et al*., 2011). Additional loci used in phylogenetic species recognition include the mitochondrial small subunit (mtSSU) (Zhou and Stanosz, 2001), protein coding genes (for translation elongation factor 1α (TEF-1α), β-tubulin, actin, calmodulin, chitin synthase, glyceraldehyde-6-phosphate dehydrogenase, histone-3 and heat shock protein) (de Wet *et al*., 2003; Inderbitzin *et al*., 2010), various unknown microsatellite-containing loci (de Wet *et al*., 2003; Pavlic *et al*., 2009) and data from the rDNA locus (small subunit (SSU), ITS and large subunit (LSU)) (Zhou and Stanosz, 2001; Crous *et al*., 2006; Phillips *et al*., 2008). Concordance among multiple loci has been used to convincingly argue for the existence of cryptic species, an approach known as genealogical concordance phylogenetic

species recognition (GCPSR), or applied as a genealogical sorting index (de Wet *et al*., 2003; Slippers *et al*., 2004b; Pavlic *et al*., 2009; Sakalidis *et al*., 2011). Regardless of the loci selected for phylogenetic analyses, most sequence data pertaining to species of the *Botryosphaeriaceae* on GenBank (the US National Institutes of Health (NIH) genetic sequence database provided by the US National Center for Biotechnology Information (NCBI)) are limited to the ITS and TEF-1α loci. Much work is therefore required to fill the gaps and to facilitate analyses that incorporate sequence data from the other loci listed.

PCR-RFLPs (restriction fragment length polymorphisms) were first developed by Jacobs (2002) to distinguish among species infecting mangoes (*Mangifera indica* L.). Typically, the ITS region is amplified, sometimes in combination with another gene region (Pavlic *et al*., 2007), and digested using the restriction endonuclease *Cfo*I or various combinations of other enzymes (Slippers *et al*., 2004b, 2007; Alves *et al*., 2005). This method, however, does not distinguish among all species using the banding pattern from a particular locus (Slippers *et al*., 2004b), thereby necessitating the amplification of other loci and their subsequent restriction. Furthermore, sequence data are initially required to test whether restriction sites for the selected endonucleases occur within the locus to be amplified. Consequently, the method has little relevance in screening plant material for which the full complement of the associated *Botryosphaeriaceae* has not been determined.

Species-specific primers, as the name implies, enable the detection of an individual species of the *Botryosphaeriaceae*. These primers have been developed based on variations in sequence data of the ITS locus and tested using nested PCR (Ma and Michailides, 2002; Flowers *et al*., 2003). One study developed primers based on data from the mtSSU locus (Smith and Stanosz, 2006), while another exploited sequence variation within the same locus to delineate among several species using melting analysis of the PCR products (Luchi *et al*., 2011). Other than the study of Ma and Michailides (2002), these studies have tested the ability of the primers developed to detect species *in planta* using wood, bark or bud samples. Primers tested are usually extremely sensitive. Real-time quantitative PCR has enabled detection at as little as 0.1 pg fungal DNA, or roughly two to three asexual spores (conidia) (Ridgway *et al*., 2011; Spagnolo *et al*., 2011). These studies have been confined to the *Botryosphaeriaceae* on pines (*Pinus* spp.) (Flowers *et al*., 2003; Luchi *et al*., 2005, 2011; Smith and Stanosz, 2006) and grapevines (Ridgway *et al*., 2011; Spagnolo *et al*., 2011), and have only concentrated on a few species: *D. pinea*, *D. seriata* De Not., *D. scrobiculata* de Wet, Slippers & M.J. Wingf. (Flowers *et al*., 2003; Luchi *et al*., 2005, 2011; Smith and Stanosz, 2006), *Neofusicoccum parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips/ *N. ribis* (Slippers, Crous & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips and *B. dothidea* (Ridgway *et al*., 2011; Spagnolo *et al*., 2011). There is a need to consider species from many other genera within the family.

The molecular tools outlined above have enabled rapid identification and delineation amongst the *Botryosphaeriaceae*. They can potentially be used for quarantine purposes to screen plant material for these fungi. However, there is a danger of obtaining false positive or negative results and the limitations of these techniques should thus be carefully considered.

14.3 Infection Biology

Based on the classification of Rodriguez *et al*. (2009), the *Botryosphaeriaceae* are best grouped with the class 3 endophytes. These are defined as non-clavicipitaceous endophytes that have a broad host range, occupy the shoots of plants, have limited *in planta* colonization, high *in planta* biodiversity, grow intercellularly and are transmitted horizontally. Like other endophytes, the *Botryosphaeriaceae* germinate epiphytically, penetrate plants and grow intercellularly (Arnold and Herre, 2003). The ability of these fungi to invade plant tissue and persist therein is competitively advantageous, especially when they later switch to a saprophytic life cycle.

The *Botryosphaeriaceae* have two main strategies for infecting plants. The first involves infection via natural apertures and endophytic persistence within asymptomatic tissue (Flowers *et al*., 2003; Bihon *et al*., 2011a). Infection generally requires the presence of free water (Rayachhetry *et al*., 1996a). Conidia can be trapped among bud scales resulting in subsequent infection of the buds (Beisel *et al*., 1984; Michailides, 1991; Michailides and Morgan, 1993). Other natural openings, such as stomata on leaves and shoots, and lenticels either on fruit or stems, as well as the inflorescences, pedicels or peduncles of fruit trees, provide avenues for infection (Weaver, 1979; Michailides, 1991; Johnson and Kotzé, 1994; Kim *et al*., 1999; Ploetz, 2003). The process begins with conidial germination: germ tubes emerge from either one or both ends of the conidia (Milholland, 1970; Kim *et al*., 1999). In the case of stomata that may be closed, germ tubes encircle stomates or grow over them (Brookhouser and Peterson, 1970; Milholland, 1970). Utilizing the peduncle, the *Botryosphaeriaceae* gain entrance to fruits to initiate stem end rots (Johnson and Kotzé, 1994) once fruit begin ripening or after harvest (Menge and Ploetz, 2003; Timmer *et al*., 2003). Direct appressorial penetration of fruit has been reported when high levels of inoculum are present, but appears to be rare (Johnson and Kotzé, 1994; Menge and Ploetz, 2003).

The second strategy for plant infection by the *Botryosphaeriaceae* is via wounds (Epstein *et al*., 2008). Wounds associated with diseases caused by these fungi include those resulting from human activities such as pruning, hand-picking of fruit, grafting, or via scars when scales, buds, petioles and fruit abscise (Michailides, 1991; Johnson, 1994; Pusey *et al*., 1995; Menge and Ploetz, 2003). Large numbers of isolates have been obtained from graft unions, suggesting transmission by grafting tools (Aroca *et al*., 2006). These high numbers could, however, result from stress arising from graft incompatibility and the presence of *Botryosphaeriaceae* occupying the tissue endophytically. Other sources of wound infections include those attributed to branch splitting resulting from wind, frost and hail damage (Swart and Wingfield, 1991; Menge and Ploetz, 2003) although even these wounds could have enabled endophytic *Botryosphaeriaceae* to emerge. Finally, wounds naturally formed during root growth can also enable infection from inoculum present on residual diseased plant material on the ground (Whitelaw-Weckert *et al*., 2006).

14.3.1 Histopathology

The infection process of the *Botryosphaeriaceae* argues against a strict classification of these fungi as class 3 endophytes. Class 3 endophytes have been reported to only grow intercellularly (Rodriguez *et al*., 2009). In contrast, the *Botryosphaeriaceae* can grow both intercellularly and intracellularly. Additionally, tissue specificity occurs among different species of class 3 endophytes, promoting the absence of niche overlap and cooperation, rather than competition, between these fungi. However, different species of the *Botryosphaeriaceae* can occupy the same tissue sample simultaneously (Pusey, 1993; Smith *et al*., 2001).

After penetration via the stomata, species of the *Botryosphaeriaceae* produce an appressorium-like hyphal mass in the stomatal pit (Brookhouser and Peterson, 1970; Milholland, 1970). Mycelium originating from this mass then grows into the mesophyll (Brookhouser and Peterson, 1970). In leaves, the mycelium continues to grow, advancing through the intercellular spaces of the mesophyll and intracellular spaces of the midrib to colonize the oil cells. Pycnidial stromata are initiated in the oil cells that develop and these stromata rupture the leaf epidermis (Rayachhetry *et al*., 1996a). In fruits, fungal progress is rapid as the fruits are used as a source of nutrition. In avocadoes (*Persea americana* Mill.), the *Botryosphaeriaceae* move via the vascular bundles to the base and stem ends to encompass the fruit (Johnson, 1994), while in citrus (*Citrus*) the centre columnella enables progression through the fruit (Timmer *et al*., 2003).

Host response to infection by the *Botryosphaeriaceae* involves the formation of barriers. In fruits, cell walls produce protuberances that block fungal growth and confine the invading mycelium. These protuberances are

marked by the formation of red halos surrounding the circular lesions caused by infection (Kim *et al*., 2001). In other plant organs, hyperplasia occurs that involves the differentiation of parenchyma cells to form new periderm and the production of gum ducts proximal or distal to a site of infection (Biggs and Britton, 1988). The new periderm comes into contact, and becomes continuous, with the old periderm. It is constituted by three to five layers of suberized phellem cells (Biggs and Britton, 1988; Rayachhetry *et al*., 1996a). The new periderm might delimit these fungi and prevent their entry into the deeper plant tissues, but is not always effective in doing so.

Formation of the new periderm produced by plants in response to infection by the *Botryosphaeriaceae* can be ineffective and allow these fungi to invade the cortex. This is as a result of defects or cracks in the periderm due to the rapid proliferation of callus (Milholland, 1972). Hyphae invade the primary phloem and vascular cambium. In the case of wounds, the cell walls of xylem vessels and tracheids adjacent to the wounds are extensively lignified, and the vascular cambium beneath the new periderm re-differentiates to xylem and phloem. These tissues are subsequently invaded via the pits, becoming necrotic and collapsing to create gaps (Milholland, 1972; Rayachhetry *et al*., 1996a). In addition, the accumulation of parenchyma cells results in disorganization of the cortex and phloem. The parenchyma cells are eventually crushed, producing large cavities that are occupied by mycelium (Milholland, 1970; Atia *et al*., 2003). The cortex collapses and is filled with a watery substance, while the periderm expands until it ruptures (Brown and Hendrix, 1981). The end result is a canker.

Entry of the *Botryosphaeriaceae* into the cortex allows invasion of the systemic tissues. The mycelium invades the xylem via the xylem rays (Milholland, 1970; Ramos *et al*., 1991; Rayachhetry *et al*., 1996a). The xylem vessels are then occupied by an abundance of tyloses (Ramos *et al*., 1991; Rayachhetry *et al*., 1996a; Atia *et al*., 2003) that either partially or completely block the vessels. Vessels can be occupied by several mycelial strands, which then branch to invade the adjacent tracheids (Milholland, 1972; Ramos *et al*., 1991; Rayachhetry *et al*., 1996a). Rapid mycelial growth occurs in the xylem as a result of the availability of nutrients (Cedeño *et al*., 1996).

The formation of the new periderm produced by plants in response to infection by the *Botryosphaeriaceae* can be successful in restricting these fungi to the epidermis. As a result, hyphae are not observed in the cortex (Milholland, 1972). However, enzymatic activity by the *Botryosphaeriaceae* results in degradation of the epidermal cell wall, thus enabling entry of the mycelium into the epidermal cells. This allows for their persistence in the bark, as latent pathogens. Swelling subsequently occurs, due to separation of the cuticular membrane from the underlying epidermal cell wall, and the cuticular membrane becomes folded and creased (Chou, 1978). This gives old cankers a characteristically swollen, crusty appearance (Brown and Britton, 1986).

14.3.2 The role of stress

Various stresses enable endophytes, including the *Botryosphaeriaceae*, to cause disease (Schulz *et al*., 1998). Some species in the family can cause disease in the absence of stress (Mullen *et al*., 1991), but disease typically only manifests once plants are stressed. Stress affects both cuticle penetrability and the persistence of endophytes in plant tissue (Arnold and Herre, 2003).

Levitt (1972) and Schoeneweiss (1975) noted that stress results in a strain on plants that is either plastic or elastic. Plastic strains result in irreversible physical or chemical change(s) in physiology as opposed to elastic strains, where physical or chemical changes are reversible when stress is removed. Knowledge of when an elastic strain transitions to a plastic strain and the implicit physiological limits differ among plants (Paoletti *et al*., 2001).

Moisture stress caused by drought is most commonly associated with disease reports resulting from infection by the *Botryosphaeriaceae*. Moisture stress contributes to nutrient stress by causing deficiencies, as plants require water to translocate nutrients. Moisture stress also impedes plant defence; wound periderm formation is halted or

inhibited (Parker, 1961; Puritch and Mullick, 1975; Paoletti *et al*., 2001), along with inhibited production of defensive compounds (Madar *et al*., 1995). Upon exposure to moisture stress, plants seemingly cope with the initial loss of turgidity and reduction of xylem water potentials. Initially, the strain remains elastic, with increased colonization of the wood near the cambium and bark by endophytic *Botryosphaeriaceae* as water potentials further decrease to a particular threshold level. Maintenance of these low water potentials for more than 3 days results in a plastic strain and predisposition to disease (Crist and Schoeneweiss, 1975; Schoeneweiss, 1975, 1981a,b; Paoletti *et al*., 2001). The water potentials differ depending on the plant pathosystem concerned (Paoletti *et al*., 2001).

Early studies by Schoeneweiss (Crist and Schoeneweiss, 1975; Schoeneweiss, 1975, 1981a,b) focused on the effects of low temperatures in plant pathosystems involving the *Botryosphaeriaceae*. Recent studies have, however, considered higher temperatures in the context of global climate change (Desprez-Loustau *et al*., 2007; Botella *et al*., 2010). For low temperatures, Rayachhetry *et al*. (1996b) found that short-term exposure (3 days) to low temperatures (6°C) in melaleuca (*Melaleuca* spp.) resulted in increased canker length and sapwood discoloration. In other treatments involving longer periods of exposure, symptoms did not manifest. Similarly, Schoeneweiss (Crist and Schoeneweiss, 1975; Schoeneweiss, 1975, 1981a,b) found increased wood colonization in mountain ash (*Sorbus* spp.), European white birch (*Betula pendula* Roth), sweetgum (*Liquidambar* spp.) and red osier dogwood (*Cornus sericea* L.) at low temperatures. He postulated that freezing results in dehydration of plant cells (Schoeneweiss, 1981a,b). Studies that considered elevated temperatures (Desprez-Loustau *et al*., 2007; Botella *et al*., 2010) have predicted that the *Botryosphaeriaceae* will increasingly become more prominent and significant. Apart from the increased likelihood of moisture stress occurring, higher temperatures favour the mycelial growth of these fungi. Typically, the temperature optima for growth are 25–30°C. Future studies should, therefore, consider what effect short-term periods of exposure to heat stress have on plant pathosystems involving the *Botryosphaeriaceae*.

A number of diseases caused by the *Botryosphaeriaceae* result in defoliation (Gerlach *et al*., 1974; Shahin and Claflin, 1980; Chakraborty *et al*., 2004). Defoliation produces stress, and results in an avenue for additional infections and in decreased starch content, in terms of both soluble and stored carbohydrates (Schoeneweiss, 1975; Old *et al*., 1990). The effect of defoliation, as with other stresses, varies depending on the pathosystem. Downy birch (*Betula pubescens* Ehrh.) stems inoculated with *B. dothidea* and defoliated for more than 4 weeks were girdled by the pathogen and died, while those defoliated for 4 weeks and allowed to recover survived (Crist and Schoeneweiss, 1975). Similarly, punktrees (*Melaleuca quinquenervia* (Cav.) S.F. Blake) inoculated with *N. ribis* and exposed to complete defoliation suffered 90% mortality within 4 weeks (Rayachhetry *et al*., 1996b). Defoliation and infection by the *Botryosphaeriaceae* initially produces an elastic strain. Sustained defoliation, however, results in a plastic strain, and potentially high levels of mortality among infected plants (Crist and Schoeneweiss, 1975; Schoeneweiss, 1975, 1981b).

There are a number of other stresses that can allow disease expression by the *Botryosphaeriaceae*. The transplanting of plants frequently necessitates extensive pruning of roots, which results in a loss of stored root food reserves and reduced root surface for nutrient uptake (Schoeneweiss, 1981b). Wounding resulting from hail, snow, insects, or combinations thereof, both produces stress and provides an avenue for further infections by the *Botryosphaeriaceae* (Nicholls and Ostry, 1990; Menge and Ploetz, 2003). Improper planting soils that are too shallow, too hard or too sandy to retain water also place stress on trees (Wright and Marks, 1970). Poor training and thinning of trees results in shading, weakening of branches, reduced vigour and overstocking (Wright and Marks, 1970; Crist and Schoeneweiss, 1975). Competition for resources, including water, can arise from neighbouring vegetation (Blodgett *et al*., 1997). High levels of nitrogen and phosphorus (Van Dijk *et al*., 1992), resulting from fertilization,

can reduce the concentrations of lignin and total soluble phenolics, rendering plants susceptible to infection by various fungi, including the *Botryosphaeriaceae* (Blodgett *et al*., 2005). Nutrient deficiencies also cause stress (Menge and Ploetz, 2003). Other diseases can weaken and stress plants that are already infected endophytically by the *Botryosphaeriaceae* (Menge and Ploetz, 2003). Damage can also arise from the application of chemicals (Menge and Ploetz, 2003). Finally, in the case of juvenile tropical palms (*Iriartea deltoidea* Ruiz & Pav.), high light intensities stress the plants, rendering them susceptible to disease caused by *Diplodia mutila* (Fr.) Mont. (syn. *Botryosphaeria stevensii* Shoemaker) (Alvarez-Loayza *et al*., 2011).

14.3.3 Insect associations

The *Botryosphaeriaceae* are principally dispersed by water and wind. They are not specifically adapted for insect dispersal and it is unlikely that insects play any significant role in their dissemination. Nevertheless, there are several reports of *Botryosphaeriaceae* associated with insect damage (Feci *et al*., 2003; Romero *et al*., 2005; Moral *et al*., 2010). In each of these cases, damage caused by these insects enabled either entry of the *Botryosphaeriaceae*, or disease expression when the pathogen was already present as an endophyte. Disease expression is also likely to facilitate the abscission of insect-damaged plant parts, as noted by Slippers and Wingfield (2007).

Several recent publications (Adair *et al*., 2009; Janson *et al*., 2009, 2010; Heath and Stireman, 2010) have reported the transmission of *B. dothidea* by gall midges. These insects possess specialized structures (mycangia) on the ovipositor that house fungal conidia of unknown origin. Evidently, oviposition includes the inoculation of a gall with conidia of the fungus. However, this is seemingly casual, as *B. dothidea* was not isolated from every gall tested and other fungi were also isolated from the same galls (Adair *et al*., 2009; Heath and Stireman, 2010). For one of the midges, *B. dothidea* is a source of sterol for developing larvae, as shown by the sterol profile consisting of ergosterol and its precursors/metabolites (Janson *et al*., 2009). This is not surprising: insects are unable to produce their own sterols so phytophagous insects typically ingest sterols derived from plants (phytosterols) and then enzymatically convert them to cholesterol (Clayton, 1964).

14.3.4 Melanization

Several genera of the *Botryosphaeriaceae* produce pigmented spores or hyaline spores that become pigmented at maturity, e.g. *Barriopsis*, *Diplodia*, *Lasiodiplodia*, *Neodeightonia* (Crous *et al*., 2006; Phillips *et al*., 2008). A number of species of the *Botryosphaeriaceae* also produce pigmented cultures. Pigmentation in fungi results from the production of melanin (Bell and Wheeler, 1986).

Melanin enables appressorial penetration of the plant host epidermis or cellular membranes (Bell and Wheeler, 1986; Butler *et al*., 2001). This is accomplished by the accumulation of glycerol in the melanized appressorium, which generates intense pressure on the epidermis or membrane under attack, owing to melanin forming a barrier that is impermeable to glycerol (Nosanchuk and Casadevall, 2003). Consequently, melanins can play a prominent role in the virulence and success of the *Botryosphaeriaceae* as pathogens.

14.3.5 Phytotoxins

Several species of the *Botryosphaeriaceae* are known to produce various non-specific phytotoxins (Evidente *et al*., 1996, 1999, 2003a, 2010; Veiga *et al*., 2007; Djoukeng *et al*., 2009) (Table 14.1). There is a strong likelihood that many other phytotoxins are also produced by the *Botryosphaeriaceae*, and are awaiting discovery and characterization.

14.4 Epidemiology

Species of the *Botryosphaeriaceae* can overwinter or survive adverse environmental conditions on a variety of substrates, often endophytically. These include infected and/or pruned wood (Epstein *et al*., 2008), bark

Table 14.1. Known toxins produced by species of the *Botryosphaeriaceae*. Listed, along with toxins, are their associated phytopathogenic effects, host species and any recorded antimycotic activity.

Species and toxin	Disease symptoms	Inoculated host(s)	Antimicrobial against phytopathogenic fungi	References
***Diplodia cupressi* A.J.L. Phillips & A. Alves**				
Sphaeropsidin A	Non-specific; produces a variety of disease symptoms, including necrosis and dieback	Various *Cupressus* spp.	Yes	Evidente *et al.*, 1996
Sphaeropsidin B Sphaeropsidin C	Yellowing, browning, necrosis and dieback	*Cupressus* and *Quercus* spp.	Yes	Evidente *et al.*, 1997
Sphaeropsidin D	Chlorosis and subsequent necrosis	*Hesperocyparis macrocarpa* (Hartw.) Bartel	NT[a]	Evidente *et al.*, 2002
Sphaeropsidin F	Chlorosis	*Cupressus sempervirens* L.	NT	Evidente *et al.*, 2003b
Sapinopyridione	Chlorosis Chlorosis and dieback	*Hesperocyparis arizonica* (Greene) Bartel *H. macrocarpa* and *C. sempervirens*	NT	Evidente *et al.*, 2006
Sphaeropsidone	Browning and necrosis Chlorosis	*H. macrocarpa* *H. arizonica*	Yes	Evidente *et al.*, 1998
Episphaeropsidone	Necrosis Browning and necrosis	*H. arizonica* and *H. macrocarpa* *C. sempervirens*	Yes	Evidente *et al.*, 1998
***Diplodia pinea* (Desm.) J. Kickx f. (syn. *Sphaeropsis sapinea* (Fr.) Dyko & B. Sutton)[b]**				
Sapinofuranone A	Browning on excised twigs, similar to sapwood staining	*Cupressus* spp.	NT	Evidente *et al.*, 1999
Sapinofuranone B	Yellowing of twigs, withering of needles	*Pinus* spp.		
Mellein (3*R*,4*R*)-4-hydroxymellein (3*R*,4*S*)-4-hydroxymellein	Chlorosis and subsequent necrosis	*Pinus radiata* D. Don (cuttings)	NT	Cabras *et al.*, 2006
***Diplodia seriata* De Not. (syn. *Botryosphaeria obtusa* (Schwein.) Shoemaker)**				
Mellein (3*R*,4*R*)-4-hydroxymellein 5-hydroxymellein 7-hydroxymellein 4,7-hydroxymellein	Necrotic lesions, characteristic of infection[c]	*Malus pumila* Mill.	NT	Venkatasubbaiah *et al.*, 1991; Djoukeng *et al.*, 2009

Continued

Table 14.1. Continued.

Species and toxin	Disease symptoms	Inoculated host(s)	Antimicrobial against phytopathogenic fungi	References
4-hydroxybenzaldehyde Tyrosol				
***Diplodia mutila* (Fr.) Mont. (syn. *Botryosphaeria stevensii* Shoemaker)**[d]				
Diplopyrone	Necrotic lesions on leaves within 4 days, wilting of cuttings within 8 days	*Quercus suber* L.	NT	Evidente *et al.*, 2003a
***Lasiodiplodia theobromae* (Pat.) Griffon & Maubl.**				
(3*S*,4*R*)-3-carboxy-2-methylene-heptan-4-olide[e] Mellein Lasiodiplodin De-*O*-methyl-lasiodiplodin (3*R*,4*R*)-hydroxymellein Botryosphaeran	Lasiodiplodin inhibits ATP synthesis by interfering with electron flow and phosphorylation	NT	NT	Aldridge *et al.*, 1971; Barbosa *et al.*, 2003; He *et al.*, 2004; Veiga *et al.*, 2007
***Neofusicoccum parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips**				
(3*R*,4*R*)-4-hydroxymellein (3*R*,4*S*)-4-hydroxymellein Isosclerone Tyrosol		Tomato (*Solanum lycopersicon* L.) (cuttings)	NT	Evidente *et al.*, 2010

[a]NT, not tested.
[b]It is unclear which morphotype(s) of the fungus were tested: whether A, C or both.
[c]Toxin production has not been correlated with virulence.
[d]The fungus also produces sphaeropsidins A and C (Evidente *et al.*, 1997).
[e]Toxicity remains doubtful as it was evaluated using bananas (*Musa*) (He *et al.*, 2004).

(Srivastava, 1972), infected rotting/mummified fruit, twigs, buds, petioles, flowers, inflorescences and rachises (Michailides, 1991). Inoculum on these substrates can be sustained for up to 6 years, while infected plant parts typically persist for longer than healthy plant parts (Michailides, 1991; Ahimera *et al*., 2003) and are not abscised. Inoculum on infected fruits can be more abundant than that on infected leaves (Ahimera *et al*., 2003). Principally, inoculum consists of conidia housed in pycnidia. Latent propagules can also be introduced into an orchard through infected seedlings, rootstocks or scions (Cysne *et al*., 2010). Inoculum may also be present on residual diseased plant material present on the ground, thereby enabling root infection (Hodges, 1983; Whitelaw-Weckert *et al*., 2006). Root-to-root transmission might also occur (Wingfield and Knox-Davies, 1980) although this has not been shown experimentally. Primary infections occur in early spring and summer, and secondary infections in late summer and autumn (Michailides and Morgan, 1993).

During spore release, conidia of the *Botryosphaeriaceae* are typically exuded from pycnidia in a gelatinous matrix as a ribbon-like mass or cirrhus (Michailides and Morgan, 1993; Úrbez-Torres *et al*., 2010). This gelatinous matrix or mucilage encompasses conidia within the pycnidia, and contains glucides and proteins. The matrix is believed to prevent conidia from desiccating, losing their viability and germinating (Fournet, 1969; Fitt and McCartney, 1986). During periods of high relative humidity, the mucilage swells to produce the ribbon-like cirrhi; when wet, it dissolves, thereby freeing conidia to germinate (Fitt and McCartney, 1986).

Dispersal of the conidia of *Botryosphaeriaceae*, including the initiation of spore release, has been correlated with rainfall, or occurs as a result of overhead irrigation events, as water dissolves the mucilage to free the conidia. Spore release usually begins within 1 hour of water beginning to fall and lasts for up to 2 hours after water has stopped falling (Amponsah *et al*., 2009; Úrbez-Torres *et al*., 2010). The release can be elevated during night-time rainfall events as the humidity is higher while temperatures are lower and wind is reduced (Holmes and Rich, 1970). It has been hypothesized that conidia are dispersed over relatively short distances because of their dependence on rain splash (Úrbez-Torres *et al*., 2010). Consequently, these conidia are involved in further disease cycles and infections of the plant concerned (Michailides *et al*., 2001), in which they can persist endophytically. Methods of dissemination other than rain splash include wind blowing during rain, infected pruning shears and insects (Swart *et al*., 1987; Epstein *et al*., 2008).

The sexual spores (ascospores) of the *Botryosphaeriaceae* are produced in perithecia (pseudothecia). Ascospore release can be stimulated by fog (Úrbez-Torres *et al*., 2010), rainfall, dew or mist (Pusey, 1989). Ascospores are generally found in much smaller numbers than conidia (Parker and Ramsdell, 1977; Michailides *et al*., 2001), but appear to be released during periods of rapid air movement or wind and optimal temperatures, in the spring (Pusey, 1989). These climatic factors can facilitate long-distance dispersal of the pathogen as the ascospores are airborne (Pusey, 1989; Michailides *et al*., 2001; Ma and Michailides, 2002).

14.5 Management Strategies and Tactics

14.5.1 Avoidance

Strategies that minimize or avoid infection by the *Botryosphaeriaceae* are the most important means of disease management and control as the fungi persist endophytically once plants are infected. Minimizing exposure to stress is an important first step to dealing with diseases of the *Botryosphaeriaceae* because stress is clearly the dominant factor inciting disease expression. In commercial forestry, site–species matching is used to anticipate the site and climatic variation requirements of different species and provenances, and thus minimize stress in clonal plantings (Little *et al*., 2003). A second strategy is to utilize appropriate planting densities and spacings to avoid potential competition between plants for nutrients and/or water. Thinning can be undertaken in situations where this is unavoidable (Dawson

and Were, 1998). Stands should also not be left to over mature (Slippers *et al*., 2009).

Some control strategies used to manage diseases of *Botryosphaeriaceae* in commercial agricultural orchards could be applied in seed orchards and nurseries. For example, grapevine rootstocks are treated with hot water or a chemical or biological drench to kill inoculum and thereby minimize infection (Fourie and Halleen, 2004). Appropriate hygiene practices, such as disinfecting grafting shears, aim to avoid potential scion-to-scion transmission of these fungi (Fourie and Halleen, 2004; Cysne *et al*., 2010). Soil treatment involving fumigation, soil solarization or sanitation can be applied to reduce inoculum on infected plant debris at the soil surface, as these sources would otherwise infect rootstocks or seedlings via planting wounds (Whitelaw-Weckert *et al*., 2006). Soil preparation may also entail the addition of any fertilizer that is required to avoid nutrient stress, and irrigation soon after planting to avoid moisture stress. Strategies for irrigation that reduce the humidity available to the *Botryosphaeriaceae* for spore release, mycelial growth and infection should, therefore, limit or eliminate a wet tree canopy and/or orchard floor. These include the use of drip or micro-jet irrigation (preferred) (Michailides and Morgan, 2004), or else sprinklers with a low trajectory angle (Michailides and Morgan, 1993, 2004), and irrigation done for short time periods, preferably early in the day, to minimize the duration of leaf wetness (Michailides and Morgan, 1992). Pressure applied in irrigation systems can also be lowered to reduce any fogging and humidity that would otherwise enable infection (Michailides and Morgan, 2004). In pistachio (*Pistacia vera* L.) orchards, it has been recommended that dead plant material be burned (Michailides and Morgan, 2004), and sanitation should be practised throughout the growing period to control and manage infection by these fungi.

Pruning in seed orchards should be done in winter as low temperatures minimize the dispersal of inoculum and infection by the *Botryosphaeriaceae* (Swart and Wingfield, 1991), and should be done so that only a moderate amount (25%) of the crown is removed. Regular (39%) or heavy (49%) pruning can produce physiological stress and result in disease expression by latent *Botryosphaeriaceae* (Chou and MacKenzie, 1988). Pruning wounds provide an additional avenue for infection by the *Botryosphaeriaceae*, so coating wounds with a fungicidal paint may reduce infection by these and other pathogens (Epstein *et al*., 2008). In commercial forestry, these strategies for pruning are impractical, both economically and in terms of labour.

Horizontal transmission of the *Botryosphaeriaceae* between trees inevitably results in newly established plantation or orchard trees being infected by these fungi. The infections originate from neighbouring flora, including windbreaks, that contribute a mix of isolates over the lifetime of a tree (Stanosz *et al*., 2005, 2007; Burgess *et al*., 2006; Pavlic *et al*., 2007; Bihon *et al*., 2011a). Some studies have shown that species of *Botryosphaeriaceae* infect related hosts (within the same plant family), while other studies show that species are not host specific and originate from unrelated neighbouring plants (Damm *et al*., 2007; Inderbitzin *et al*., 2010). Clearly, neighbouring trees and plants will need to be considered as an extension in any management strategy against the *Botryosphaeriaceae*. This is especially important in nursery settings where young plants can be heavily infected by inoculum originating from surrounding plants (Stanosz *et al*., 2005, 2007).

14.5.2 Exclusion

The global movement of people and products continues to facilitate the movement of fungal pathogens, including the *Botryosphaeriaceae*, across geographic or ecological borders (Anderson *et al*., 2004). Global trade has increased the frequency of introductions of exotic species and is expected to continue rising (Cunningham *et al*., 2003; Anderson *et al*., 2004). Pathogens, including the *Botryosphaeriaceae*, are typically introduced via infected plant material, including imported planting or nursery material. In some cases, such pathogens can then shift or jump hosts to cause devastating diseases on native vegetation (Slippers *et al*., 2005). There are already several examples of such diseases caused by

the *Botryosphaeriaceae* in which non-native trees are planted in new areas in close proximity to native trees that are broadly related to the non-natives, albeit not at the scale of epidemics (Burgess *et al*., 2006; Pavlic *et al*., 2007; Pérez *et al*., 2010).

Seed infection by the *Botryosphaeriaceae* and the possibility of disseminating more virulent and pathogenic genotypes of these fungi through international seed trade is a concern (Burgess *et al*., 2001; Burgess and Wingfield, 2002). A species that is suspected to have been spread via seed and associated material is *D. pinea*, which infects and causes diseases of pine trees. This fungus infects both immature cones (Smith *et al*., 1996), from which it has been isolated from the outer scales of second-year cones, and the pith tissue of third-year seed cones, ovuliferous scales, seeds and seed wings (Smith *et al*., 2002). While the fungus sporulates prolifically on cones (Smith *et al*., 2002), conidia are passed on at low frequencies (2.3%) to the seed (Bihon *et al*., 2011b). Conidia may also be present in coarse woody debris collected with cones and seeds (Burgess and Wingfield, 2002; Bihon *et al*., 2011b). Seed infection is also caused by early harvesting of the cones (Fraedrich, 1996). The emerging seedlings are not systematically infected, although overall germination is decreased (Bihon *et al*., 2011b). In seeds infected by either *D. pinea* or *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl., both the embryo and gametophyte tissues are destroyed, effectively killing the seeds (Miller and Bramlett, 1979; Rees, 1988; Rees and Webber, 1988; Fraedrich, 1996). Consequently, quarantine for the *Botryosphaeriaceae* should consider the global spread of seeds, along with any plant debris accompanying those seeds.

Quarantine will prove challenging in limiting the global movement of the *Botryosphaeriaceae*, because of the extensive dissemination of propagation material for nurseries – such as rootstocks, scions and other nursery material. The *Botryosphaeriaceae* are noticeably conspicuous in their absence among lists of introduced pathogens (Brasier, 2008), probably as a result both of their ability to remain latent within visibly symptomless material and of a poor understanding of their native ranges. Molecular tools such as those outlined in this chapter will enable quarantine authorities to detect these fungi within apparently healthy plant material.

14.5.3 Eradication

Eradication for the *Botryosphaeriaceae* is most probably impossible. No eradication programme has been attempted for these fungi. The fact that they persist endophytically means that complete detection and eradication within either a plantation or orchard is impossible. Also, inoculum frequently originates from neighbouring flora (including plantations, orchards, windbreaks, individual plants and trees) (Burgess *et al*., 2006; Pavlic *et al*., 2007), and the removal of these sources, including native flora, is obviously not possible.

14.5.4 Protection

Fungicidal applications have been evaluated in the control of the *Botryosphaeriaceae* in forestry, but have been shown to be ineffective. These trials have demonstrated that fungicides, while effective *in vitro*, are ineffective *in vivo* (Peterson, 1977; Palmer *et al*., 1986; Hartman *et al*., 2009). Their application is also impractical, when the labour, risks to the people involved and costs are considered. This is because trees host a multitude of isolates that infect over the lifetime of a tree (Bihon *et al*., 2011a) and fungicide application over the entire lifetime of a tree is simply impossible. Nevertheless, fungicides can be applied to manage these fungi in young plants in nurseries (Swart and Wingfield, 1991). In agriculture, in contrast, several fungicides have been registered for application to various crops to control diseases of the *Botryosphaeriaceae* (Michailides and Morgan, 2004; Kim *et al*., 2005; van Niekerk *et al*., 2006).

14.5.5 Resistance

Plant breeding provides a basis for evaluating the resistance and tolerance to species of the *Botryosphaeriaceae* in clonal forestry

and agriculture. These are easily evaluated using pathogenicity trials involving stem inoculations with agar plugs of species of *Botryosphaeriaceae*, which provide an opportunity to screen for resistance before commercialization and field planting (Slippers *et al*., 2009). In two studies (Mohali *et al*., 2009; Rodas *et al*., 2009), variation was evident both among species that were inoculated and in clonal tolerance to infection. Clonal tolerance, however, should be consistent regardless of the site where a stand is established (Rodas *et al*., 2009).

There can be considerable variation in pathogenicity among isolates of species of the *Botryosphaeriaceae*, so much so that this can influence virulence on the trees that are inoculated. This has been evident from pathogenicity trials undertaken on a variety of plant hosts (Pavlic *et al*., 2007; Stanosz *et al*., 2007; Mohali *et al*., 2009; Mehl *et al*., 2011). The variation can be confounded by both site factors and other environmental factors when a trial is undertaken (Rodas *et al*., 2009). Consequently, a large number of isolates should be used in trials in both commercial forestry and agricultural planting materials. Challenging a clone, clonal rootstock or scion with multiple isolates, some potentially highly pathogenic, will provide a better indication of resistance and tolerance mechanisms than simply using one or two isolates, even if their pathogenicity is well established.

14.5.6 Integrated disease management

An integrated approach is recommended to ensure that diseases of the *Botryosphaeriaceae* are controlled to an acceptable level. Strategies for management in plantations include site–species matching (Little *et al*., 2003), the use of appropriate planting densities and spacing (Dawson and Were, 1998) and consideration of neighbouring plants that are sources of inoculum (Burgess *et al*., 2006; Pavlic *et al*., 2007). In seed orchards and nurseries, strategies include sanitation, hygiene practices such as the disinfection of grafting shears and pruning tools (Fourie and Halleen, 2004; Cysne *et al*., 2010), wound treatment with a fungicidal paint (Epstein *et al*., 2008), pruning at an appropriate time and at a moderate level (Swart and Wingfield, 1991), and irrigation strategies that minimize exposure to the water or humidity that are available for spore release and mycelial growth (Michailides and Morgan, 2004). Chemical control through fungicide use is restricted to nurseries, owing to the practical impossibilities of application to plantations and seed orchards (Swart and Wingfield, 1991). Trials on nursery stock and planting material and field trials on forestry stock should be undertaken at multiple locations to avoid the confounding of results (Rodas *et al*., 2009) and should utilize multiple isolates.

References

Adair, R.J., Burgess, T., Serdani, M. and Barber, P. (2009) Fungal associations in Asphondylia (Diptera: Cecidomyiidae) galls from Australia and South Africa: implications for biological control of invasive acacias. *Fungal Ecology* 2, 121–134.

Ahimera, N., Driever, G.F. and Michailides, T.J. (2003) Relationships among propagule numbers of *Botryosphaeria dothidea*, latent infections, and severity of panicle and shoot blight in pistachio orchards. *Plant Disease* 87, 846–853.

Aldridge, D.C., Galt, S., Giles, D. and Turner, W.B. (1971) Metabolites of *Lasiodiplodia theobromae*. *Journal of the Chemical Society C: Organic*, 1623–1627.

Alvarez-Loayza, P., White, J.F. Jr, Bergen, M. and Cadenas, C. (2008) *Diplodia mutila* causing seedling mortality of the palm *Iriartea deltoidea*. *Plant Pathology* 57, 382.

Alvarez-Loayza, P., White, J.F. Jr, Torres, M.S., Balslev, H., Kristiansen, T., Svenning, J.-C. and Gil, N. (2011) Light converts endosymbiotic fungus to pathogen, influencing seedling survival and niche-space filling of a common tropical tree, *Iriartea deltoidea*. *PLoS One* 6(1): e16386.

Alves, A., Phillips, A.J.L., Henriques, I. and Correia, A. (2005) Evaluation of amplified ribosomal DNA restriction analysis as a method for the identification of *Botryosphaeria* species. *FEMS Microbiology Letters* 245, 221–229.

Amponsah, N.T., Jones, E.E., Ridgway, H.J. and Jaspers, M.V. (2009) Rainwater dispersal of *Botryosphaeria* conidia from infected grapevines. *New Zealand Plant Protection* 62, 228–233.

Anderson, P.K., Cunningham, A.A., Patel, N.G., Morales, F.J., Epstein, P.R. and Daszak, P. (2004) Emerging infectious diseases of plants: pathogen pollution, climate change and agrotechnology drivers. *Trends in Ecology and Evolution* 19, 535–544.

Arnold, A.E. and Herre, E.A. (2003) Canopy cover and leaf age affect colonization by tropical fungal endophytes: ecological pattern and process in *Theobroma cacao* (*Malvaceae*). *Mycologia* 95, 388–398.

Aroca, A., García-Figueres, F., Bracamonte, L., Luque, J. and Raposo, R. (2006) A survey of trunk disease pathogens within rootstocks of grapevines in Spain. *European Journal of Plant Pathology* 115, 195–202.

Atia, M.M.M., Aly, A.Z., Tohamy, M.R.A., El-Shimy, H. and Kamhawy, M.A. (2003) Histopathological studies on grapevine die-back. *Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz* 110, 131–142.

Barbosa, A.M., Steluti, R.M., Dekker, R.F.H., Cardoso, M.S. and Corradi da Silva, M.L. (2003) Structural characterization of Botryosphaeran: α (1→3;1→6)-β-D-glucan produced by the ascomyceteous fungus, *Botryosphaeria* sp. *Carbohydrate Research* 338, 1691–1698.

Beisel, M., Hendrix, F.F. Jr and Starkey, T.E. (1984) Natural inoculation of apple buds by *Botryosphaeria obtusa*. *Phytopathology* 74, 335–338.

Bell, A.A. and Wheeler, M.H. (1986) Biosynthesis and functions of fungal melanins. *Annual Review of Phytopathology* 24, 411–451.

Biggs, A.R. and Britton, K.O. (1988) Presymptom histopathology of peach trees inoculated with *Botryosphaeria obtusa* and *B. dothidea*. *Phytopathology* 78, 1109–1118.

Bihon, W., Burgess, T.I., Slippers, B., Wingfield, M.J. and Wingfield, B.D. (2011a) Distribution of *Diplodia pinea* and its genotypic diversity within asymptomatic *Pinus patula* trees. *Australasian Plant Pathology* 40, 540–548.

Bihon, W., Slippers, B., Burgess, T.I., Wingfield, M.J. and Wingfield, B.D. (2011b) Sources of *Diplodia pinea* endophytic infections in *Pinus patula* and *P. radiata* seedlings in South Africa. *Forest Pathology* 41, 370–375.

Blodgett, J.T., Kruger, E.L. and Stanosz, G.R. (1997) *Sphaeropsis sapinea* and water stress in a red pine plantation in central Wisconsin. *Phytopathology* 87, 429–434.

Blodgett, J.T., Herms, D.A. and Bonello, P. (2005) Effects of fertilization on red pine defense chemistry and resistance to *Sphaeropsis sapinea*. *Forest Ecology and Management* 208, 373–382.

Botella, L., Santamaría, O. and Diez, J.J. (2010) Fungi associated with the decline of *Pinus halepensis* in Spain. *Fungal Diversity* 40, 1–11.

Brasier, C.M. (2008) The biosecurity threat to the UK and global environment from international trade in plants. *Plant Pathology* 57, 792–808.

Brookhouser, L.W. and Peterson, G.W. (1970) Infection of Austrian, Scots, and ponderosa pines by *Diplodia pinea*. *Phytopathology* 61, 409–414.

Brown, E.A. and Britton, K.O. (1986) *Botryosphaeria* diseases of apple and peach in the southeastern United States. *Plant Disease* 70, 480–484.

Brown, E.A. and Hendrix, F.F. (1981) Pathogenicity and histopathology of *Botryosphaeria dothidea* on apple stems. *Phytopathology* 71, 375–379.

Burgess, T.I. and Wingfield, M.J. (2002) Quarantine is important in restricting the spread of exotic seed-borne tree pathogens in the southern hemisphere. *International Forestry Review* 4, 56–65.

Burgess, T.I., Wingfield, B.D. and Wingfield, M.J. (2001) Comparison of genotypic diversity in native and introduced populations of *Sphaeropsis sapinea* isolated from *Pinus radiata*. *Mycological Research* 105, 1331–1339.

Burgess, T.I., Sakalidis, M.L. and Hardy, G.E.St.J. (2006) Gene flow of the canker pathogen *Botryosphaeria australis* between *Eucalyptus globulus* plantations and native eucalypt forests in Western Australia. *Austral Ecology* 31, 559–566.

Butler, M.J., Day, A.W., Henson, J.M. and Money, N.P. (2001) Pathogenic properties of fungal melanins. *Mycologia* 93, 1–8.

Cabras, A., Mannoni, M.A., Serra, S., Andolfi, A., Fiore, M. and Evidente, A. (2006) Occurrence, isolation and biological activity of phytotoxic metabolites produced *in vitro* by *Sphaeropsis sapinea*, pathogenic fungus of *Pinus radiata*. *European Journal of Plant Pathology* 115, 187–193.

Cedeño, L., Mohali, S.R. and Palacios-Prü, E. (1996) Ultrastructure of *Lasiodiplodia theobromae* causal agent of Caribbean pine blue stain in Venezuela. *Interciencia* 21, 264–271.

Chakraborty, M.R., Dutta, S. and Chatterjee, N.C. (2004) Dieback disease of bottle brush – a new record from India. *Journal of Mycology and Plant Pathology* 34, 146.

Chou, C.K.S. (1978) Penetration of young stems of *Pinus radiata* by *Diplodia pinea*. *Physiological Plant Pathology* 13, 189–192.

Chou, C.K.S. and MacKenzie, M. (1988) Effect of pruning intensity and season on *Diplodia pinea* infection of *Pinus radiata* stem through pruning wounds. *European Journal of Forest Pathology* 18, 437–444.

Clayton, R.B. (1964) The utilization of sterols by insects. *Journal of Lipid Research* 5, 3–19.

Crist, C.R. and Schoeneweiss, D.F. (1975) The influence of controlled stresses on susceptibility of European white birch stems to attack by *Botryosphaeria dothidea*. *Phytopathology* 65, 369–373.

Crous, P.W., Slippers, B., Wingfield, M.J., Rheeder, J., Marasas, W.F.O., Phillips, A.J.L., Alves, A., Burgess, T.I., Barber, P. and Groenewald, J.Z. (2006) Phylogenetic lineages in the *Botryosphaeriaceae*. *Studies in Mycology* 55, 235–253.

Cunningham, A.A., Daszak, P. and Rodriguez, J.P. (2003) Pathogen pollution: defining a parasitological threat to biodiversity conservation. *Journal of Parasitology* 89, S78–S83.

Cysne, A.Q., Cardoso, J.E., Aline de Holanda, N.M. and Farias, F.C. (2010) Spatial-temporal analysis of gummosis in three cashew clones at northeastern Brazil. *Journal of Phytopathology* 158, 676–682.

Dakin, N., White, D., Hardy, G.E.St.J. and Burgess, T.I. (2010) The opportunistic pathogen, *Neofusicoccum australe*, is responsible for crown dieback of peppermint (*Agonis flexuosa*) in Western Australia. *Australasian Plant Pathology* 39, 202–206.

Damm, U., Crous, P.W. and Fourie, P.H. (2007) *Botryosphaeriaceae* as potential pathogens of *Prunus* species in South Africa, with descriptions of *Diplodia africana* and *Lasiodiplodia plurivora* sp. nov. *Mycologia* 99, 664–680.

Dawson, I. and Were, J. (1998) Multiplication, that's the name of the game: guidelines for seed production of agroforestry trees. *Agroforestry Today* 10, 19–22.

Desprez-Loustau, M.-L., Robin, C., Reynaud, G., Déqué, M., Badeau, V., Piou, D., Husson, C. and Marçais, B. (2007) Simulating the effects of a climate-change scenario on the geographical range and activity of forest-pathogenic fungi. *Canadian Journal of Plant Pathology* 29, 101–120.

de Wet, J., Burgess, T.I., Slippers, B., Preisig, O., Wingfield, B.D. and Wingfield, M.J. (2003) Multiple gene genealogies and microsatellite markers reflect relationships between morphotypes of *Sphaeropsis sapinea* and distinguish a new species of *Diplodia*. *Mycological Research* 107, 557–566.

Djoukeng, J.D., Polli, S., Larignon, P. and Abou-Mansour, E. (2009) Identification of phytotoxins from *Botryosphaeria obtusa*, a pathogen of black dead arm disease of grapevine. *European Journal of Plant Pathology* 124, 303–308.

Epstein, L., Sukhwinder, K. and VanderGheynst, J.S. (2008) *Botryosphaeria*-related dieback and control investigated in noncoastal California grapevines. *California Agriculture* 62, 161–166.

Evidente, A., Sparapano, L., Motta, A., Giordano, F., Fierro, O. and Frisullo, S. (1996) A phytotoxic pimarane diterpene of *Sphaeropsis sapinea* f. sp. *cupressi*, the pathogen of a canker disease of cypress. *Phytochemistry* 42, 1541–1546.

Evidente, A., Sparapano, L., Fierro, O., Bruno, G., Giordano, F. and Motta, A. (1997) Sphaeropsidins B and C, phytotoxic pimarane diterpenes from *Sphaeropsis sapinea* f. sp. *cupressi* and *Diplodia mutila*. *Phytochemistry* 45, 705–713.

Evidente, A., Sparapano, L., Fierro, O., Bruno, G., Giordano, F. and Motta, A. (1998) Sphaeropsidone and episphaeropsidone, phytotoxic dimedone methylethers produced by *Sphaeropsis sapinea* f. sp. *cupressi* grown in liquid culture. *Phytochemistry* 48, 1139–1143.

Evidente, A., Sparapano, L., Fierro, O., Bruno, G. and Motta, A. (1999) Sapinofuranones A and B, two new 2(3H)-dihydrofuranones produced by *Sphaeropsis sapinea*, a common pathogen of conifers. *Journal of Natural Products* 62, 253–256.

Evidente, A., Sparapano, L., Bruno, G. and Motta, A. (2002) Sphaeropsidins D and E, two other pimarane diterpenes, produced *in vitro* by the plant pathogenic fungus *Sphaeropsis sapinea* f. sp. *cupressi*. *Phytochemistry* 59, 817–823.

Evidente, A., Maddau, L., Spanu, E., Franceschini, A., Lazzaroni, S. and Motta, A. (2003a) Diplopyrone, a new phytotoxic tetrahydropyranpyran-2-one produced by *Diplodia mutila*, a fungus pathogen of cork oak. *Journal of Natural Products* 66, 313–315.

Evidente, A., Sparapano, L., Andolfi, A., Bruno, G. and Motta, A. (2003b) Sphaeropsidin F, a new pimarane diterpene produced *in vitro* by the cypress pathogen *Sphaeropsis sapinea* f. sp. *cupressi*. *Australian Journal of Chemistry* 56, 615–619.

Evidente, A., Fiore, M., Bruno, G., Sparapano, L. and Motta, A. (2006) Chemical and biological characterisation of sapinopyridione, a phytotoxic 3,3,6-trisubstituted-2,4-pyridione produced by *Sphaeropsis sapinea*, a toxigenic pathogen of native and exotic conifers, and its derivatives. *Phytochemistry* 67, 1019–1028.

Evidente, A., Punzo, B., Andolfi, A., Cimmino, A., Melck, D. and Luque, J. (2010) Lipophilic phytotoxins produced by *Neofusicoccum parvum*, a grapevine canker agent. *Phytopathologia Mediterranea* 49, 74–79.

Feci, E., Smith, D. and Stanosz, G.R. (2003) Association of *Sphaeropsis sapinea* with insect-damaged red pine shoots and cones. *Forest Pathology* 33, 7–13.

Fisher, P.J., Petrini, O. and Sutton, B.C. (1993) A comparative study of fungal endophytes in leaves, xylem and bark of *Eucalyptus* in Australia and England. *Sydowia* 45, 338–345.

Fitt, B.D.L. and McCartney, H.A. (1986) Spore dispersal in splash droplets. In: Ayres, P.G. and Boddy, L. (eds) *Water, Fungi and Plants*. Cambridge University Press, Cambridge, UK, pp. 87–104.

Flowers, J.L., Hartman, J.R. and Vaillancourt, L.J. (2003) Detection of latent *Sphaeropsis sapinea* infections in Austrian pine tissues using nested-polymerase chain reaction. *Phytopathology* 93, 1471–1523.

Fourie, P.H. and Halleen, F. (2004) Occurrence of grapevine trunk disease pathogens in rootstock mother plants in South Africa. *Australasian Plant Pathology* 33, 313–315.

Fournet, J. (1969) Propriétés et rôle du cirrhe du *Septoria nodorum* Berk. [Properties and role of cirrhus of *Septoria nodorum* Berk.]. *Annales de Phytopathologie* 1, 87–94.

Fraedrich, S. (1996) Seedborne diseases of southern pines and developing strategies for their control. In: Landis, T.D. and South, D.B. (eds) *National Proceedings: Forest and Conservation Nursery Associations, 1996*. General Technical Report PNW 389, US Department of Agriculture, Forest Service, Pacific Northwest Research Station, Portland, Oregon, pp. 75–81.

Gerlach, W.W.P., Hoitink, H.A.J. and Ellett, C.W. (1974) Shoot blight and stem dieback of *Pieris japonica* caused by *Phytophthora citricola*, *P. citrophthora* and *Botryosphaeria dothidea*. *Phytopathology* 64, 1368–1370.

Giraud, M. (2009) Le black rot du pommier [Apple black rot]. *Infos-Ctifl* 257, 36–41.

Hartman, J.R., Vaillancourt, L.J., Flowers, J.L. and Bateman, A.M. (2009) Managing Diplodia tip blight of landscape Austrian pines. *Arboriculture and Urban Forestry* 35, 27–32.

He, G., Matsuura, H. and Yoshihara, T. (2004) Isolation of an α-methylene-γ-butyrolactone derivative, a toxin from the plant pathogen *Lasiodiplodia theobromae*. *Phytochemistry* 65, 2803–2807.

Heath, J.J. and Stireman, J.O. III (2010) Dissecting the association between a gall midge, *Asteromyia carbonifera*, and its symbiotic fungus, *Botryosphaeria dothidea*. *Entomologia Experimentalis et Applicata* 137, 36–49.

Hodges, C.S. (1983) Pine mortality in Hawaii associated with *Botryosphaeria dothidea*. *Plant Disease* 67, 555–556.

Holmes, J. and Rich, A.E. (1970) Factors affecting release and dissemination of *Physalospora obtusa* spores in a New Hampshire apple orchard. *Phytopathology* 60, 1052–1054.

Inderbitzin, P., Bostock, R.M., Trouillas, F.P. and Michailides, T.J. (2010) A six locus phylogeny reveals high species diversity in *Botryosphaeriaceae* from California almond. *Mycologia* 102, 1350–1368.

Jacobs, K.A. and Rehner, S.A. (1998) Comparison of cultural and morphological characters and ITS sequences in anamorphs of *Botryosphaeria* and related taxa. *Mycologia* 90, 601–610.

Jacobs, R. (2002) Characterisation of *Botryosphaeria* species from mango in South Africa. MSc thesis, University of Pretoria, Pretoria, South Africa.

Janson, E.M., Grebenok, R.J., Behmer, S.T. and Abbot, P. (2009) Same host–plant, different sterols: variation in sterol metabolism in an insect herbivore community. *Journal of Chemical Ecology* 35, 1309–1319.

Janson, E.M., Peeden, E.R., Stireman, J.O. III and Abbot, P. (2010) Symbiont-mediated phenotypic variation without co-evolution in an insect–fungus association. *Journal of Evolutionary Biology* 23, 2212–2228.

Johnson, G.I. (1994) *Dothiorella* stem canker and fruit rot. In: Ploetz, R.C., Zentmyer, G.A., Nishijima, W.T., Rohrbach, K.G. and Ohr, H.D. (eds) *Compendium of Tropical Fruit Diseases*. APS Press, St Paul, Minnesota, p. 76.

Johnson, G.I. and Kotzé, J.M. (1994) Stem-end rot. In: Ploetz, R.C., Zentmyer, G.A., Nishijima, W.T., Rohrbach, K.G. and Ohr, H.D. (eds) *Compendium of Tropical Fruit Diseases*. APS Press, St Paul, Minnesota, pp. 81–83.

Kim, K.W., Park, E.W. and Ahn, K.K. (1999) Pre-penetration behavior of *Botryosphaeria dothidea* on apple fruits. *Plant Pathology Journal* 15, 223–227.

Kim, K.W., Park, E.W., Kim, Y.H., Ahn, K.K., Kim, P.G. and Kim, K.S. (2001) Latency- and defense-related ultrastructural characteristics of apple fruit tissues infected with *Botryosphaeria dothidea*. *Phytopathology* 91, 165–172.

Kim, K.W., Kim, K.R. and Park, E.W. (2005) An infection model of apple white rot based on conidial germination and appressorium formation of *Botryosphaeria dothidea*. *Plant Pathology Journal* 21, 322–327.

Levitt, J. (1972) *Responses of Plants to Environmental Stresses*. Academic Press, New York.

Little, K.M., van Staden, J. and Clarke, G.P.Y. (2003) *Eucalyptus grandis* × *E. camaldulensis* variability and intra-genotypic competition as a function of different vegetation management treatments. *New Forests* 25, 227–242.

Luchi, N., Capretti, P., Surico, G., Orlando, C., Pazzagli, M. and Pinzani, P. (2005) A real-time quantitative PCR assay for the detection of *Sphaeropsis sapinea* from inoculated *Pinus nigra* shoots. *Journal of Phytopathology* 153, 37–42.

Luchi, N., Pratesi, N., Simi, L., Pazzagli, M., Capretti, P., Scala, A., Slippers, B. and Pinzani, P. (2011) High-resolution melting analysis: a new molecular approach for the early detection of *Diplodia pinea* in Austrian pine. *Fungal Biology* 115, 715–723.

Ma, Z. and Michailides, T.J. (2002) Characterization of *Botryosphaeria dothidea* isolates collected from pistachio and other plant hosts in California. *Phytopathology* 92, 519–526.

Madar, Z., Solel, Z., Riov, J. and Sztejnberg, A. (1995) Phytoalexin production by cypress in response to infection by *Diplodia pinea* f. sp. *cupressi* and its relation to water stress. *Physiological and Molecular Plant Pathology* 47, 29–38.

Mehl, J.W.M., Slippers, B., Roux, J. and Wingfield, M.J. (2011) *Botryosphaeriaceae* associated with *Pterocarpus angolensis* (kiaat) in South Africa. *Mycologia* 103, 534–553.

Menge, J.A. and Ploetz, R.C. (2003) Diseases of avocado. In: Ploetz, R.C. (ed.) *Diseases of Tropical Fruit Crops*. CAB International, Wallingford, UK, pp. 35–71.

Michailides, T.J. (1991) Pathogenicity, distribution, sources of inoculum, and infection courts of *Botryosphaeria dothidea* on pistachio. *Phytopathology* 81, 566–573.

Michailides, T.J. and Morgan, D.P. (1992) Effects of temperature and wetness duration on infection of pistachio by *Botryosphaeria dothidea* and management of disease by reducing duration of irrigation. *Phytopathology* 82, 1399–1406.

Michailides, T.J. and Morgan, D.P. (1993) Spore release by *Botryosphaeria dothidea* in pistachio orchards and disease control by altering the trajectory angle of sprinklers. *Phytopathology* 83, 145–152.

Michailides, T.J. and Morgan, D.P. (2004) Panicle and shoot blight of pistachio: a major threat to the California pistachio industry. Available at: http://www.apsnet.org/publications/apsnetfeatures/Pages/Pistachio.aspx (accessed 6 December 2012).

Michailides, T.J., Morgan, D.P. and Felts, D. (2001) Collection and characterization of *Botryosphaeria dothidea* from various hosts and pathogenicity studies on pistachio. *KAC Plant Protection Quarterly* 11, 3–8.

Milholland, R.D. (1970) Histology of *Botryosphaeria* canker of susceptible and resistant highbush blueberries. *Phytopathology* 60, 70–74.

Milholland, R.D. (1972) Histopathology and pathogenicity of *Botryosphaeria dothidea* on blueberry stems. *Phytopathology* 62, 654–660.

Miller, T. and Bramlett, D.L. (1979) Damage to reproductive structures of slash pine by two seed-borne pathogens: *Diplodia gossypina* and *Fusarium moniliforme* var. *subglutinans*. In: Bonner, F. (ed.) *Proceedings, IUFRO/USFS/Mississippi State University Symposium on Flowering and Seed Development in Trees, May 15–18, 1978*. Mississippi State University, Starkville, Mississippi, pp. 347–355.

Mohali, S.R., Slippers, B. and Wingfield, M.J. (2009) Pathogenicity of seven species of the *Botryosphaeriaceae* on *Eucalyptus* clones in Venezuela. *Australasian Plant Pathology* 38, 135–140.

Moral, J., Muñoz-Díez, C., González, N., Trapero, A. and Michailides, T.J. (2010) Characterization and pathogenicity of *Botryosphaeriaceae* species collected from olive and other hosts in Spain and California. *Phytopathology* 100, 1340–1351.

Mullen, J.M., Gilliam, C.H., Hagan, A.K. and Morgan-Jones, G. (1991) Canker of dogwood caused by *Lasiodiplodia theobromae*, a disease influenced by drought stress or cultivar selection. *Plant Disease* 75, 886–889.

Nicholls, T.H. and Ostry, M.E. (1990) *Sphaeropsis sapinea* cankers on stressed red and jack pines in Minnesota and Wisconsin. *Plant Disease* 74, 54–56.

Nosanchuk, J.D. and Casadevall, A. (2003) The contribution of melanin to microbial pathogenesis. *Cellular Microbiology* 5, 203–223.

Old, K.M., Gibbs, R., Craig, I., Myers, B.J. and Yuan, Z.Q. (1990) Effect of drought and defoliation on the susceptibility of eucalypts to cankers caused by *Endothia gyrosa* and *Botryosphaeria ribis*. *Australian Journal of Botany* 38, 571–581.

Palmer, M.A., Nicholls, T.H. and Croghan, C.F. (1986) Fungicidal control of shoot blight caused by *Sphaeropsis sapinea* on red pine nursery seedlings. *Plant Disease* 70, 194–196.

Paoletti, E., Danti, R. and Strati, S. (2001) Pre- and post-inoculation water stress affects *Sphaeropsis sapinea* canker length in *Pinus halepensis* seedlings. *Forest Pathology* 31, 209–218.

Parker, A.K. (1961) Bark moisture relations in disease development: present status and future needs. *Recent Advances in Botany* 2, 1535–1537.

Parker, P.E. and Ramsdell, D.C. (1977) Epidemiology and chemical control of *Godronia* (*Fusicoccum*) canker of highbush blueberry. *Phytopathology* 67, 1475–1480.

Pavlic, D., Slippers, B. Coutinho, T.A., Gryzenhout, M. and Wingfield, M.J. (2004) *Lasiodiplodia gonubiensis* sp. nov., a new *Botryosphaeria* anamorph from native *Syzygium cordatum* in South Africa. *Studies in Mycology* 50, 313–322.

Pavlic, D., Slippers, B., Coutinho, T.A. and Wingfield, M.J. (2007) *Botryosphaeriaceae* occurring on native *Syzygium cordatum* in South Africa and their potential threat to *Eucalyptus*. *Plant Pathology* 56, 624–636.

Pavlic, D., Wingfield, M.J., Barber, P., Slippers, B., Hardy, G.E.St.J. and Burgess, T.I. (2008) Seven new species of the *Botryosphaeriaceae* from baobab and other native trees in Western Australia. *Mycologia* 100, 851–866.

Pavlic, D., Slippers, B., Coutinho, T.A. and Wingfield, M.J. (2009) Multiple gene genealogies and phenotypic data reveal cryptic species of the *Botryosphaeriaceae*: a case study on the *Neofusicoccum parvum/N. ribis* complex. *Molecular Phylogenetics and Evolution* 51, 259–268.

Pérez, C.A., Wingfield, M.J., Slippers, B., Altier, N.A. and Blanchette, R.A. (2010) Endophytic and canker-associated *Botryosphaeriaceae* occurring on non-native *Eucalyptus* and native *Myrtaceae* trees in Uruguay. *Fungal Diversity* 41, 53–69.

Peterson, G.W. (1977) Infection, epidemiology, and control of Diplodia blight of Austrian, ponderosa and Scots pines. *Phytopathology* 67, 511–514.

Petrini, O. and Fisher, P.J. (1988) A comparative study of fungal endophytes in xylem and whole stem of *Pinus sylvestris* and *Fagus sylvatica*. *Transactions of the British Mycological Society* 91, 233–238.

Phillips, A.J.L. (2002) *Botryosphaeria* species associated with diseases of grapevines in Portugal. *Phytopathologia Mediterranea* 41, 3–18.

Phillips, A.J.L., Alves, A., Correia, A. and Luque, J. (2005) Two new species of *Botryosphaeria* with brown, 1-septate ascospores and *Dothiorella* anamorphs. *Mycologia* 97, 513–529.

Phillips, A.J.L., Oudemans, P.V., Correia, A. and Alves, A. (2006) Characterisation and epitypification of *Botryosphaeria corticis*, the cause of blueberry cane canker. *Fungal Diversity* 21, 141–155.

Phillips, A.J.L., Alves, A., Pennycook, S.R., Johnston, P.R., Ramaley, A., Akulov, A. and Crous, P.W. (2008) Resolving the phylogenetic and taxonomic status of dark-spored teleomorph genera in the *Botryosphaeriaceae*. *Persoonia* 21, 29–55.

Piškur, B., Pavlic, D., Slippers, B., Ogris, N., Maresi, G., Wingfield, M.J. and Jurc, D. (2011) Diversity and pathogenicity of *Botryosphaeriaceae* on declining *Ostrya carpinifolia* in Slovenia and Italy following extreme weather conditions. *European Journal of Forest Research* 130, 235–249.

Ploetz, R.C. (2003) Diseases of mango. In: Ploetz, R.C. (ed.) *Diseases of Tropical Fruit Crops*. CAB International, Wallingford, UK, pp. 327–363.

Puritch, G.S. and Mullick, D.B. (1975) Effect of water stress on the rate of non-suberized impervious tissue formation following wounding in *Abies grandis*. *Journal of Experimental Botany* 26, 903–910.

Pusey, P.L. (1989) Availability and dispersal of ascospores and conidia of *Botryosphaeria* in peach orchards. *Phytopathology* 79, 635–639.

Pusey, P.L. (1993) Role of *Botryosphaeria* species in peach tree gummosis on the basis of differential isolation from outer and inner bark. *Plant Disease* 77, 170–174.

Pusey, P.L., Kitajima, H. and Wu, Y. (1995) Fungal gummosis. In: Ogawa, J.M., Zehr, E.I., Bird, G.W., Ritchie, D.F., Uriu, K. and Uyemoto, J.K. (eds) *Compendium of Stone Fruit Diseases*. APS Press, St Paul, Minnesota, pp. 33–34.

Ramos, L.J., Lara, S.P., McMillan, R.T. Jr and Narayanan, K.R. (1991) Tip dieback of mango (*Mangifera indica*) caused by *Botryosphaeria ribis*. *Plant Disease* 75, 315–318.

Rayachhetry, M.B., Blakeslee, G.M. and Miller, T. (1996a) Histopathology of *Botryosphaeria ribis* in *Melaleuca quinquenervia*: pathogen invasion and host response. *International Journal of Plant Sciences* 157, 219–227.

Rayachhetry, M.B., Blakeslee, G.M. and Center, T.D. (1996b) Predisposition of melaleuca (*Melaleuca quinquenervia*) to invasion by the potential biological control agent *Botryosphaeria ribis*. *Weed Science* 44, 603–608.

Rees, A.A. (1988) Infection of *Pinus caribaea* seed by *Lasiodiplodia theobromae*. *Transactions of the British Mycological Society* 90, 321–324.

Rees, A.A. and Webber, J.F. (1988) Pathogenicity of *Sphaeropsis sapinea* to seed, seedlings and saplings of some central American pines. *Transactions of the British Mycological Society* 91, 273–277.

Ridgway, H.J., Amponsah, N.T., Brown, D.S., Baskarathevan, J., Jones, E.E. and Jaspers, M.V. (2011) Detection of botryosphaeriaceous species in environmental samples using a multi-species primer pair. *Plant Pathology* 60, 1118–1127.

Rodas, C.A., Slippers, B., Gryzenhout, M. and Wingfield, M.J. (2009) *Botryosphaeriaceae* associated with *Eucalyptus* canker diseases in Colombia. *Forest Pathology* 39, 110–123.

Rodriguez, R.J., White J.F. Jr, Arnold, A.E. and Redman, R.S. (2009) Fungal endophytes: diversity and functional roles. *New Phytologist* 182, 314–330.

Romero, M.A., Sánchez, M.E. and Trapero, A. (2005) First report of *Botryosphaeria ribis* as a branch dieback pathogen of olive trees in Spain. *Plant Disease* 89, 208.

Sakalidis, M.L., Hardy, G.E.St.J. and Burgess, T.I. (2011) Use of the genealogical sorting index (GSI) to delineate species boundaries in the *Neofusicoccum parvum–Neofusicoccum ribis* species complex. *Molecular Phylogenetics and Evolution* 60, 333–344.

Schoeneweiss, D.F. (1975) Predisposition, stress, and plant disease. *Annual Review of Phytopathology* 13, 193–211.

Schoeneweiss, D.F. (1981a) Infectious diseases of trees associated with water and freezing stress. *Journal of Arboriculture* 7, 13–18.

Schoeneweiss, D.F. (1981b) The role of environmental stress in diseases of woody plants. *Plant Disease* 65, 308–314.

Schulz, B., Guske, S., Dammann, U. and Boyle, C. (1998) Endophyte–host interactions II: defining symbiosis of the endophyte–host interaction. *Symbiosis* 25, 213–227.

Shahin, E.A. and Claflin, L.E. (1980) Twig blight of Douglas-fir: a new disease caused by *Dothiorella dothidea*. *Plant Disease* 64, 47–50.

Shearer, B.L., Tippett, J.T. and Bartle, J.R. (1987) *Botryosphaeria ribis* infection associated with death of *Eucalyptus radiata* in species selection trials. *Plant Disease* 71, 140–145.

Slippers, B. and Wingfield, M.J. (2007) *Botryosphaeriaceae* as endophytes and latent pathogens of woody plants: diversity, ecology and impact. *Fungal Biology Reviews* 21, 90–106.

Slippers, B., Crous, P.W., Denman, S., Coutinho, T.A., Wingfield, B.D. and Wingfield, M.J. (2004a) Combined multiple gene genealogies and phenotypic characters differentiate several species previously identified as *Botryosphaeria dothidea*. *Mycologia* 96, 83–101.

Slippers, B., Fourie, G., Crous, P.W., Coutinho, T.A., Wingfield, B.D., Carnegie, A.J. and Wingfield, M.J. (2004b) Speciation and distribution of *Botryosphaeria* spp. on native and introduced *Eucalyptus* trees in Australia and South Africa. *Studies in Mycology* 50, 343–358.

Slippers, B., Stenlid, J. and Wingfield, M.J. (2005) Emerging pathogens: fungal host jumps following anthropogenic introduction. *Trends in Ecology and Evolution* 20, 420–421.

Slippers, B., Smit, W.A., Crous, P.W., Coutinho, T.A., Wingfield, B.D. and Wingfield, M.J. (2007) Taxonomy, phylogeny and identification of *Botryosphaeriaceae* associated with pome and stone fruit trees in South Africa and other regions of the world. *Plant Pathology* 56, 128–139.

Slippers, B., Burgess, T.I., Pavlic, D., Ahumada, R., Maleme, H., Mohali, S., Rodas, C.A. and Wingfield, M.J. (2009) A diverse assemblage of *Botryosphaeriaceae* infect *Eucalyptus* in native and non-native environments. *Southern Forests* 71, 101–110.

Smith, D.R. and Stanosz, G.R. (2006) A species-specific PCR assay for detection of *Diplodia pinea* and *D. scrobiculata* in dead red and jack pines with collar rot symptoms. *Plant Disease* 90, 307–313.

Smith, H., Wingfield, M.J., Crous, P.W. and Coutinho, T.A. (1996) *Sphaeropsis sapinea* and *Botryosphaeria dothidea* endophytic in *Pinus* spp. and *Eucalyptus* spp. in South Africa. *South African Journal of Botany* 62, 86–88.

Smith, H., Crous, P.W., Wingfield, M.J., Coutinho, T.A. and Wingfield, B.D. (2001) *Botryosphaeria eucalyptorum* sp. nov., a new species in the *B. dothidea*-complex on *Eucalyptus* in South Africa. *Mycologia* 93, 277–285.

Smith, H., Wingfield, M.J. and Coutinho, T.A. (2002) The role of latent *Sphaeropsis sapinea* infections in post-hail associated dieback of *Pinus patula*. *Forest Ecology and Management* 164, 177–184.

Spagnolo, A., Marchi, G., Peduto, F., Phillips, A.J.L. and Surico, G. (2011) Detection of *Botryosphaeriaceae* species within grapevine woody tissues by nested PCR, with particular emphasis on the *Neofusicoccum parvum/N. ribis* complex. *European Journal of Plant Pathology* 129, 485–500.

Srivastava, D.N. (1972) Epidemiology and prevention of *Diplodia* stem-end rot of ripe mango fruits. *Acta Horticulturae* 24, 235–236.

Stanosz, G.R., Smith, D.R. and Albers, J.S. (2005) Surveys for asymptomatic persistence of *Sphaeropsis sapinea* on or in stems of red pine seedlings from seven Great Lakes region nurseries. *Forest Pathology* 35, 233–244.

Stanosz, G.R., Smith, D.R. and Leisso, R. (2007) Diplodia shoot blight and asymptomatic persistence of *Diplodia pinea* on or in stems of jack pine nursery seedlings. *Forest Pathology* 37, 145–154.
Swart, W.J. and Wingfield, M.J. (1991) Biology and control of *Sphaeropsis sapinea* on *Pinus* species in South Africa. *Plant Disease* 75, 761–766.
Swart, W.J., Wingfield, M.J. and Knox-Davies, P.S. (1987) Conidial dispersal of *Sphaeropsis sapinea* in three climatic regions of South Africa. *Plant Disease* 71, 1038–1040.
Thompson, S., Alvarez-Loayza, P., Terborgh, J. and Katul, G. (2010) The effects of plant pathogens on tree recruitment in the Western Amazon under a projected future climate: a dynamical systems analysis. *Journal of Ecology* 98, 1434–1446.
Timmer, L.W., Garnsey, S.M. and Broadbent, P. (2003) Diseases of citrus. In: Ploetz, R.C. (ed.) *Diseases of Tropical Fruit Crops*. CAB International, Wallingford, UK, pp. 163–195.
Úrbez-Torres, J.R., Battany, M., Bettiga, L.J., Gispert, C., McGourty, G., Roncoroni, J., Smith, R.J., Verdegaal, P. and Gubler, W.D. (2010) *Botryosphaeriaceae* species spore-trapping studies in California vineyards. *Plant Disease* 94, 717–724.
Van Dijk, H.F.G., Van der Gaag, M., Perik, P.J.M. and Roelofs, J.G.M. (1992) Nutrient availability in Corsican pine stands in the Netherlands and the occurrence of *Sphaeropsis sapinea*: a field study. *Canadian Journal of Botany* 70, 870–875.
van Niekerk, J.M., Fourie, P.H., Halleen, F. and Crous, P.W. (2006) *Botryosphaeria* spp. as grapevine trunk disease pathogens. *Phytopathologia Mediterranea* 45, S43–S54.
Veiga, T.A.M., Silva, S.C., Francisco, A.-C., Filho, E.R., Vieira, P.C., Fernandes, J.B., Silva, M.F.G.F., Müller, M.W. and Lotina-Hennsen, B. (2007) Inhibition of photophosphorylation and electron transport chain in thylakoids by lasiodiplodin, a natural product from *Botryosphaeria rhodina*. *Journal of Agricultural and Food Chemistry* 55, 4217–4221.
Venkatasubbaiah, P., Sutton, T.B. and Chilton, W.S. (1991) Effect of phytotoxins produced by *Botryosphaeria obtusa*, the cause of black rot of apple fruit and frogeye leaf spot. *Phytopathology* 81, 243–247.
Weaver, D.J. (1979) Role of conidia of *Botryosphaeria dothidea* in the natural spread of peach tree gummosis. *Phytopathology* 69, 330–334.
Webb, R.S. (1983) Seed capsule abortion and twig dieback of *Eucalyptus camaldulensis* in south Florida induced by *Botryosphaeria ribis*. *Plant Disease* 67, 108–109.
Whitelaw-Weckert, M.A., Sergeeva, V. and Priest, M.J. (2006) *Botryosphaeria stevensii* infection of Pinot Noir grapevines by soil–root transmission. *Australasian Plant Pathology* 35, 369–371.
Wingfield, M.J. and Knox-Davies, P.S. (1980) Association of *Diplodia pinea* with a root disease of pines in South Africa. *Plant Disease* 64, 221–223.
Wright, J.P. and Marks, G.C. (1970) Loss of merchantable wood in radiata pine associated with infection by *Diplodia pinea*. *Australian Forestry* 34, 107–119.
Zhou, S. and Stanosz, G.R. (2001) Relationships among *Botryosphaeria* species and associated anamorphic fungi inferred from the analyses of ITS and 5.8S rDNA sequences. *Mycologia* 93, 516–527.