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Sphaeropsis sapinea and *Botryosphaeria dothidea* endophytic in *Pinus* spp. and *Eucalyptus* spp. in South Africa

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Sphaeropsis sapinea (Fr.: Fr.) Dyko & B. Sutton and the anamorph of *Botryosphaeria dothidea* (Moug.) Ces. et De Not. are morphologically and ecologically similar fungi that cause serious canker and die-back diseases of *Pinus* and *Eucalyptus* spp. respectively in South Africa. In this article, the presence of both these fungi as symptomless endophytes in healthy pine and eucalypt tissue was demonstrated. *Sphaeropsis sapinea* was present in 50% of young, green *P. patula* Schl. et Cham., and 90% of *P. radiata* D. Don cones. In contrast, it was virtually absent from the cones of *P. elliottii* Engalm. et Vasey and *P. taeda* L. *Botryosphaeria dothidea*, on the other hand, was found to be common in all the *Eucalyptus* spp. tested, occurring in 93% of *E. smithii* R. T. Bak., 77% of *E. camaldulensis* Dehnh., 63% of *E. grandis* Hill ex Maid. and 57% of *E. nitens* (Deane et Maid.) Maid. leaves tested. The enigma of the rapid ingress of both these fungi in stressed or damaged trees might therefore be explained by their endophytic habit.

Keywords: *Botryosphaeria dothidea*, *Eucalyptus*, fungal endophytes, *Pinus*, *Sphaeropsis sapinea*.

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Introduction

The forestry industry in South Africa is economically dynamic and rapidly expanding. Currently, approximately 1 400 000 ha are planted to exotic *Eucalyptus* and *Pinus* species. As the estimated production of wood and fiber is expected to double by the year 2005 and the land area suitable for establishing plantations is limited to less than 2 000 000 ha, the industry is challenged to optimization (Denison & Kietzka 1993). Plantations are concentrated in the eastern parts of the country and include various climatic areas. Invariably, some plantations are established in marginal areas where the impact of stress-related pathogens is accentuated. The impact of fungal diseases on the industry has been ignored in the past but is rapidly gaining recognition (Wingfield 1987; Wingfield *et al.* 1991).

Many fungal pathogens are well established and cause diseases of *Pinus* and *Eucalyptus* spp. in South Africa (Wingfield 1987; Wingfield *et al.* 1991). These can account for millions of rands of losses due to reduced wood quality, loss of volume, and tree mortality (Zwolinski *et al.* 1990). Two important pathogens that are very similar both in ecology and morphology are *Sphaeropsis sapinea* (Fr.:Fr.) Dyko & B. Sutton and the anamorph of *Botryosphaeria dothidea* (Moug.) Ces. et De Not. *Sphaeropsis sapinea* is generally considered to be an opportunistic wound- and stress-related die-back and canker pathogen of *Pinus* (Swart & Wingfield 1991), and *B. dothidea* has a similar ecology on *Eucalyptus* (Smith *et al.* 1994).

Sphaeropsis sapinea is one of the most common fungi occurring on *Pinus* spp. and was first described under the name *Sphaeria pinea* Desm. in 1842 (Sutton 1980). Many synonyms exist for this fungus, with *Diplodia pinea* (Desm.) J. Kickx f. probably the most widely used (Punithalingam & Waterston 1970). Although this pathogen has been reported from many countries, it is most notorious in South Africa, where it causes extensive infection and mortality of *Pinus radiata* D. Don and *Pinus patula* Schl. et Cham. after hail damage (Laughton 1937; Swart *et al.* 1987). Many disease symptoms are associated with infections by *S. sapinea*, but shoot blight and top die-back are most common (Swart & Wingfield 1991). Frequent hailstorms and drought contribute largely to the extensive nature of die-back caused by this fungus in South Africa. Management of losses due to this pathogen in plantations is difficult and largely restricted to selection of *Pinus* spp. for disease tolerance (Swart & Wingfield 1991).

Like *S. sapinea, B. dothidea* has been known on woody plants for many years and has had a confused taxonomic history. The cosmopolitan nature and wide host range of this pathogen has been recognized for many years (Smith 1934). On *Eucalyptus*, *B. dothidea* causes a wide range of symptoms, including leaf spots, shoot die-back, branch and stem cankers (Barnard *et al.* 1987; Crous *et al.*, 1989; Davison & Tay 1983; Shearer *et al.* 1987; Smith *et al.*, 1994; Webb 1983). Infection and subsequent symptom development associated with this fungus is aided by the presence of wounds (Witcher & Clayton 1963) and environmental stress (Crist & Schoeneweiss 1975).

In South Africa, *B. dothidea* is associated with many important disease symptoms on *Eucalyptus* spp. In most cases, symptom development is associated with trees under stress (Smith *et al.* 1994). There is, however, good evidence for variation in susceptibility of various *Eucalyptus* spp. to this pathogen.

Recently, *B. dothidea* has been found to occur as a symptomless endophyte in *Eucalyptus nitens* (Deane *et* Maid.) Maid. in England (Fisher *et al.* 1993). The latter study prompted us to consider whether this pathogen might also occur in healthy *Eucalyptus* trees in this country. Given the ecological and taxonomic similarities of *B. dothidea* and *S. sapinea*, we also questioned whether the latter fungus might similarly exist in healthy pine tissue. The aim of this study was therefore to consider the possible existence of these important pine and eucalypt pathogens as symptomless endophytes. Implications to the South African forestry industry are also considered.

Materials and Methods

Pine tissue

Healthy, green, mature, but unopened cones (Gifford & Foster 1988) of four *Pinus* spp. were collected from commercial stands during March 1995. These included *Pinus elliottii* Engalm. *et* Vasey (Kwambomanbi area, northern KwaZulu-Natal), *P. patula* (Kwambonambi area, northern KwaZulu-Natal), *P. radiata* (Humansdorp area, Eastern Cape) and *Pinus taeda* L. (Kwambonambi area, northern KwaZulu-Natal). Ten cones from each pine species (two trees with five cones per tree) were surface sterilized by an immersion sequence in 96% ethanol for 1 min; undiluted bleach (3.5–5% available chlorine) for 5 min; 96% ethanol for 30 sec and rinsed in sterile water. All cones were opened under sterile conditions and eight seeds, eight seed wings, eight tissue segments from ovuliferous scales and eight segments of pith tissue from each cone were placed in Petri dishes containing MEA (2% Biolab, malt extract agar), supplemented with 200 mg l^{-1} chloramphenicol to suppress bacterial growth. Plates were incubated at 20°C in the dark for 10 days. Dark, fast-growing fungi were transferred to agar plates containing water agar (WA) on which sterile pine needles had been placed, and allowed to form pycnidia. Isolates of *S. sapinea* were identified based on their characteristic conidia, using light microscopy.

Eucalypt tissue

Healthy leaves of four *Eucalyptus* spp. were collected from commercial stands during February 1995. These included *Eucalyptus camaldulensis* Dehnh. (Kwambonambi area, northern KwaZulu-Natal), *Eucalyptus grandis* Hill ex Maid. (Kwambonambi area, northern KwaZulu-Natal), *E. nitens* (Piet Retief area, Mpumalanga) and *Eucalyptus smithii* R. T. Bak (Piet Retief area, Mpumalanga). Thirty leaves from each of the four species (two trees with 15 leaves per tree) were surface sterilized as previously described. Following surface sterilization, each leaf was divided into five pieces and these were placed in Petri dishes containing MEA. Plates were incubated at 20°C in the dark for 10 days. Dark, fast-growing fungi were transferred to 2% MEA and incubated under continuous cool fluorescent light to induce sporulation. Isolates of *B. dothidea* were identified on the basis of their characteristic conidia, using light microscopy.

Results and Discussion

Sphaeropsis sapinea was a common inhabitant of various parts of healthy, asymptomatic cones of *P. radiata* and *P. patula* but was virtually absent in similar tissue from *P. elliottii* and *P. taeda* (Table 1). The relative abundance of this important pathogen in the four *Pinus* spp. tested closely matches their relative susceptibility to this pathogen (Swart & Wingfield 1991). Therefore, amongst *Pinus* spp. commonly propagated in South Africa, *P. radiata* and *P. patula* are known to be the most susceptible and *P. elliottii* and *P. taeda* the most resistant to infection by *S. sapinea*.

Sphaeropsis sapinea has been found to be able to survive as endophytic infections in the wood of *Pinus sylvestris* (Petrini & Fisher 1988), but the fact that *S. sapinea* can live as a symptomless endophyte in healthy pine cone tissue has not previously been recognized. Rather, the fungus is generally thought to primarily infect wounded and stressed tissue and its occurrence on dying or dead tissue has been considered an indication of a primarily saprotrophic habit. Results of this study might imply that the fungus preferentially infects healthy cone tissue, remaining latent until the onset of stress. Proliferation in pine tissue other than cones would then originate from existing infections, rather than from new infections of wounded or stressed tissue.

Sphaeropsis sapinea is one of the most important pathogens of pines in South Africa, causing extensive die-back in plantations of susceptible species after hail (Swart *et al.* 1987). Trees are rapidly colonized by the fungus and become blue-stained and die within a few weeks. The speed at which this colonization takes place has always been enigmatic. The fact that this fungus is most likely extensively present within living tissues of trees suggests that it is stress due to hail, rather than infection of hail wounds alone, that leads to rapid colonization and tree death. This intriguing aspect of the biology of *S. sapinea* is currently being investigated.

Botryosphaeria dothidea was commonly isolated from leaves of all four *Eucalyptus* spp. considered in this study (Table 2). In some species, such as *E. smithii*, virtually every leaf examined was infected with this fungus. *B. dothidea* is evidently a common inhabitant of asymptomatic *Eucalyptus* leaves in South Africa, thus supporting the results of Fisher *et al.* (1993), who investigated *E. nitens* in England. Based on these results, it seems likely that the occurrence of *B. dothidea* on leaf spots and on dead and dying *Eucalyptus* leaves might well be derived from earlier latent endophytic infections of healthy leaves. This would be in contrast to the dogma suggesting that the fungus is a saprophyte that colonizes dead tissue.

Like *S. sapinea*, infections of *Eucalyptus* spp. associated with *B. dothidea* in South Africa develop rapidly after the onset of environmental stress such as frost, hot winds or drought. The rapid development of these disease symptoms, particularly under conditions not likely to be conducive to spore germination, has been a source of confusion. The presence of latent endophytic infections of the fungus in healthy tissue would provide a logical explanation for symptom development, as it is observed under field conditions.

Endophytic fungi are those with the ability to colonize healthy plant tissue without exhibiting virulence or symptoms (Carroll 1990; Rollinger & Langenheim 1993), thus not causing obvious damage (McCutcheon *et al.* 1993). These fungi occur entirely within the host tissue (Todd 1988) and include latent pathogens and fungi with mycorrhizal associations (Petrini 1991). The

Table 1 Number of cones or cone parts with endophytic infections of *S. sapinea* in four species of *Pinus*

	% *S. sapinea* isolates[1]				
Pinus spp.	% cones infected	% seed infected	% seed blades infected	% pith tissue infected	% ovuliferous scales infected
P. elliottii	0	0	0	0	0
P. patula	50	19	20	30	18
P. radiata	90	81	85	89	74
P. taeda	10	0	0	1	0

[1]10 cones, 80 seeds (8 per cone), 80 seed blades (8 per cone), 80 pith tissues (8 per cone) and 80 ovuliferous scales (8 per cone) were sampled for each *Pinus* sp.

Table 2 Number of leaves and leaf segments with *B. dothidea* endophytic infections in four species of *Eucalyptus*

Eucalyptus spp.	No. of leaves tested	% of leaves infected[1]	% of leaf segments infected[2]
E. camaldulensis	30	77	60
E. grandis	30	63	58
E. nitens	30	57	47
E. smithii	30	93	77

[1]Percentage of leaves infected with *B. dothidea* in each species of *Eucalyptus*

[2]Percentage of leaf segments that yielded isolates of *B. dothidea*. Each leaf sampled was cut into five pieces, yielding a total of 150 segments per species

nature of endophytic relationships is variable, with latent pathogens also sharing an endophytic relationship with their hosts. Pathogens can thus remain latent for long periods of time, causing symptoms only when the physiological or ecological conditions favour virulence (Carroll 1986; Bettucci & Saravay 1993; Kulik 1984; McOnie 1967; Nataniels & Taylor 1983; Tokunaga & Ohira 1973). Based on the results of this study, we suggest that *S. sapinea* and *B. dothidea* be considered as latent pathogens capable of endophytic infections. This is in contrast to the view that they are primarily opportunists that preferentially colonize wounded and stressed tissue.

The fact that *S. sapinea* and *B. dothidea* exist endophytically in healthy pine and eucalypt trees, respectively, has important implications for the South African forestry industry. For example, the presence and relative abundance of these fungi in healthy tissue might provide a reflection of the inherent susceptibility of species to these pathogens. This seems to be the case with *S. sapinea,* which was primarily present in species known to be most susceptible to it. It may also be possible to eliminate endophytic infections in high-value trees, such as those in valuable seed orchards, through chemical treatments, consequently reducing losses in these situations. Clearly, the endophytic nature of *S. sapinea* and *B. dothidea* in pines and eucalypts deserves further intensive investigation.

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