NEW OR UNUSUAL RECORDS

Canker and die-back of *Eucalyptus* in South Africa caused by *Botryosphaeria dothidea*

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Die-back and canker of several *Eucalyptus* species and clones were observed during a survey of the most important forestry areas of South Africa. Disease was often associated with extreme environmental conditions such as drought, hot winds and frost. *Botryosphaeria dothidea*, which is well known as a cause of canker and die-back of *Eucalyptus* in other countries, was consistently isolated from symptomatic trees. Artificial inoculations of *Eucalyptus nitens* resulted in lesion development, confirming the pathogenicity of *B. dothidea* to *Eucalyptus* in South Africa. This is the first report of this disease in South Africa.

The forestry industry in South Africa depends largely on species of *Pinus* and *Eucalyptus* from which it produces pulp, paper and structural timber. Commercial plantations cover approximately 1·2 million ha, and about half of this area is planted with *Eucalyptus* spp. (Anonymous, 1990). Despite the importance of eucalypts in South Africa, very little attention has been given to the possibility that diseases might be affecting the value of the crop or the success of establishing plantations. Concern has been expressed regarding the potential impact of diseases on the industry in view of the extensive monoculture system practised (Wingfield *et al.*, 1991).

In a survey of *Eucalyptus* plantations during 1991 and 1992, twig die-back and stem cankers were found to be widespread on various *Eucalyptus* spp. (*E. grandis, E. nitens, E. macarthurii* and *E. smithii*), clones of *E. grandis* and hybrids of *E. grandis* with *E. camaldulensis* and *E. urophylla*. Twig die-back was the most common symptom and occurred on young current-year shoots and terminal leader shoots. Trees most commonly affected ranged in age from 1 to 2 years. Dead shoots were black, often curled and retained dead leaves (Fig. 1a). Visible die-back was preceded by a dark discoloration of the pith and immediate surrounding sapwood in proximate stem portions (Fig. 1b).

Cankers were commonly observed on twigs, branches and main stems. On young twigs and branches they were often associated with hail damage. The first sign of canker formation was the presence of reddish-brown necrotic lesions in the bark of young shoots. With the extension of lesions to the wood, small irregular cankers were produced on the branches, causing deformity (Fig. 1c), and often became sites of wind breakage. In severe cases, large deformed cankers developed which exceeded the diameter of the trunk. The cankers were also associated with a characteristic blackish discoloration of the heartwood and pith. These types of cankers were most commonly associated with younger branches and main stems of up to 3 years of age.

Main stem cankers were evident as cracks in the bark exuding kino. The location and degree of canker formation appeared to vary between trees, especially in seedling populations, suggesting differences in host resistance. In trees that appeared relatively resistant, cankers were small and localized around the point of infection, causing irregular zones of cracks. Cankers were distributed much more widely on the trunks of apparently less resistant trees, often extending to the apex of the tree. Extensive cankering was associated with general discoloration of the heartwood in mature trees (Fig. 2a).

The bark of severely infected trees appeared reddish in color due to the extensive production of kino, which is water soluble and spreads down the stems after rain. Cankers caused the formation of kino pockets in the sapwood (Fig. 2b) which were sometimes sufficiently large and numerous to form kino rings (Fig. 2c). Trees most commonly affected ranged in age from
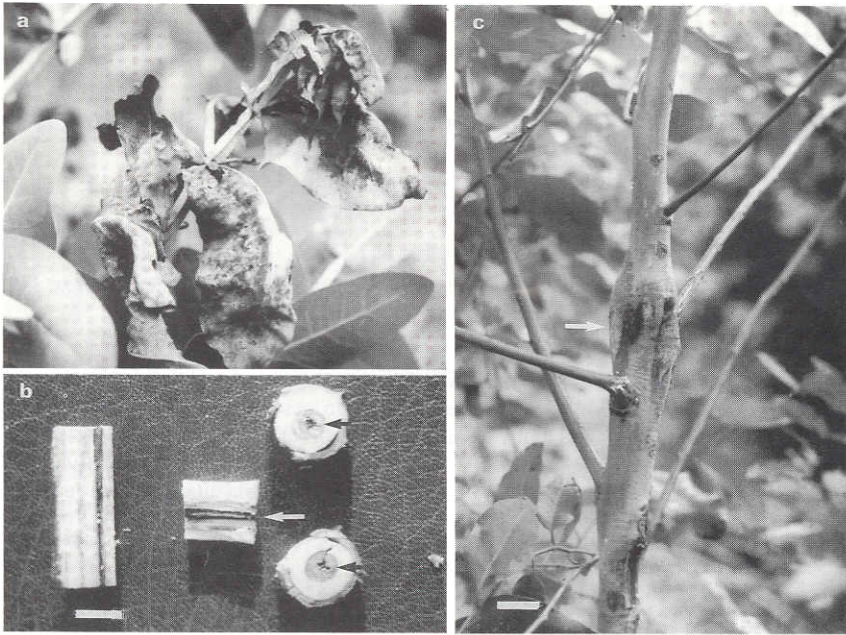
Fig. 1. Symptoms on *Eucalyptus* associated with infection by *Botryosphaeria dothidea*. (a) Shoot die-back of *Eucalyptus nitens* (bar = 30 mm). (b) Dark discoloration of the pith and immediate surrounding sapwood (arrow) associated with die-back of *E. nitens* (bar = 5 mm). (c) Stem-deforming cankers (arrow) on an *E. grandis* clone (bar = 30 mm).

3 to 12 years. Such extensive kino production renders the wood unacceptable to pulp mills, as it would necessitate extended periods of bleaching. The kino rings also weaken the wood causing it to split when used as structural timber.

In most cases where die-back due to *Botryosphaeria dothidea* leads to mortality, there was apparently an association with environmental conditions stressful to the trees. Winds associated with hot temperatures, hail, frost and drought often occur in certain areas and probably served as predisposing factors for infection by *B. dothidea*. Therefore *B. dothidea* infections on predisposed trees were often conspicuous, leading to severe die-back, cankering and in some instances mortality, especially in the case of 1–2 year-old transplants. There were, however, some clones and hybrids that tended to tolerate damage by regenerating terminal leader shoots, and showed only reduced growth, forking and stem deformation.

Perithecia of *B. dothidea* (Moug.) Ces. et de Not (= *B. ribis* (Tode. : Fr.) Grossenb. & Dugger) were occasionally observed on dead twigs. These were dark and short necked. Asci were bitunicate (70–120 × 16–24 µm) and each contained eight ascospores. Ascospores (16–25 × 7–10 µm) were unicellular, hyaline and ovoid. Conidiomata were frequently observed on dead and dying tissue. These were dark, ostiolate pycnidia. Conidiogenous cells developed from the inner walls of pycnidia and the first conidia developed holoblastically. Conidia exuded from the pycnidia in white masses during periods of high relative humidity. Conidia (17–25 × 5–7 µm) were unicellular, hyaline and varied from ellipsoidal to fusiform with distinctly truncate bases. The conidia of some isolates became darker with age and developed two to three septa prior to germination. Germinating conidia had one or two germ tubes originating either from or near the apices.

Single-spore isolates were made on water agar from spore masses emerging from perithecia and pycnidia formed on dead shoots in the field. Germinating ascospores and conidia were transferred to 2% malt extract agar (Biolab) plates and incubated at 20°C under continuous cool fluorescent light to stimulate sporulation. Colonies were initially white and fluffy, becoming dark brown to black with age. Conidiomata were generally formed after 2 weeks but
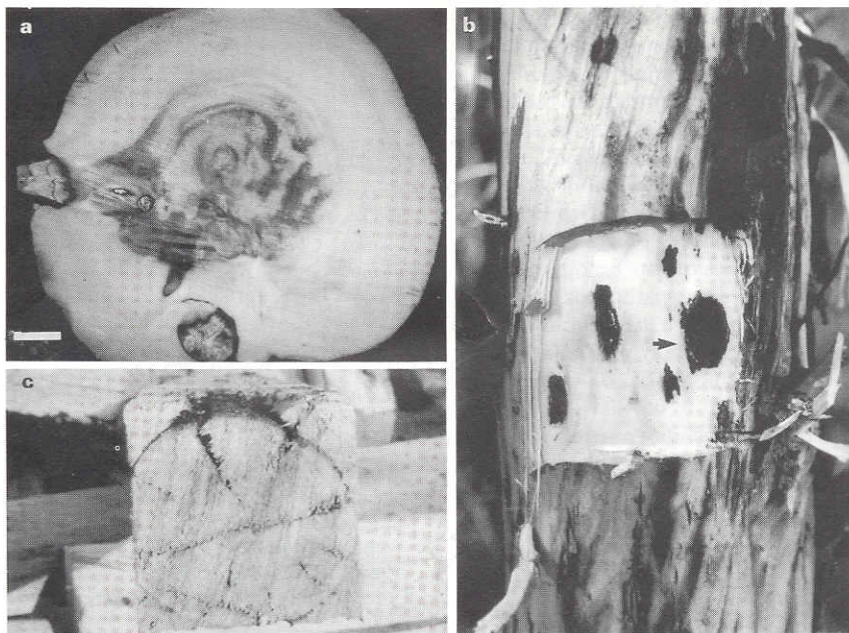
Fig. 2. Symptoms on *Eucalyptus* associated with infection by *Botryosphaeria dothidea*. (a) Extensive heartwood discoloration of a mature *E. grandis* clone (bar = 35 mm). (b) Kino (resin) pockets formed in the sapwood (arrow) associated with cankers on *E. nitens* (bar = 40 mm). (c) Ring of kino in the sapwood (arrows) of an *E. grandis* clone (bar = 40 mm).

occasionally colonies lost their pigmentation and failed to sporulate, even after 30 days of incubation. The teleomorph was never observed in culture and attempts to induce teleomorph formation by pairing isolates were unsuccessful.

Pathogenicity tests were conducted on 2-year-old *E. nitens* during February 1989 in the Piet Retief area, South Africa, using an isolate from *E. nitens* growing in the same area. Twenty trees (approximately 50 mm in diameter) were inoculated by removing a single 6-mm diameter piece of bark (1 m above soil level) on each tree with a cork-borer. The bark discs were replaced with discs of MEA from a 1-week-old culture of *B. dothidea*. Twenty control trees were inoculated with sterile MEA disks. Wounds were sealed with masking tape. After 3 months lesions had developed on all trees inoculated with *B. dothidea*, whereas all of the control inoculation wounds had healed. Lesions were in the range 32–64 mm long with a mean of 42·3 mm. *B. dothidea* was re-isolated from all lesions resulting from inoculation.

In South Africa, *B. dothidea* has been previously isolated from leaves of *Eucalyptus* species (Crous *et al.*, 1989) but this is the first report of it being associated with twig die-back, cankers and mortality of eucalypts. The fungus has previously been reported to cause canker and die-back diseases of *Eucalyptus* species in Australia and the USA (Davison & Tay, 1983; Webb, 1983; Barnard *et al.*, 1987; Shearer *et al.*, 1987). Webb (1983) reported the abandonment of commercial seed production of *E. camaldulensis* because of the impact of diseases caused by *B. dothidea*, but all other reports were of isolated problems. Shearer *et al.* (1987) reported the death of *E. radiata* in selection trails, Davison & Tay (1983) reported the natural occurrence of *B. dothidea* cankers in *E. marginata* forests and Barnard *et al.* (1987) reported *B. dothidea* to be involved in a complex of organisms that cause coppice failure of *E. grandis*. The damage in South Africa is widespread and may be more severe, with more significant mortality, than elsewhere.

B. dothidea is the most economically important and well-documented species in the genus and causes diseases on various woody hosts (Smith,

1934; Punithalingam & Holliday, 1973). Stress is known as a major factor contributing to disease development caused by *Botryosphaeria* spp. Stresses that have been reported to predispose plants to invasion by *Botryosphaeria* spp. and to subsequent disease development include drought, freezing and defoliation (Crist & Schoeneweiss, 1975; Wene & Schoeneweiss, 1980; Pusey, 1989). Indeed, *Botryosphaeria* spp. are, in general, considered to be opportunistic stress and wound related pathogens. As noted above, South Africa has a harsh and extreme climate, and these factors may play an important role in the epidemiology of *B. dothidea* on eucalypts.

B. dothidea may become one of the most important pathogens of *Eucalyptus* in South Africa. Strategies to avoid losses, including screening species, clones and hybrids for tolerance of this disease, are therefore currently being implemented.

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