Short communications

Eucalyptus die-back in South Africa associated with *Colletotrichum gloeosporioides*

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Die-back of members of several *Eucalyptus* species, clones and hybrids was observed during a survey of forest plantations in the Mpumalanga and KwaZulu-Natal provinces, South Africa. This symptom was often associated with agents of environmental stress such as drought, frost and hot winds. *Botryosphaeria dothidea*, a well-known pathogen of *Eucalyptus* was frequently isolated from twigs showing die-back symptoms. In some cases, *Colletotrichum gloeosporioides* was isolated together with *B. dothidea*. Artificial inoculations of members of a *Eucalyptus grandis* clone with both fungi resulted in lesion development. Although *C. gloeosporioides* was isolated much less frequently and only in the Mpumalanga Province, it gave rise to larger lesions after inoculation than did *B. dothidea*. This is the first report of die-back of *Eucalyptus* trees caused by *C. gloeosporioides* in South Africa.

Keywords: Stress, anthracnose, *Botryosphaeria dothidea*, *Colletotrichum gloeosporioides*.

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Commercial forestry in South Africa largely relies on plantations of exotic *Eucalyptus* and *Pinus* that cover approximately 1 400 000 ha (Denison & Kietzka 1993a). The impact of various fungal pathogens (Wingfield, Swart & Kemp 1991) on the industry as measured by tree mortality, potential yield loss and reduced wood quality accounts for millions of Rands of loss (Zwolinski, Swart & Wingfield 1990). The commercial forestry industry in South Africa has, therefore, tended to move towards the intensive use of *Eucalyptus* clones and hybrids (Denison & Kietzka 1993a; Denison & Kietzka 1993b). Disease tolerance, as a desirable trait, has unfortunately not been incorporated into screening and selection programs from the start. This scenario provided opportunity for disease avoidance, but also resulted in serious losses, where susceptible planting stock was used.

Surveys of *Eucalyptus* plantations in the Mpumalanga and KwaZulu-Natal provinces have revealed that twig die-back is a wide spread and common symptom on various clones of *Eucalyptus grandis* Hill: Maid. and hybrids of *E. grandis* with *E. camaldulensis* Dehnh. Smith, Kemp and Wingfield (1994) reported that *Botryosphaeria dothidea* (Moug.) Ces et de Not. was responsible for wide spread die-back and canker symptoms on members of various *Eucalyptus* species, clones and hybrids. The aim of this paper is to discuss the role of *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. as a pathogen causing die-back, in some *E. grandis* clones and *E. grandis* × *E. camaldulensis* hybrids. Twig die-back occurred on young current year shoots and terminal leader shoots of trees ranging from 1–2-years-old (Figure 1a). Dead shoots were black and fungal fruiting structures were abundant on their surfaces. *Botryosphaeria dothidea* and, to a lesser extent *C. gloeosporioides*, was isolated from the lesion margins, as well as from erumpent fruiting structures. *Botryosphaeria dothidea* was wide spread (Smith, Kemp & Wingfield 1994), whereas *C. gloeosporioides* was confined to the Mpumalanga Province on clones of *E. grandis* and *E. grandis* × *E. camaldulensis* hybrids. In most cases where die-back due to *C. gloeosporioides* was observed, there was a direct association with stress conditions. Damage by hot wind appeared to be the major predisposing factor that contributed to die-back caused by *C. gloeosporioides*.

Table 1 Mean bark and wood lesion lenghts of a *E. grandis* clone innoculated with *Botryosphaeria dothidea* and *Colletotrichum gloeosporioides*

	Mean length [1]	
	Lesion in bark and wood (mm) [2]	
Treatment	Bark	Wood
Botryosphaeria dothidea	45.6 d	143.1 b
Colletotrichum gloeosporioides	63.1 c	235.6 a
Control	8.1 e	8.6 e

[1] Data represent the means of 20 trees innoculated with each fungus and the control.

[2] Letters specify significant differences at a cinfidence level of 5%.

Conidiomata of *C. gloeosporioides* were frequently observed on dead and dying tissue. These were typical dark acervuli exuding large masses of pale pink to pale orange conidia. Conidia were unicellular, hyaline, straight cylindrical with an obtuse apex and truncate base [10–(16)–22 × 3–(5)–6 µm] (50 conidia measured). Setae were commonly observed on host material, often partly submerged in the conidial masses, brown and septate [38–(61)–104 × 1–(4)–7 µm] (50 setae measured). Appressoria were not observed on the host material.

Single conidial isolates were made on water agar (WA, Biolab) from spore masses emerging from acervuli on dead shoots. Germinating conidia were transferred to 2% malt extract agar (MEA, Biolab) in Petri dishes and incubated at 20°C under continuous cool fluorescent light to stimulate sporulation. Colonies were initially white, becoming mouse grey to light olive green, with concentric rings associated with sporulation (Baxter, van der Westhuizen & Eicker 1983). Conidiomata were generally formed after 2 weeks. The formation of setae was variable in culture with the majority of isolates not producing these structures after 3 weeks of incubation.

Pathogenicity tests were conducted on 3-year-old trees of an *E. grandis* clone in the White River area, Mpumalanga Province. Twenty trees (approximately 50 mm in diameter) were prepared for inoculation by drilling a hole (2 cm deep, 5 mm in diameter) into the main stem 1 m above the ground. The holes were injected with a suspension of conidia obtained from conidiomata. Twenty control trees were inoculated with sterile water. Wounds were sealed with masking tape. After 3 months, lesions had developed on all trees inoculated with *B. dothidea* and *C. gloeosporioides*, whereas the control inoculation wounds either had healed or showed no lesion development. Lesion lengths were measured as visible damaged areas on the bark (Figure 1b) as well as the discoloration within the wood (Figure 1c). Discolored lesions in the wood extended upward and downward from the point of inoculation (Figure 1c). All results were analyzed by means of a two factorial analysis of variance, and for significant differences using Tukey's procedure for the compar-

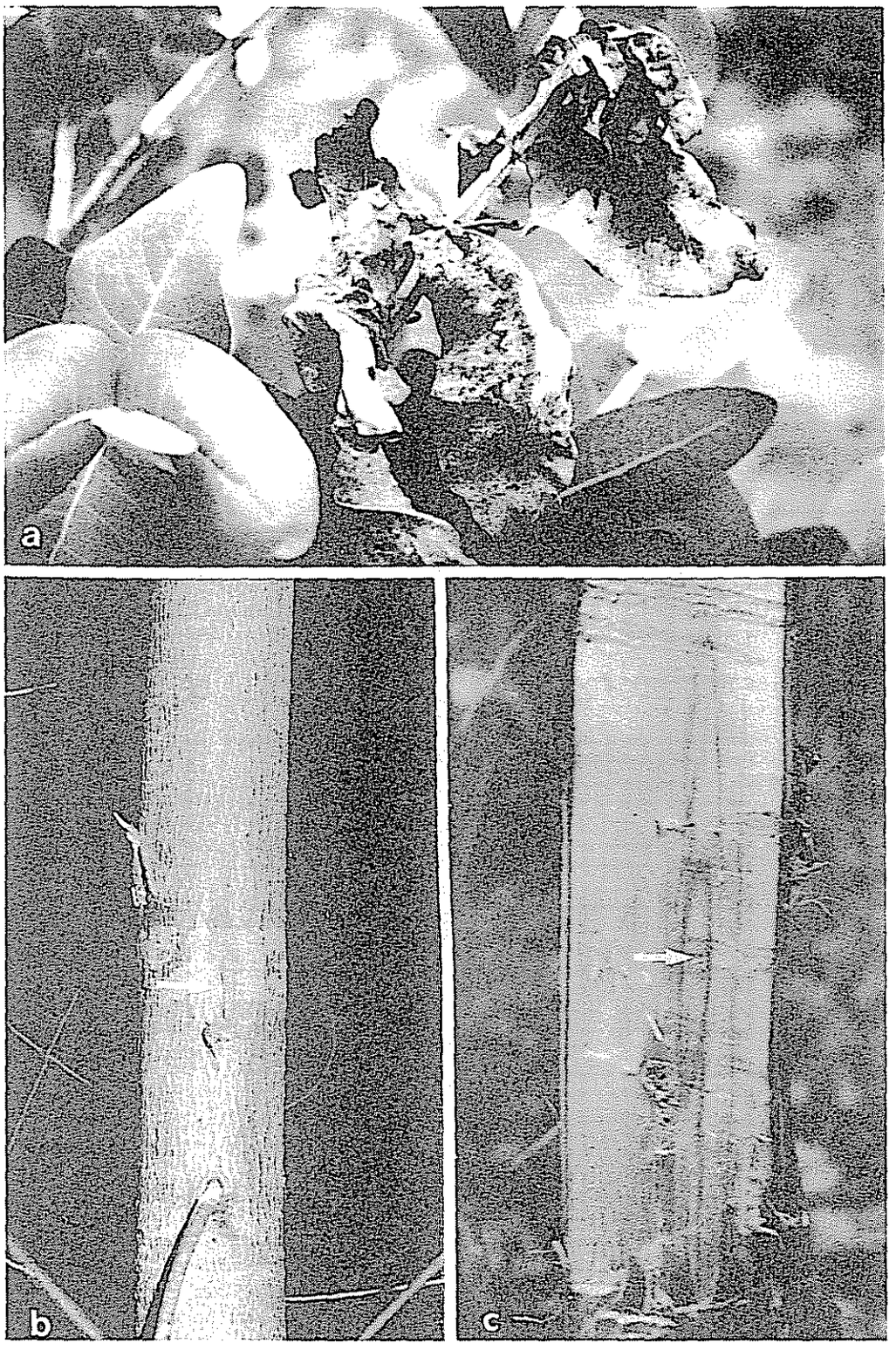

Figure 1 Symptoms of infection by *C. gloeosporioides* and *B. dothidea* and lesions associated with innoculation with *C. gloeosporioides*. (**a**) Typical twig die-back associated with natural infections by *C. gloeosporioides* and *B. dothidea* (**b**) Lesions on the bark of *E. grandis* clone inoculated with *C. gloeosporioides* (**c**) Lesion in the wood of inoculated tree (arrow = point of inoculation).

ison of means at a 5% confidence level. Significant differences were found to occur between the bark and wood lesions, with the latter being the most extensive (Table 1). *Colletotrichum gloeosporioides* (mean wood lesion length 235.6 mm) was found to cause significantly larger lesions than *B. dothidea* (mean wood lesion length 143.1 mm). These data are, however, based on the inoculation of only one isolate for each fungus, and more variation within each fungus population is possible.

Colletotrichum gloeosporioides is known as an ubiquitous polyphage, occurring as a saprotroph or pathogen on a wide variety of plants (Sutton 1980) causing symptoms such as leaf, shoot and fruit anthracnose, post-bloom fruit drop, leaf spot and postharvest fruit rot (Waller 1992). *Colletotrichum gloeosporioides* is also known to be associated with leaf (Farr *et al.* 1989)

and branch lesions (Dianese, Ribeiro & Moraos 1985) of *Eucalyptus*. In South Africa, this fungus seems to be well established, causing post harvest fruit rot of avocado (Darvas & Kotze 1987) and mango (Darvas 1991), as well as die-back of indigenous *Protea* spp. (Benic & Knox-Davies 1983, Serfontein & Knox-Davies 1990).

Colletotrichum gloeosporioides preferentially infects young succulent tissue (Dodd *et al* 1991), which is consistent with field observations on young *Eucalyptus* shoots in South Africa. It has been shown to be present as quiescent infections in avocado and mango (Prusky & Plumbley 1992) and also to occur in asymptomatic leaves and twigs of Citrus and Rhododendron (Von Arx 1957) and leaves of *Eucalyptus nitens* (Deane et Maid.) Maid. and *E. grandis* (Smith, Wingfield & Petrini 1996). Such latent infections could give rise to the die-back of stressed shoots observed in this study. Although the impact of *C. gloeosporioides* on the *Eucalyptus* industry in South Africa seems to be relatively insignificant at present, we consider this fungus to be a pathogen worth noting in disease surveys.

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In vitro propagation of some *Cyrtanthus* species

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Shoots and roots were initiated on bulb explants of *Cyrtanthus brachyscyphus*, *C. elatus*, *C. falcatus*, *C. guthrieae*, and *C. mackenii* var. *mackenii*. *C. breviflorus* produced small amounts of wound callus only. The species differed in their response to the different levels of plant growth regulators used. In general shoot formation was most favourable with high concentrations BA (2 mgl^{-1}) and lower concentrations NAA (1 mgl^{-1}). Best root formation was obtained with low BA and NAA (0–0.5 mgl^{-1}) concentrations. *Cyrtanthus brachyscyphus* was the most prolific shoot producer, with a 3-fold increase at every sub-culture. *C. elatus*, *C. guthrieae*, and *C. mackenii* var. *mackenii* were less vigorous and on average showed a 1.5-fold increase at every sub-culture. *C. falcatus* produced a low number of shoots from the explants and this did not increase with subsequent sub-cultures. Rooted plantlets were successfully acclimatized in vermiculite in a mist house (100% survival).

Keywords: Bulb explants, *Cyrtanthus*, *in vitro* propagation, shoot and root formation.

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Cyrtanthus L.f. is a member of the family Amaryllidaceae and is mainly a southern Africa genus (Olivier 1980; Du Plessis & Duncan 1989). There are fifty-one species in southern Africa (Dyer 1976; Du Plessis & Duncan 1989). This bulbous herb may be evergreen, winter- or summer growing. The leaves differ significantly among the species, from slender to strap-shaped. The flowers are single to many and umbellate, tubular and pendulous to widely bell shaped. The colour of the flowers ranges from white and cream to shades of pink, red, orange, and dark maroon (Figure 1 A–C). The seeds are black, flattened and somewhat winged.

The six species studied in this paper are described (Duncan 1990a, 1990b; Du Plessis & Duncan 1989) as follows: *Cyrtanthus guthrieae* L. Bol. is endemic to Bredasdorp. This plant is deciduous and mainly winter-growing. Flowering occurs in March to April. The plant is 10–12 cm in height and the large