Short communications

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

H. Smith*, M.J. Wingfield† and T.A. Coutinho‡

*ARC-Institute for Tropical and subtropical Crops, Private Bag X 11208, Pretoria, 1001 Republic of South Africa
†Tree Pathology Co-operative Programme, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, 0001 Republic of South Africa

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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 granis* 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*.

*To whom correspondence should be addressed.

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 Rand s of loss (Zwolinski, Swart & 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*.

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 × (5)–6 μm] (50 conidia measured). Septa were commonly observed on host material, often partly submerged in the conidial masses, brown and septate [38–(61)–104 × (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 conidiotama. 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-

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Bark</th>
<th>Wood</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Botryosphaeria dothidea</em></td>
<td>45.6 e</td>
<td>143.1 b</td>
</tr>
<tr>
<td><em>Colletotrichum gloeosporioides</em></td>
<td>63.1 e</td>
<td>235.6 a</td>
</tr>
<tr>
<td>Control</td>
<td>8.1 e</td>
<td>8.6 e</td>
</tr>
</tbody>
</table>

Data represent the means of 20 trees inoculated with each fungus and the control.

Letters specify significant differences at a confidence level of 5%.

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*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*.

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*Figure 1b* Lesions were measured as visible damaged areas on the bark. The formation of setae was variable in culture with the majority of isolates not producing these structures after 3 weeks of incubation.

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*Figure 1c* Discolored lesions in the wood extended upward and downward from the point of inoculation.
Figure 1 Symptoms of infection by *C. gloeosporioides* and *B. dothidea* and lesions associated with inoculation 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).

Reason 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)