

## A serious canker disease of *Eucalyptus* in South Africa caused by a new species of *Coniothyrium*

Michael J. Wingfield,<sup>1</sup> Pedro W. Crous<sup>2</sup> and Teresa A. Coutinho<sup>1</sup>

<sup>1</sup>Tree Pathology Cooperative Programme, Department of Microbiology and Biochemistry, University of the Orange Free State, P.O. Box 339, Bloemfontein 9300;

<sup>2</sup>Department of Plant Pathology, University of Stellenbosch, P. Bag XI, Matieland 7602, South Africa

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### Abstract

*Eucalyptus* spp. are being propagated extensively as exotics in plantations in South Africa, and many other parts of the world. In South Africa, a number of diseases result in serious losses to this resource. This paper describes a new and very damaging stem canker disease, which has recently appeared on plantation-grown eucalyptus in South Africa. The disease, first noted in an isolated location in Zululand is now common in other parts of the country, and is typified by discrete necrotic lesions on stems. These lesions coalesce to form large, gum-impregnated cankers and malformed stems. The causal agent of the disease, as inferred from pathogenicity tests, is a new species of *Coniothyrium* described here as *C. zuluense*. This fungus is a serious impediment to eucalypt propagation in South Africa, and is most likely a threat to similar forest industries elsewhere in the world.

**Key words:** *Eucalyptus* stem canker, *Coniothyrium zuluense*, tree disease, South Africa.

### Introduction

The forestry industry of South Africa relies almost exclusively on monocultures of *Pinus* and *Eucalyptus* species. Species of these genera are planted in approximately equal proportions and 1.5 million ha of land are currently afforested. *Eucalyptus* plantations comprise numerous species with *E. grandis* Hill ex Maid. the most common species planted. A contemporary approach is to raise *Eucalyptus* trees vegetatively from cuttings, as opposed to seedling propagation. Clones of *E. grandis* and hybrids of this and numerous other species such as *E. camaldulensis* Dehnh., *E. urophylla* S.T. Blake, *E. tereticornis* Sm. and *E. nitens* (Deane et Maid.) Maid., are thus being propagated on a relatively large scale.

Diseases pose one of the greatest threats to the South African forestry industry [1]. Various diseases have already had a profound effect on the industry and have either made the planting of certain species impossible or have limited planting of desirable species to specific areas. Management practices have also had to be adjusted to accommodate the ravages of certain dis-

eases [2]. Clonal propagation of *Eucalyptus* has raised fears that the impact of diseases could increase due to increased genetic uniformity of plantations. Strategies to ensure that large numbers of clones are planted, and that a high degree of genetic diversity is maintained in clonal plantations, have, therefore, been implemented [3].

A number of diseases have been reported to occur on various species and clones of *Eucalyptus* in South Africa for the first time during the course of the past decade. Amongst the most serious of these are *Cryphonectria* canker caused by *Cryphonectria cubensis* (Bruner) Hodges [4] and *Botryosphaeria* canker and die-back caused by *Botryosphaeria dothidea* (Moug.: Fr.) Ces. & De Not [5]. Amongst the new diseases that have appeared on eucalypt trees in recent years is a serious stem canker, apparently unknown elsewhere in the world. The aim of this paper is to describe the disease and the fungus responsible for it.

## Materials and methods

### Pathogen description

A species of *Coniothyrium* Corda was found to sporulate abundantly on the surface of necrotic lesions on the stems of diseased trees. This is only fungus consistently associated with the disease. Single-celled dematiaceous conidia were collected in sterile distilled water and allowed to germinate on the surface of 1% malt extract agar (MEA) (10 g Merck malt extract, 20 g Merck agar and 1000 ml distilled H<sub>2</sub>O). Single germinating conidia were then transferred to MEA plates in order to observe growth characteristics in culture. Growth studies using a single-conidial culture of the *Coniothyrium* sp. were conducted on MEA for 21 days in the dark at temperatures ranging from 5 to 35 °C at 5° intervals, with three plates per temperature. The experiment was repeated once.

In order to clearly determine the mode of conidium development, conidiogenous cells were viewed using scanning electron microscopy (SEM). Pycnidia formed on colonized *E. grandis* leaves on water agar were torn open using a sharp scalpel blade. Small pieces of leaf tissue approximately 5 mm<sup>2</sup> bearing these pycnidia were prepared for SEM. Leaf material was fixed in 3% glutaraldehyde in 0.05 M sodium cacodylate buffer and post-fixed in 2% osmium tetroxide in the same buffer. The tissue was then dehydrated in a graded acetone series, critical point-dried and sputter-coated with gold palladium. The material was viewed using a Joel JSM 6400 scanning electron microscope.

### Pathogenicity tests

Pathogenicity tests were conducted on 6-month-old *E. grandis* trees of the clone ZG14 in the Kwambonambi area of Zululand. Fifteen trees were inoculated with MEA plugs derived from a single-conidial isolate of the *Coniothyrium* sp. from infected trees in the area. The same number of trees was also inoculated with sterile MEA discs to serve as controls. Inoculations were done in the field during early summer, by removing a 10 mm diam. disc of bark from the trees at breast height and replacing this with a disc of agar bearing the fungus, or an uninoculated disc in the case of the controls. Inoculation sites were covered with masking tape to prevent desiccation of the inoculum. Trees were inspected for disease development 6 weeks after inoculation.

## Results

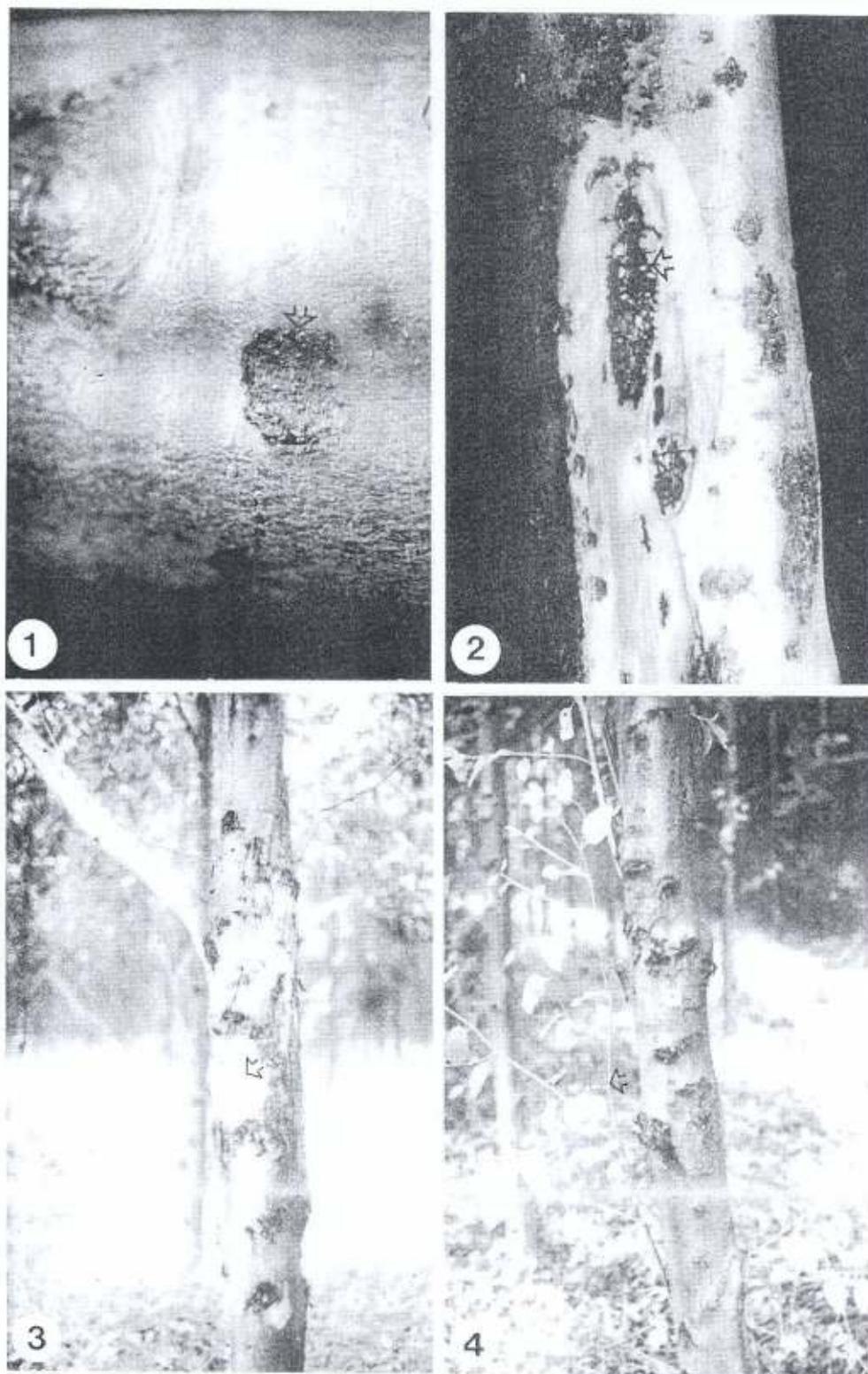
### Disease development

The earliest signs of natural infection on *E. grandis* clones are small (2–5 mm diam.), discrete, necrotic lesions on the young green bark (Figure 1). These lesions coalesce to form large necrotic patches on the stems from which copious amounts of red/brown gum exude (Figure 2). Infection sites form in a discrete area of the green stem (Figure 3) suggesting that infections occur in a specific period during the growing season. These infection areas on stems become swollen and spindle-shaped (Figure 3). Epicormic shoots are commonly produced in the areas of the cankers, indicative of a partial girdling of the stems (Figure 4). New infections occur on young green tissue annually which, in severe cases, leads to the development of a series of cankers on stems representing annual infection events. In severely infected clones, the tops of trees die due to the girdling effect of cankers and the production of epicormic shoots or branches, resulting in a resumption of apical dominance. These branches subsequently also become diseased and die at the apices, with the result that height growth virtually ceases.

### Pathogen description

The *Coniothyrium* sp. consistently associated with *Eucalyptus* stem cankers is typical of this genus. Pycnidia of the fungus are produced below the epidermis on necrotic tissue and give rise to abundant, single-celled, dark conidia (Figure 5). These conidia are produced percurrently from conidiogenous cells that are distinctly annellated. The fungus is slow growing in culture (Figure 6) reaching only 40.5 mm in 21 days at 30 °C, which is indicative of an apparently biotrophic habit. A comparison of this *Coniothyrium* sp. with other species of the genus from *Eucalyptus*, suggests that the species is new to science.

Several species of *Coniothyrium* are known from *Eucalyptus* leaves and stems [6–8]. Based on conidium size and shape, the South African species can easily be distinguished from species such as *C. eucalypticola* B. Sutton, *C. ahmadii* B. Sutton and *C. kallangurensis* B. Sutton & Alcorn. Morphologically, the canker pathogen is similar to two species reported from *Eucalyptus* leaves, namely *C. parvum* Swart and *C. ovatum* Swart. Conidia of the South African strain (4–) 4.5–5(–6) × 2–2.5(–3.5) µm are similar in size to those of *C. parvum* 4.5–6(–7) × 2–3.5 µm, but smaller than



Figures 1-4. Symptoms associated with *Coniothyrium* canker on *Eucalyptus grandis* in South Africa. Figure 1. Discrete necrotic lesion on green tissue (arrow), typical of early infections. Figure 2. Gum pockets (arrow) found below the bark and in the sapwood of infected trees. Figure 3. Spindle-shaped malformation on infected tree, also showing discrete necrotic lesions on infected tissue. Figure 4. Epicormic shoots (arrow) produced from cankered stem.

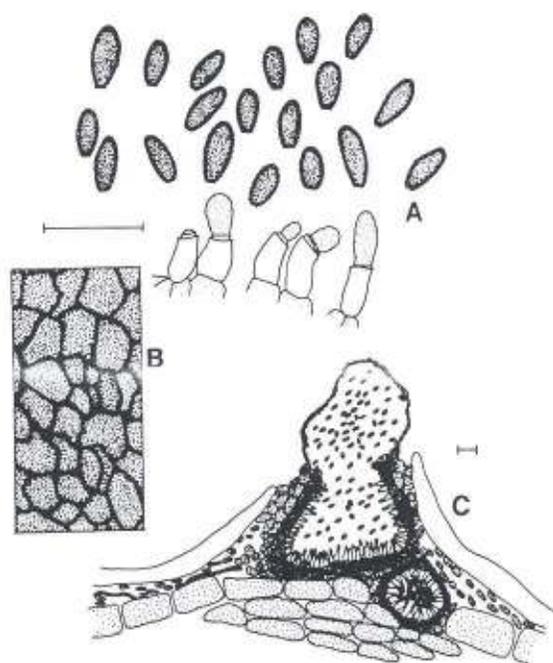


Figure 5. *Coniothyrium zuluense*. A: Thick-walled conidia and conidiogenous cells. B: Surface view of outer pycnidium wall layer. C: Vertical section through a pycnidium (bars = 10  $\mu\text{m}$ ).

those of *C. ovatum* (6–)7–11  $\times$  3–4.5(–5)  $\mu\text{m}$ . However, unlike the latter two species, conidia of the canker pathogen lack a marginal frill, and also have longer or narrower conidiogenous cells (4–8  $\times$  2.5–3.5  $\mu\text{m}$ ) (Figures 5, 7, 8) than respectively *C. parvum* (up to 6  $\times$  3  $\mu\text{m}$ ) or *C. ovatum* (4–7  $\times$  4–5  $\mu\text{m}$ ) [8].

*Coniothyrium zuluense* Wingfield, Crous and Coutinho, *sp. nov.*

*Mycelium* internum in textura hospitis, medio-brunneum vel fuscum, ramosum, septatum crassitunicatum, level vel verruculosum, 1.5–3  $\mu\text{m}$  diametro. *Pycnidia* solitaria vel aggregata in necroticis, fuscis, elevatis laesionibus, 2–15  $\mu\text{m}$  diametro; intra- vel subepidermalia, globosa vel depressa, 60–120  $\mu\text{m}$  lata, 60–80  $\mu\text{m}$  alta, exsudentia sporas fuscis massis; pycnidii parietes constituti ex 2–3 stratis texturae angularis fuscae. Cellae conidiogenae annellidicae, subbrunneae, leves, doliiformes vel reniformes, 4–8  $\times$  2.5–3.5  $\mu\text{m}$ , formatae ex interiore parietis strato pycnidii. *Conidia* medio-brunnea, crassotunicata, levia vel verruculosa, late ellipsoidea, apex obtusus, basis subtruncata vel obtuse rotundata, (4–)4.5–5(–6)  $\times$  2–2.5(–3.5)  $\mu\text{m}$ . *Spermogonia* frequenter evenientia commixta pycnidi-

is in laesionibus et concatenata per fila hyphalia. *Spermatia* hyalina, bacillaria, 2–4  $\times$  1.5–2  $\mu\text{m}$ .

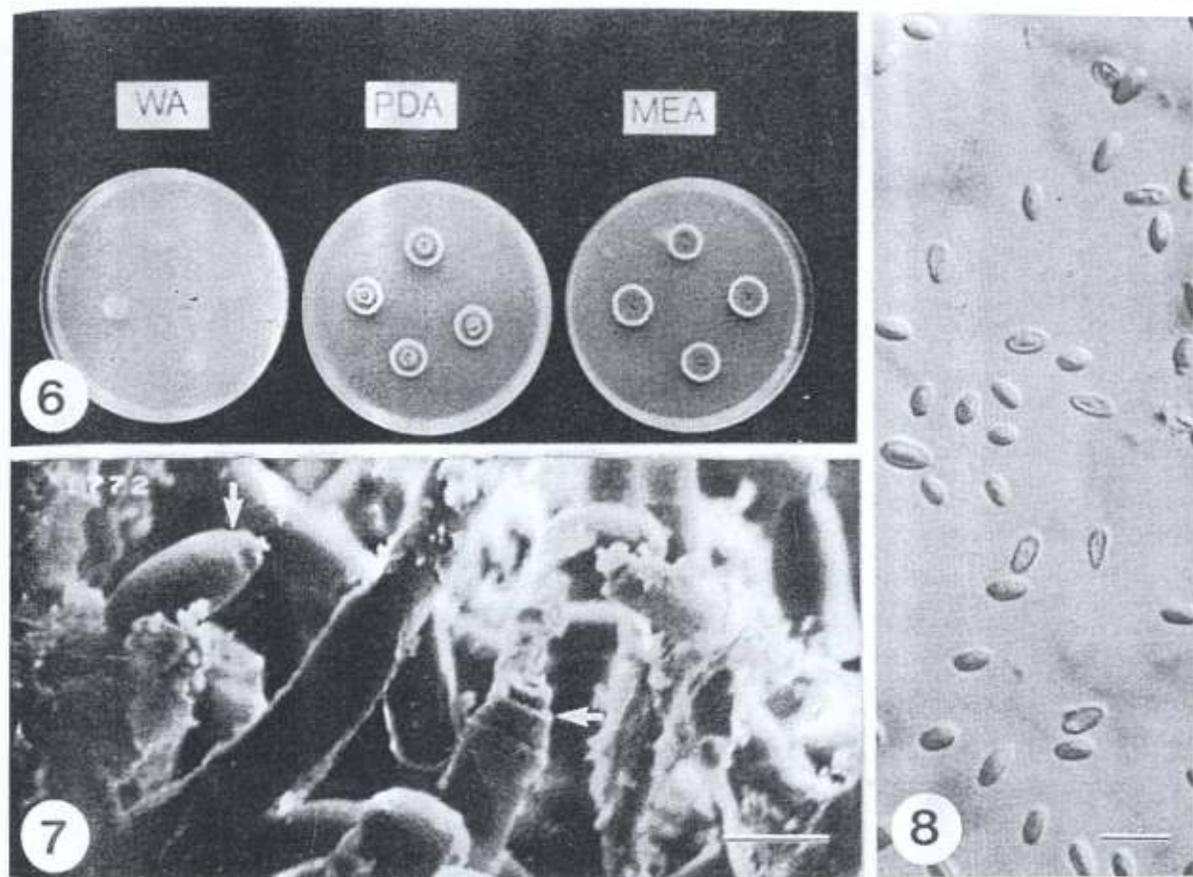
*Holotype*: Kwazulu-Natal, Kwambonambi, Teza nursery, bark of 1-year-old *E. grandis* trees, M.J. Wingfield, Jan. 1996, IMI 370886. *PARATYPES*: Kwazulu-Natal, Kwambonambi, Trust plantation, bark of 4-year-old *E. grandis* trees, M.J. Wingfield, Jan. 1996, IMI 370887; Kwazulu-Natal, Kwambonambi, Futulu plantation, bark of 2-year-old *E. grandis* trees, M.J. Wingfield, Jan. 1996, IMI 370888; Kwazulu-Natal, Kwambonambi, Terranera plantation, bark of 2-year-old *E. grandis* trees, M.J. Wingfield, Jan. 1996, IMI 370889.

*Etymology*: The name *zuluense* refers to the area in which the fungus is most common and which is home to the Zulu nation.

*Mycelium* internal in host tissue, medium to dark brown, branched, septate, thick-walled, smooth to verruculose, 1.5–3  $\mu\text{m}$  in diam. *Pycnidia* single or aggregated in necrotic, dark brown, raised lesions, 2–15  $\mu\text{m}$  in diam.; intra- or subepidermal, globose or depressed, 60–120  $\mu\text{m}$  wide, 60–80  $\mu\text{m}$  high, exuding spores in dark brown masses; pycnidial walls composed of 2–3 layers of dark brown *textura angularis*. *Conidiogenous cells* annellidic, pale brown, smooth, doliiform to reniform, 4–8  $\times$  2.5–3.5  $\mu\text{m}$ , formed from the inner wall layer of the pycnidium. *Conidia* medium brown, thick-walled, smooth to verruculose, broadly ellipsoid, apex obtuse, base subtruncate to bluntly rounded, (4–)4.5–5(–6)  $\times$  2–2.5(–3.5)  $\mu\text{m}$ . *Spermogonia* frequently occurring mixed with pycnidia on lesions, and linked via hyphal strands. *Spermatia* hyaline, rod-shaped, 3–4  $\times$  1.5–2  $\mu\text{m}$ .

#### *Colonies on PDA*

At 30  $^{\circ}\text{C}$ , colonies appear irregular, pale olivaceous (surface), with an outer olivaceous grey band of mycelium, and a pale mouse grey margin. Colony margins, however, tend to be smooth at lower temperatures. Colonies viewed from below at 30  $^{\circ}\text{C}$  exhibit four bands of colour. The outer two bands are olivaceous, the second band is greenish-black and the centre of the colony is rust in colour.



Figures 6-8. Cultures, conidia and conidiogenous cells of *Coniothyrium zuluense*. Figure 6. Single-conidial cultures on water agar, potato dextrose agar and malt extract agar. Figure 7. Conidiogenous cell with percurrent proliferations (arrows; bar = 4  $\mu$ m). Figure 8. Single-celled ovoid conidia (bar = 10  $\mu$ m).

#### Cardinal temperature requirements for growth

The fungus failed to grow at 5, 10 and 35 °C. At 15 °C, the mean colony diameter measured 6 mm after 21 day's incubation. At higher temperatures, 20, 25 and 30 °C, the colony diameter was 22, 28 and 40 mm, respectively.

#### Pathogenicity tests

Stems of trees inoculated with *C. zuluense* developed distinct lesions up to 20 mm diam. after 6 weeks. The most notable symptom observed was a distinct swelling of the stem tissue around the site of inoculation (Figures 9, 10). This was indicative of the stem swelling observed in the case of natural infections. Areas immediately around the inoculation points were necrotic (Figures 9, 10), and pycnidia of *C. zuluense* occurred abundantly on this tissue. Removal of the bark

exposed the presence of a number of discrete necrotic lesions in the cambium (Figure 10). Control inoculations developed no symptoms and inoculation points were closed by callus tissue (Figure 9).

#### Discussion

*Coniothyrium* canker was first observed in the Zululand forestry region of the Kwazulu-Natal Province in September 1988, where it occurred on a single clone of *E. grandis*. It has subsequently become widespread in the area and occurs, not only on a wide range of *E. grandis* clones, but also on hybrids of this and other species. This disease has rapidly become one of the most serious problems affecting the *Eucalyptus* industry in South Africa. It is most serious in the Zululand forestry area, which is typified by a sub-tropical climate, and all indications are that it is substantially less



Figures 9–10. Lesions associated with inoculations of *Coniothyrium zuluense* on stems of young trees. Figure 9. Stem (left) inoculated with sterile agar (control) showing callus development, and stem (right) inoculated with *C. zuluense* showing malformation and development of necrotic tissue. Figure 10. Extensive lesion development and necrotic tissue associated with inoculation.

severe in those areas of the country with temperate climates such as in the Mpumalanga Province. Areas in Zululand with higher rainfall appear to be more conducive to disease development. The disease is common and damaging in all *E. grandis* stands derived from seed, as well as on many clones of *E. grandis* in the Zululand area. In addition, hybrid clones of *E. grandis* with *E. urophylla* and *E. camaldulensis* can be very seriously affected.

The initial occurrence of *C. zuluense* in a limited area of Zululand, and the fact that the disease associated with it has become progressively more damaging and widespread during the course of the last 4 years, suggests that it is of recent origin in the country. The disease is not known in Australia where *Eucalyptus* is native, which might suggest that the causal

agent is native in South Africa, possibly on native *Myrtaceae*, and has developed the capacity to infect *Eucalyptus*. This would thus be similar to the situation with *Eucalyptus* rust caused by *Puccinia psidii* which is not known in Australia, but is common and damaging in South and Central America where it apparently originated from native *Myrtaceae* [9, 10]. It is, however, possible that the fungus occurs in Australia, but due to ecological homeostasis, is uncommon and has thus not yet been recognized.

Variation in the susceptibility of different *Eucalyptus* clones and hybrids to *C. zuluense* is clearly evident and provides a promising strategy to avoid the associated disease. Considerable efforts are, therefore, currently being made to select clones and hybrids that are not susceptible to this disease. In this respect, a

difficulty encountered is the fact that an inordinately high number of clones that are currently available for planting show susceptibility to infection. There is also evidence to suggest that clones previously believed to be tolerant to this disease, are beginning to show signs of infection. This would lend credence to the hypothesis that virulence in the pathogen is changing with time.

As far as we are aware, *C. zuluense* has no sexual state, and it appears to propagate only asexually. The presence of spermatogonia suggests, however, that such a state may occur, but has yet to be discovered. At present very little is known of the population structure of the fungus, and such information is critically important to programmes aimed at reducing the impact of this disease [11]. A study of the population diversity of *C. zuluense* is underway, but is hampered somewhat by the fact that the fungus grows poorly in culture. Population diversity, which should also give some indication as to the probable origin of the pathogen, will only be accurately resolved using sophisticated molecular fingerprinting techniques.

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*Address for correspondence:* Prof. M.J. Wingfield, Department of Microbiology and Biochemistry, University of the Orange Free State, P.O. Box 339, Bloemfontein 9300, South Africa.  
Phone: (27)51-4012581; Fax: (27)51-4482004;  
E-mail: Wingfjmj@micro.nw.uovs.ac.za