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First report of Sphaeropsis canker on cypress in South Africa

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Summary

In this study a *Sphaeropsis* sp. is recorded as causing cankers on cypress in South Africa. These cankers are either found alone or on trees which are infected with the well-known cypress canker pathogen, *Seiridium unicorne*. When the two fungi occurred together, lesions could not be distinguished from each other, although the greater number of lesions were caused by the *Sphaeropsis* sp. The fungus appears to be identical to that described as *Sphaeropsis sapinea* f.sp. *cupressi*, in Israel, which is a fungus very different from the pine pathogen, *Sphaeropsis sapinea*. Pathogenicity tests showed that the *Sphaeropsis* sp. is significantly more pathogenic to *Cupressus lusitanica* than to *Pinus roxburgii* and *Pinus elliottii*. *S. sapinea* was pathogenic only to the *Pinus* spp. tested, and not to *C. lusitanica*. In contrast, the cypress pathogen *Seiridium unicorne*, was pathogenic to *C. lusitanica* and also to the two *Pinus* spp. tested.

1 Introduction

Cypress trees (*Cupressus* spp.) are not native to South Africa and frequently planted as ornamentals and as windbreaks. The cypress canker pathogens *Seiridium unicorne* (Cooke and Ell.) Sutton and *Seiridium cardinale* (Wagener) Sutton and Gibson have been recorded in South Africa (WINGFIELD and DU TOIT 1986). Cankers associated with the *Seiridium* spp. are frequently encountered in South Africa in areas where susceptible Cupressaceae are planted, and contribute to regular mortalities of ornamental Cuppressaceae trees. Seiridium canker is the best known disease of Cupressaceae and has a wide geographical distribution (BOESEWINKEL 1983; SOLEL et al. 1983; VAN DER WERFF 1988); it is the only fungal disease recorded on *Cupressus* spp. in South Africa, whereas various other fungal diseases of these trees have been recorded in Israel. These diseases include Pestalotiopsis canker caused by *Pestalotiopsis funerea* (Desm.) Steyaert (MADAR et al. 1991), crown wilt, stem canker and seedling blight caused by *Botryodiplodia theobromae* Pat. (=*Lasiodiplodia theobromae* (Pat.) Griffon and Maubl.) (BRUCK et al. 1990) and also a Diplodia canker of *Cupressus sempervirens* L. caused by *Sphaeropsis sapinea* (Fr.) Dyko and Sutton (*Diplodia pinea* (DESM.) KICKX) f.sp. *cupressi* (SOLEL et al. 1987).

Sphaeropsis sapinea is a serious and well-known opportunistic pathogen of pines (PETERSON 1977). In South Africa, it is particularly important on *Pinus radiata* D. Don and *P. patula* Schlecht. and Cham. where the pathogen causes die-back of hail-damaged trees (ZWOLINSKI et al. 1990; SWART and WINGFIELD 1991). There is also a relationship between infection and colonization of pine trees by *S. sapinea* and infestation by cambiphagous insects following hail injury (ZWOLINSKI et al. 1995). Shoot blight is, however, the most common symptom associated with *S. sapinea* infection and it occurs on seedlings and on mature trees (WATERMAN 1943; CHOU 1976; PETERSON 1977; SWART et al. 1987).

During routine isolation from cankers on *Cupressus* spp. in 1991 and 1992, a fungus other

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than *Seiridium* spp. was consistently isolated from lesions. The aim of this study was to identify this fungus and determine whether it is a pathogen of *Cupressus* spp. Because of its marginal similarity to *S. sapinea* and the importance of the latter fungus as a forest pathogen in South Africa, the pathogenicity of the two fungi was compared on two *Pinus* spp. and a *Cupressus* sp.

2 Materials and methods

2.1 Occurrence and isolation

Cankers on *Cupressus lusitanica* Miller in the Mac Mac area (Mpumalanga, South Africa) and on *C. sempervirens* at Bloemfontein (Free State, South Africa) were observed. These diseased trees were planted as ornamentals in gardens and alongside roads. The cankers on diseased trees were characterized by bark discoloration, fissuring and resin exudation on the stems, which led to branch die-back and/or tree mortality.

Diseased trees from which isolations were made were selected in both the Mac Mac and Bloemfontein areas. At least five lesions were examined from 10 trees in each area. Isolations from lesions were made by removing the outer bark, after which 10 pieces (2 × 5 mm) of inner bark from canker margins of each tree were transferred aseptically to PDA (potato dextrose agar). Erumpent acervuli from diseased bark surfaces were also transferred to PDA (39 g/l, Biolab Diagnostics (Pty) Ltd., Midrand, South Africa). The plates were incubated at 25°C in the dark. The resultant mycelial growth was again transferred to PDA and incubated at 25°C under near-ultraviolet light (320 nm). A piece of sterile cypress wood was added to each plate to stimulate sporulation. Fifty conidia were measured from the two species consistently isolated from cankers, using a light microscope.

2.2 Pathogenicity

Pathogenicity of the unknown fungus, tentatively identified as a *Sphaeropsis* sp., and also *Seiridium unicorne* and *Sphaeropsis sapinea* was compared on 2-year-old *P. elliottii* Engelm., 5-year-old *P. roxburgii*, and 10-year-old *C. lusitanica* trees, in a trial site at Bloemfontein. Test fungi were grown on PDA for 7 days at 25°C. Mycelial discs were inoculated into 10-mm diameter wounds, made with a cork borer, by removing the cambium on branches of 10 trees for each test species. The same number of trees were inoculated using a sterile disc of PDA for controls. Inoculation wounds were sealed with parafilm to prevent desiccation. Lesions were examined after 2 months reisolations of inoculated fungi were made onto PDA from all developing lesions.

3 Results

3.1 Isolations

Two probable pathogens, a fungus identified as a *Sphaeropsis* sp. (Fig. 1a) and *Seiridium unicorne* (Fig. 1b), were isolated from cankers on *Cupressus* sp. from both locations studied. At Mac Mac, 70% of the trees were infected with *Seiridium unicorne* and 40% with the *Sphaeropsis* sp. At Bloemfontein, 80% and 50% of the trees were infected with *Seiridium unicorne* and the *Sphaeropsis* sp., respectively. Although both fungi commonly occurred on the same tree, they were never isolated from the same lesion. When both fungi were found on the same tree, 60% of the lesions were associated with the *Sphaeropsis* sp., and 40% of the lesions with *Seiridium unicorne*. Lesions caused by the two fungi were very similar and it was not possible to distinguish between them without microscopic analysis.

Conidia of the *Sphaeropsis* sp. were euseptate, brown and an average of 26.5 (range 24–

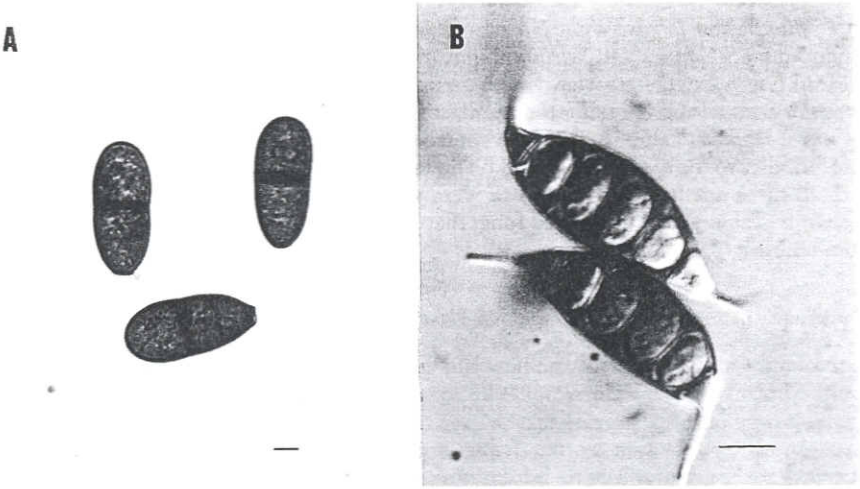

Fig. 1. Conidia of the *Sphaeropsis* sp. (a) and *Seiridium unicorne* (b); bars = 5 μm

30) μm long and 12.5 (range 10–16) μm wide. Based on morphological characteristics, this fungus was identical to that identified as *S. sapinea* f.sp. *cupressi* in Israel (SOLEL et al. 1987). It was consequently compared with an isolate of *S. sapinea* f.sp. *cupressi* supplied by Dr Z. Solel and this identification was confirmed.

3.2 Pathogenicity

Lesions produced by the *Sphaeropsis* sp. inoculated into *C. lusitanica* were significantly (p = 0.01) longer than those of *Seiridium unicorne*. *Sphaeropsis sapinea* did not produce lesions on *C. lusitanica* (Table 1). Lesions associated with the *Sphaeropsis* sp. were also significantly ($P = 0.01$) smaller than those associated with *S. sapinea* and *Seiridium unicorne*

Table 1. Lesion lengths on *Pinus elliotii* and *Pinus roxburgii* seedlings and also on established *Cupressus lusitanica* trees 2 months after inoculation with *Sphaeropsis sapinea*, *Sphaeropsis* sp. and *Seiridium unicorne*

Species[1]	Lesion length (mm)[2]			
	P. elliotti	*P. roxburgii*	*C. lusitanica*	Mean
Sphaeropsis sp. CMW 3784	4^{a}	16^{a}	45^{c}	22^{a}
Sphaeropsis sapinea CMW 1191	16^{b}	39^{b}	0^{a}	18^{a}
Seiridium unicorne CMW 3783	23^{b}	40^{b}	30^{b}	31^{b}
Mean	14^{r}	32^{s}	25^{t}	

[1] CMW, Culture collection of the Tree Pathology Cooperative Programme, University of the Free State, Bloemfontein, South Africa

[2] Each value is the average of 10 replicate trees per treatment. Values in each column followed by different letters, differ significantly (p = 0.01) according to Tuckey's procedure for comparison of means (STEEL and TORRIE 1980)

[3] Values in rows followed by different letters, differ significantly (p = 0.01) according to Tuckey's procedure for comparison of means (STEEL and TORRIE 1980)

on *P. elliottii* and *P. roxburgii*. No significant differences could be found between lesions produced by *S. sapinea* and *Seiridium unicorne* on the *Pinus* spp. tested (Table 1).

Mean lesion lengths associated with *Seiridium unicorne* on all three tree species tested, were the greatest and they differed significantly from those associated with the *Sphaeropsis* sp. and *S. sapinea* (Table 1). The different trees inoculated differed significantly ($p = 0.01$) in susceptibility to the three fungi, based on mean lesion lengths. Mean lesion lengths associated with all three test fungi on *P. elliottii* were the smallest, followed by *C. lusitanica* and *P. roxburgii* (Table 1). All inoculated fungi that produced lesions were successfully re-isolated from lesions.

4 Discussion

In this study we have identified a new and apparently important pathogen of *Cupressus* in South Africa. While Seiridium canker has undoubtedly had a dramatic effect on ornamental *Cupressus* spp., it now appears that at least some of the damage attributed to it could have been caused by infection by the *Sphaeropsis* sp. The relative importance of this newly recorded pathogen has yet to be determined, but results of this study suggest that it contributes to tree disease in South Africa.

The *Sphaeropsis* sp. identified in this study is similar to the fungus known as *S. sapinea* f.sp. *cupressi* in Israel, the identification of which has been questioned by SWART et al. (1993). The acceptability of the name S. *sapinea* f. sp. *cupressi* is questioned on the grounds that the fungus is very different from *S. sapinea*, both in terms of morphology and isozyme patterns (SWART et al. 1993). These authors also suggested that *S. sapinea* f.sp. *cupressi* should be referred to as a *Sphaeropsis* sp. until its taxonomy can be fully investigated. The discovery of the fungus in South Africa has allowed us an opportunity to compare it further with *S. sapinea*, and we support the view that it would be erroneous to link it in name to the latter.

Pathogenicity tests in this study showed pathogenicity of the *Sphaeropsis* sp. to *C. lusitanica* and not to pine. These results thus confirm those of SOLEL et al. (1987) who were unable to show lesion development when *S. sapinea* f.sp. *cupressi* was inoculated to *P. pinea* L.

Seiridium unicorne is a well-known cypress pathogen, but has been isolated from various other hosts (GUBA 1961; BOESEWINKEL 1983). This is, nevertheless, the first report of the fungus being pathogenic to pines. The result is based on artificial inoculations and may not necessarily relate to natural conditions. However, if *S. unicorne* is able to infect pines under natural conditions, this could have serious implications for South African forestry.

In recent years the annual rainfall has been exceptionally low in the areas where Sphaeropsis canker was noted in this study. In Israel, it has been shown that water stress induces and enhances cankers caused by *Sphaeropsis* sp. on stems and branches of cypress trees (MADAR et al. 1989). It is therefore likely that the Sphaeropsis canker in South Africa has also been enhanced by drought stress. This is in contrast with cankers caused by *Seiridium cardinale* that are apparently not associated with stress (MADAR et al. 1989).

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Résumé

Première mention d'un chancre à Sphaeropsis sur cyprès en Afrique du Sud

Dans ce travail, un *Sphaeropsis* sp. est mentionné comme agent de chancre sur cyprès en Afrique du Sud. Les chancres ont été trouvés soit de façon isolée soit sur des arbres infectés par le parasite bien