*Scientific paper*

# Infection Studies with *Phaeoseptoria eucalypti* and *Coniothyrium ovatum* on *Eucalyptus* spp.*

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**SYNOPSIS**

*Phaeoseptoria eucalypti* and *Coniothyrium ovatum* have recently been associated with leaf lesions on *Eucalyptus* spp. in South Africa. *P. eucalypti* caused severe defoliation of *E. camaldulensis* and *E. grandis*, prominent lesions on shoots of *E. camaldulensis*, and retarded the growth of *E. nitens*. It infected all the species tested in the subgenus *Symphyomyrtus*, but not *E. citriodora* of the subgenus *Corymbia*. Conidia of *C. ovatum* germinated on all species tested, but no infection occurred. This study has shown that *P. eucalypti* can be a serious nursery pathogen, but that *C. ovatum* would pose no threat to *Eucalyptus* spp. important in this country.

## INTRODUCTION

*Phaeoseptoria eucalypti* Hansf. *emend* Walker and *Coniothyrium ovatum* Swart were first recorded in South Africa in 1987 (Wingfield, 1987). A subsequent survey showed that *P. eucalypti* is widespread on various locally important *Eucalyptus* spp. and clones, whereas *C. ovatum* was found only on *E. cladocalyx* F. Muell. and *E. lehmannii* (Preiss: Shau.) Benth. *C. ovatum* seems to be restricted to the Western Cape (Crous, Knox-Davies and Wingfield, 1988).

In other countries *P. eucalypti* causes severe damage to seedlings of various *Eucalyptus* spp. (Walker, 1962; Heather, 1967b; Sharma, Mohanan and Maria Florence, 1984; Chipompha, 1987). On the other hand, *C. ovatum* has been recorded only from Australia, but there is no mention of its pathogenicity (Swart, 1986).

With the current emphasis being placed locally on *Eucalyptus* spp. (Directorate National Forestry Planning, 1988) and their commercial propagation (Donald, 1986), nursery pathogens are posing an increasing threat to the forest industry. The present study was therefore conducted to evaluate the potential of *P. eucalypti* and *C. ovatum* as nursery pathogens on some of the more important eucalypts.

## MATERIALS AND METHODS

### Inoculum

Inoculum of both fungi was prepared by rinsing fresh, naturally-infected leaves with distinct lesions in distilled water containing two drops of Tween 80. Spores were counted with a haemacytometer and the suspensions diluted to $1 \times 10^6$ conidia/ml. To establish spore viability, suspensions were sprayed onto malt-extract agar (MEA) in petri dishes and incubated at 25 °C under near-ultraviolet light. Dishes were examined at intervals under a dissecting microscope for spore germination.

### Petri-dish inoculations

Juvenile and mature leaves were collected from *E. grandis* and *E. cladocalyx* trees, rinsed for 20 minutes in running tap water and blotted dry. Some leaves were partially dewaxed by agitating for two seconds in chloroform (Silva Fernandes, 1965). Partially dewaxed leaves were rinsed and blotted dry. Two sets of leaves, one of which was partially dewaxed, were sprayed with spore suspensions, and placed on moist filter paper in petri dishes. The dishes were sealed with Parafilm and incubated at 25 °C under near-ultraviolet light. Uninoculated leaves were sprayed with sterile distilled water. After being incubated for 100 h, leaf sections (3 mm²) were excised, prepared for scanning electron microscopy (SEM) and examined under SEM.

### Growth room inoculations

A previous study indicated that *P. eucalypti* occurred predominantly on species of the subgenus *Symphyomyrtus*, whereas *C. ovatum* has been found only on relatively unimportant hosts such as *E. cladocalyx* and *E. lehmannii* (Crous, Knox-Davies and Wingfield, 1988). *Eucalyptus* spp. belonging to the subgenera *Symphyomyrtus* and *Corymbia* (*Table 1*) were therefore inoculated with both fungi.

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* *Part of an M.Sc. Agric. thesis submitted by the first author to the University of Stellenbosch*

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Plants were raised from seed as described by Donald and Lundquist (1984). Seedlings were planted in pots and kept in a growth chamber at a constant temperature (25 °C) and 12 h cycles of light/dark. There were 30 plants of each *Eucalyptus* sp. A randomised block design was used, with 10 plants per treatment. The 150 seedlings were divided into five blocks. Another five plants of each *Eucalyptus* sp. were included for SEM studies.

Five-month-old seedlings were inoculated by spraying with spore suspensions till run-off. Controls were sprayed with sterile distilled water. The plants were then covered with plastic bags and held in a growth chamber at a constant temperature (25 °C) and 12 h cycles of light/dark. The plastic bags were removed after five days and selected leaves collected for SEM examination. Plants were inspected at weekly intervals for symptom development.

### Disease assessment

Disease severity was determined by estimating the per cent leaf spot and the defoliation rating. Per cent leaf spot was rated by comparing infected leaves with disease assessment diagrams representing 0, 3, 6, 12, 25, 50, and 75 % of the leaf surface area. The defoliation score was determined by assigning a disease score to the defoliated area where 1 = 0–3 %, 2 = >3–6 %, 3 = >6–12 %, 4 = >12–25 % 5 = >25–38 %, 6 = >38–50 %, 7 = >50–63 %, 8 = >63–75 %, 9 = >75 % of the crown defoliated. The scale used in both cases was that devised for Mycosphaerella leaf spot by Lundquist and Purnell (1987). Defoliation was rated after 4, 8 and 12 weeks, whereas the percentage leaf spot was rated after 12 weeks.

### Scanning electron microscopy

Squares were cut from the laminas of inoculated leaves, fixed in 5 % glutaraldehyde, taken through a graded alcohol series, dried in a LADD critical point drier, mounted and examined under an SEM.

## RESULTS

### Spore viability

Conidia of both fungi showed a high viablity. After 30 h incubation, 99 % of the spores of *P. eucalypti* examined on MEA had germinated, whereas 90 % of the spores of *C. ovatum* had germinated after 52 h.

### Petri-dish inoculations

*C. ovatum*: Spores had germinated on leaves of both *E. cladocalyx* and *E. grandis* after 100 h. Germ tubes penetrated young and mature leaves of *E. cladocalyx* through stomata (*Figures 1* and *2*), but failed to penetrate any of the *E. grandis* leaves (young, mature, or those partially dewaxed). On these leaves germ tubes were frequently seen growing across or around the stomata without penetrating them.

*P. eucalypti:* Although spores germinated after 30 h on MEA, no germination was observed on any of the *Eucalyptus* leaves after 48 h incubation (*Figure 3*). *P. eucalypti* conidia germinated poorly on young *E. grandis* leaves. Germination was better on the older leaves, and best on young leaves which had been partially dewaxed. Conidia failed to germinate on untreated *E. cladocalyx* leaves, and only limited germination occurred on partially dewaxed leaves (*Figure 4*).

### Growth room inoculations

*C. ovatum*: Although conidia had germinated on leaves of all five *Eucalyptus* spp., no penetration was evident on any of the leaves examined.

*P. eucalypti:* Conidia had germinated on leaves of all the species in the subgenus *Symphyomyrtus*, and germ tubes were regularly seen to penetrate the stomata (*Figure 5*). On the other hand, there was only limited germination and no penetration on *E. citriodora* (subgenus *Corymbia*).

Disease severity of the different *Eucalyptus* spp. at different periods after inoculation is given in *Table 1*.

*TABLE 1. Disease severity of* Eucalyptus *seedlings*[a] *at three periods after inoculation with* P. eucalypti

| *Eucalyptus* sp. | Defoliation[b] after (wks) | | | Per cent leaf spot[c] |
|---|---|---|---|---|
| | 4 | 8 | 12 | |
| *E. camaldulensis* | 4 | 4 | 4,0 | 55,75 |
| *E. citriodora* | 0 | 0 | 0 | 0 |
| *E. cladocalyx* | 0 | 4 | 2,1 | 6,1 |
| *E. grandis* | 0 | 4 | 4,5 | 40,0 |
| *E. nitens* | 0 | 4 | 3,1 | 38,0 |

[a] Seedlings kept in a growth room at ± 25 °C

[b] Based on a disease score where 1 = 0–3 % and 9 = > 75 % of the crown defoliated

[c] Assessment made after 12 wks; based on disease assessment diagram representing the percentage diseased leaf surface area

Symptoms developed three to four weeks after inoculation on potted plants of *E. camaldulensis*, and were at first limited to the lowest three leaf nodes (*Figures 6, 7, 8 and 9*). Prominent lesions formed six to seven weeks after inoculation on older leaves of this species. Eight to 12 weeks after inoculation, the plants had 10 to 15 leaf nodes, but only the lowest five or six leaf pairs (which had been inoculated) were infected. The leaves remained green but were covered with pycnidia. They soon shrivelled (*Figure 10*) and dropped prematurely. All controls were healthy, and no premature defoliation occurred. Because spores were splash-dispersed during watering, newer growth eventually also developed symptoms. A final reading was taken six months after inoculation. By this time all *Eucalyptus* spp. ex-

cept *E. nitens* had shed their infected leaves. In the case of *E. nitens*, the inoculated plants showed 50–75 % leaf spot throughout, and growth averaged 200 mm less than the healthy controls.

## DISCUSSION

This study has shown that *P. eucalypti* can cause a severe leaf infection and defoliation of seedlings of the most important *Eucalyptus* spp. in the subgenus *Symphyomyrtus* cultivated locally. On *E. grandis* the defoliation score after 12 weeks was 18,5 %. This is near the critical value of 25 %, after which plant growth is retarded (Lundquist and Purnell, 1987). These results confirm those of other workers (Heather, 1967b; Sharma *et al.*, 1984) on the importance of *P. eucalypti* as a pathogen in nurseries. On the other hand, infection studies with *C. ovatum* affirmed the limited host range observed in field surveys (Crous, Knox-Davies and Wingfield, 1988). It is therefore unlikely that this fungus will infect important species such as *E. grandis* in South Africa.

Of the four species in the subgenus *Symphyomyrtus*, symptoms were initially seen only on *E. camaldulensis*. They differed from those recorded in the field, where no leaf shrivelling occurred (Crous, Knox-Davies and Wingfield, 1988). At 12 weeks, the diseased leaf area of *E. grandis* and *E. nitens* was high compared with that of *E. cladocalyx*, but less severe than that of *E. camaldulensis*. Prominent lesions also developed on shoots of *E. camaldulensis*, even though *P. eucalypti* is regarded as being primarily a leaf pathogen. Defoliation was first observed on *E. camaldulensis*, but seedlings of this host grew more vigorously than those of *E. grandis*. Therefore, when seedlings were finally rated for defoliation after 12 weeks, the effect of the disease was less pronounced on *E. camaldulensis*. At six months after inoculation, all species (except *E. nitens*) had recovered, and no obvious difference in height growth occurred between plants inoculated with *P. eucalypti* and their controls. The heavy infection of *E. nitens* can be explained by the fact that seedlings of this host were not defoliated as readily as the other species inoculated, and that older infected leaves served as a source of inoculum for secondary infection.

Although *P. eucalypti* showed limited infection on *E. cladocalyx* seedlings (*Table 1*), leaves in petri dishes were not infected, and infection has rarely been observed in nature (Crous, Knox-Davies and Wingfield, 1988).

Heather (1967a) reports that the wax layer on younger leaves of *E. bicostata* Maid. *et al.* has 21 % more ether-soluble materials than older leaves, and that the water-soluble fraction of the ether-soluble moiety in the wax inhibits *in vitro* germination of *P. eucalypti* spores (Heather, 1967b). Results in the present study suggest that wax might also play a role in the infection of *E. cladocalyx* and *E. grandis* by *P. eucalypti*. An SEM examination showed the plate wax layers of mature leaves of *E. cladocalyx* (known host of *C. ovatum*) (*Figure 11*) to be more granular and thicker than those of *E. grandis*, which is not a host (*Figure 12*). Much variation in orientation of wax plates and thickness of wax layers was also observed on juvenile and mature leaves of the different species (results not given). However, a detailed study must be made before any definite conclusions can be drawn.

Appropriate control measures for *P. eucalypti* are to breed for disease resistance. In Australia (Heather, 1967a) and South Africa (Crous, Knox-Davies and Wingfield, 1988) the host range of *P. eucalypti* seems to be limited to the subgenus *Symphyomyrtus*. There are also varying degrees of resistance within this subgenus. For instance, *E. cladocalyx* is rarely infected, and some clones of *E. grandis* seem to be more resistant than others (Crous, unpublished). However, care should be taken not to use reductionist techniques at the expense of other criteria (Buddenhagen, 1983), and resistance to other stress situations should also be considered in a breeding programme.

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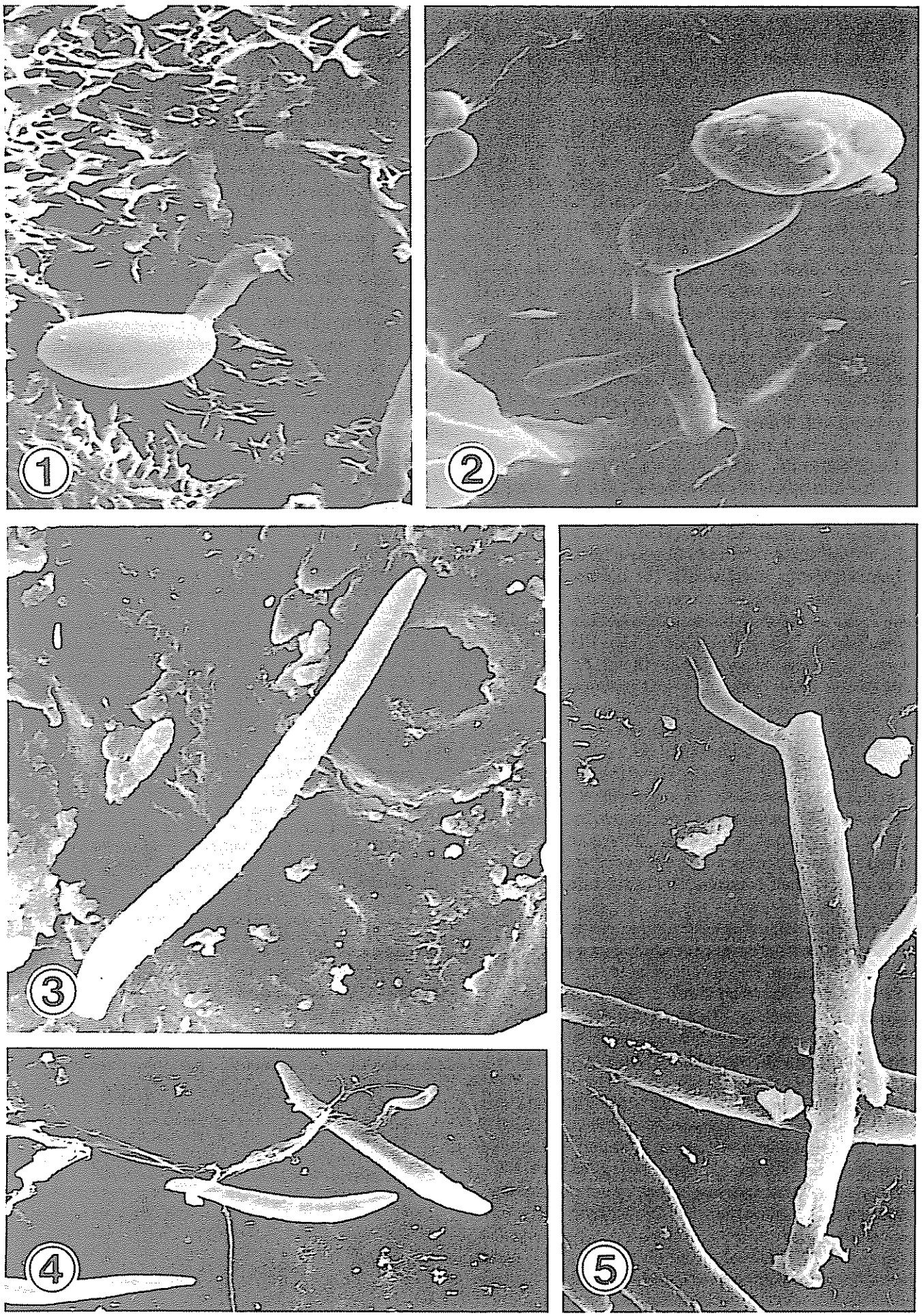
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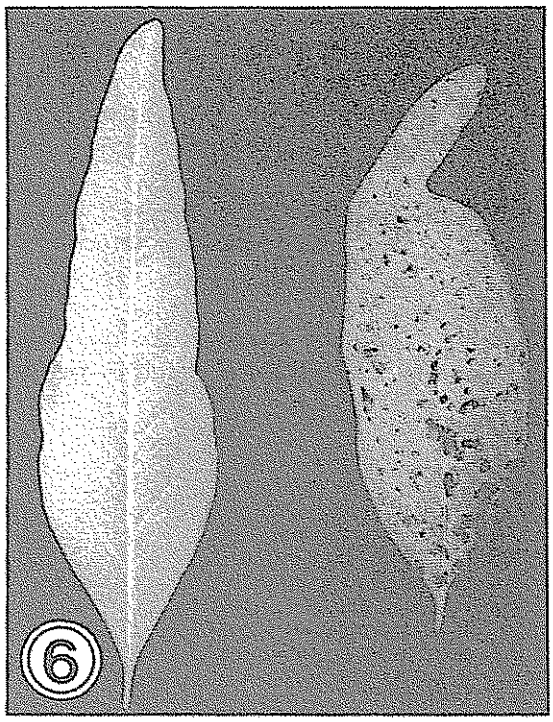
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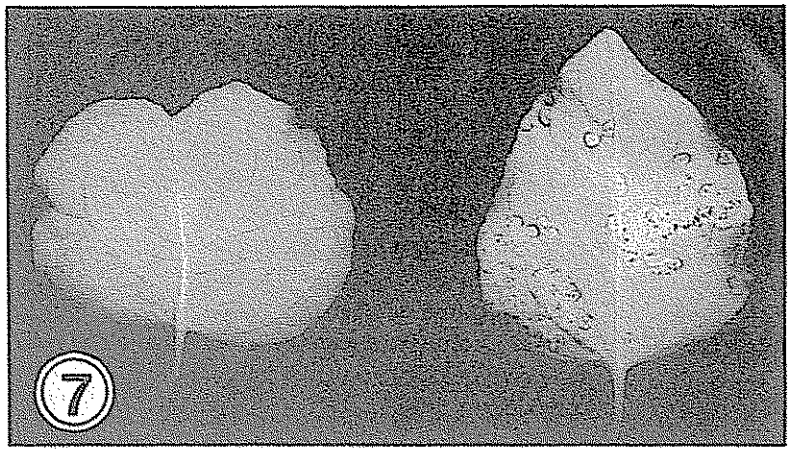
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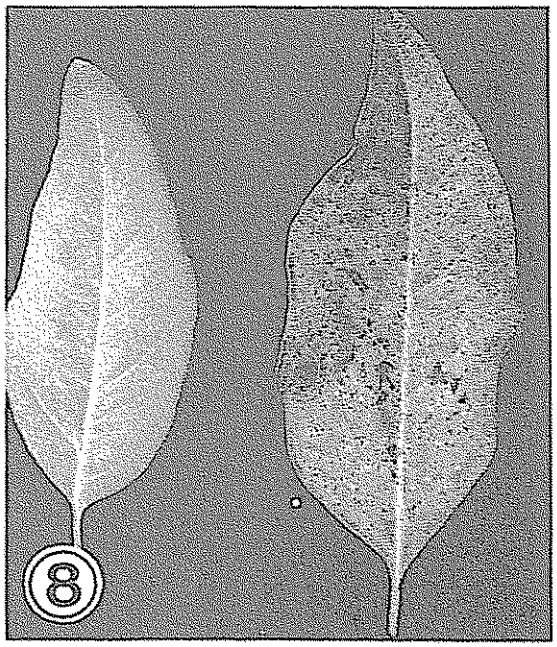
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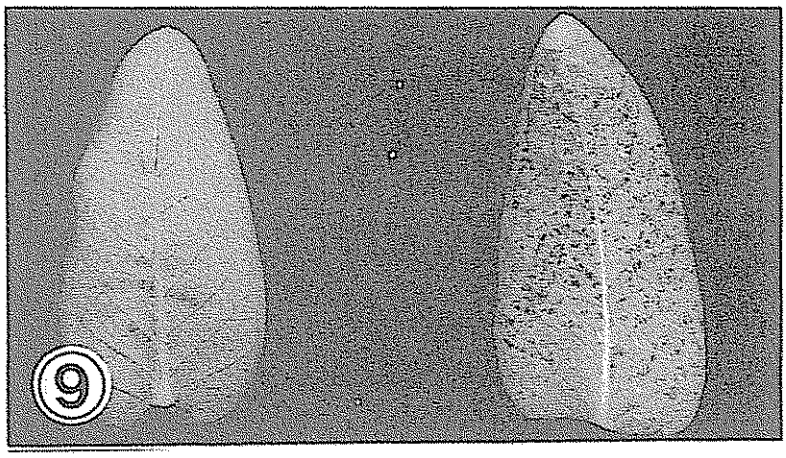
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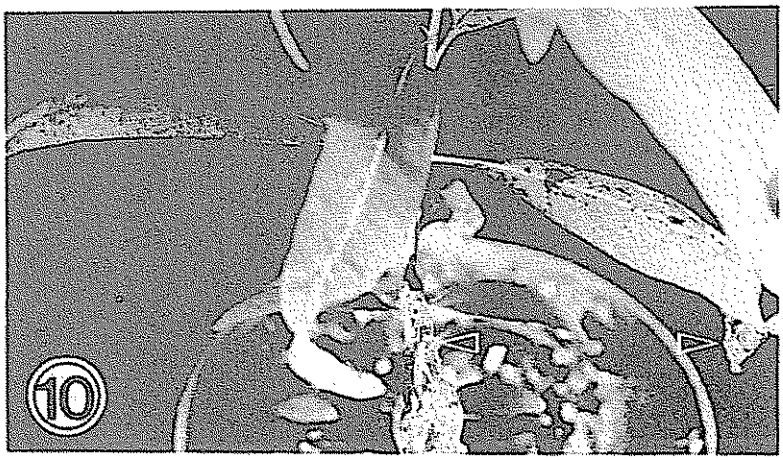
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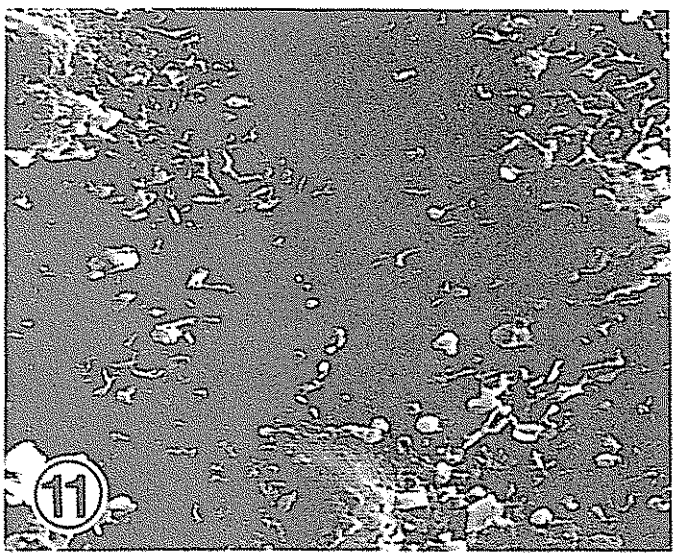
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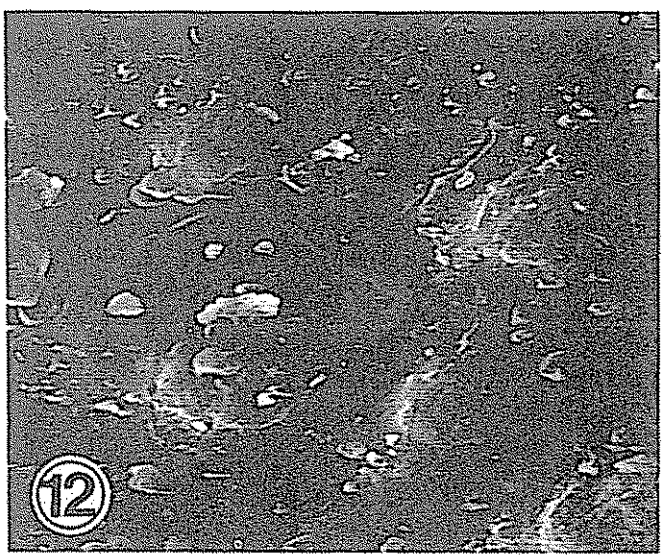
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*FIGURES 1, 2. Germ tubes from* C. ovatum *conidia penetrating stomata of young (x 3 000) and mature (x 3 600)* E. cladocalyx *leaves.*

*FIGURE 3. Non-germinating conidium of* P. eucalypti *on an* E. grandis *leaf after 48 h incubation (x 1 500).*

*FIGURE 4.: Limited germination of* P. eucalypti *conidia on an* E. cladocalyx *leaf. Note absence of wax (x 800).*

*FIGURE 5. Germ tube from* P. eucalypti *conidium penetrating a leaf stoma of* E. grandis *(x 1 000).*

*FIGURES 6, 7, 8, 9. Control treatment (left) and leaf lesions (right) caused by* P. eucalypti *on* E. camaldulensis *(Figure 6),* E. cladocalyx *(Figure 7),* E. grandis *(Figure 8) and* E. nitens *(Figure 9).*

*FIGURE 10. Shrivelling leaves (arrowed) of* E. camaldulensis, *5 weeks after inoculation with* P. eucalypti.

*FIGURES 11, 12. Plate wax layers on the upper surfaces of* E. cladocalyx *and* E. grandis *(x 1 000).*