Two Newly Reported Leaf Pathogens of *Eucalyptus grandis* in South Africa

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SYNOPSIS

Pilidium acerinum and *Ceuthospora lauri* are newly reported pathogens from *Eucalyptus grandis* leaves in South Africa. Symptoms occurred on leaves of lower branches towards the end of winter. Although occurring together on the same lesion, both fungi were individually pathogenic. Inoculation studies showed that *P. acerinum* was the more virulent pathogen.

INTRODUCTION

During observation of a *Eucalyptus* stand at Stellenbosch in the western Cape Province over successive seasons, distinct, light-brown lesions were noticed on leaves of the lower branches of eight-year-old *E. grandis* Hill: Maid. trees. Although foliage from higher branches showed the presence of *Phaeoseptoria eucalypti* Hansf. *emend.* Walker (Crous, Knox-Davies and Wingfield, 1988), the lower branch leaf lesions were not typical of this fungus (Crous, 1989). Severity of the disease on the lower branches increased during July–November, i.e. towards the end of the winter. The aim of the work described here was to isolate and identify the causal organism and to establish pathogenicity.

MATERIALS AND METHODS

Isolations and cultural descriptions

Affected leaves were examined after incubation for 24 h at 25 °C in moist chambers under mixed fluorescent and near-ultraviolet lighting. Single-spore isolations were made (Hansen and Smith, 1932) on malt-extract agar (MEA) plates consisting of 15 g Oxoid malt extract, 20 g Difco agar and 1 000 ml H_2O. Plates were sealed with Parafilm and then incubated at 25 °C under mixed lighting (12 h/day) for 14 days to induce sporulation. Morphological characters were determined by light microscopy, following the methods of Sutton (1980).

Temperature and germination studies

Optimum growth temperature (reflected as colony diameter) was determined for three isolates of both fungi. Isolates were grown on MEA for six days in the dark, at seven temperatures ranging from 5 to 35 °C (5 ° intervals). Each treatment had three replications and the experiment was repeated.

In an attempt to explain the pattern of disease development of the fungi, the effect of temperature on conidial germination and germ tube development was determined. A conidial suspension was sprayed onto MEA plates and three replicate plates were incubated in the dark at the same temperatures used for growth studies. Spore densities on the agar surface ranged from 1–10 spores/mm^2. Plates were examined at 1 h intervals to determine when germination started. Percentage germination was calculated after 9 and 24 h by counting conidia (100 per plate), and determining the number that germinated (at X200 magnification). After 9 h, the lengths of 25 germ tubes on each plate were measured with an ocular micrometer. Any further germ tube development was stopped by adding 1,0 ml of 40 % formaldehyde to each plate.

Inoculum

Inoculum of both fungi was prepared by adding sterile distilled water (containing two drops Tween 80/500 ml) to 21-day-old cultures, and dislodging the spores by lightly scraping the culture surface with a sterile blade. Mycelial fragments were removed by straining through sterile muslin. Spore concentrations were determined with a haemacytometer and diluted to give a final concentration of $1x10^5$ conidia/ml. To establish inoculum viability, spore suspensions were sprayed onto MEA plates and incubated at 25 °C. After 24 h dishes were examined under a dissecting microcope to determine percentage germination.

Glasshouse inoculations

The fungi were inoculated separately on both wounded and unwounded leaves of five-month-old *E. grandis*

plants. Three branches in the upper, mid, and lower sections of each tree were selected for inoculations. For the wound inoculations, each leaf was stabbed with four sterile pins tied to the end of a pencil. Wounded and unwounded leaves were inoculated by spraying with the spore suspension until run-off. Wounded and unwounded controls were sprayed with sterile distilled water. The branches were enclosed for three days in plastic bags to ensure high humidity, and the plants kept in a greenhouse maintained at 25 °C. Experimental design was a split-plot with five leaves per branch, three branches per plant and six plants per pathogen. Controls consisted of six plants divided equally between wounded and unwounded treatments.

Disease assessment

A photocopy was made of each symptomatic leaf to assess disease severity. The leaf images were cut precisely from the paper sheets and the weight of the entire paper-leaf was determined to three decimal places. Diseased areas were excised and the paper-leaf weighed again. Leaf spot area was calculated as a percentage weight of the whole leaf.

To confirm pathogenicity, 10 symptomatic leaves were selected at random from each treatment, and surface sterilised for 5 min. in 1 % NaOCℓ. The zone between the lesions and healthy tissue was excised, placed on MEA, incubated at 25 °C, and re-isolations made.

RESULTS

Description of isolates

Two fungi previously unreported in South Africa were found on living leaves and on leaf litter. These were *Ceuthospora lauri* (Grev.) Grev. and *Pilidium acerinum* Kunze *apud* Kunze & Schmidt, which usually occurred together. Conidiomata of *C. lauri* and *P. acerinum* exuded spore masses after 24 h of incubation at 25 °C. Both fungi grew well on MEA.

Pilidium acerinum

Cultures light brown, sporulating after one week at 25 °C, later becoming darker brown. *Conidiomata* in concentric circles around inoculation point, dark brown, exuding a creamy mass of spores; unilocular, separate, epidermal to subepidermal on leaves, wrinkled, flattened, opening by an irregular rupture. *Conidiophores* cylindrical, hyaline, formed at base of conidioma, 1–2 septate. *Conidiogenous cells* enteroblastic, phialidic, tapering towards the apex, with an inconspicuous collarette. *Conidia* falcate, hyaline, aseptate, apex acute, with obtuse base, 13–17 × 1,6–2 μm *in vivo*, and 12,5–14 × 1,3–2 μm *in vitro* (*Figure 1a*).

Ceuthospora lauri

Cultures creamy-light brown, sporulating after 2–3 weeks at 25 °C, later becoming grey. *Conidiomata* light

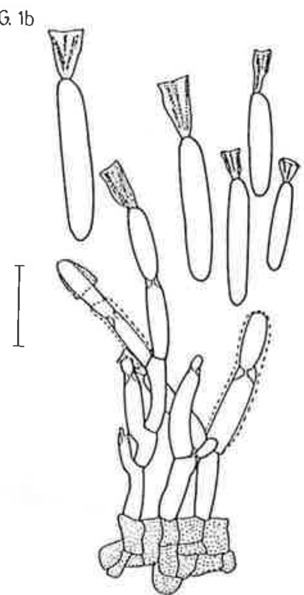


FIGURE 1a, b. Conidia, conidiogenous cells and conidioma base. a, Pilidium acerinum *(PREM 50458); b,* Ceuthospora lauri *(PREM 50459). Bar = 10 μm.*

brown on leaves, grey-green in culture, irregular, multilocular, with a raised edge separating it from the host tissue; locules with separate or communal, furfuraceous ostioles. *Conidiophores* hyaline, branched, and septate, *Conidiogenous cells* enteroblastic, phialidic, cylindrical, channel and collarette minute; frequently attached to the conidium by the mucous sheath surrounding both, creating a false catenulate impression. *Conidia* hyaline, cylindrical, aseptate, base obtuse, apex tapered, with an apical, fan-shaped mucilaginous appendage. Conidia 13–17 × 2–2,5 μm *in vivo*, and 9–16 × 2–2,5 μm *in vitro* (*Figure 1b*).

Specimens examined: *Ceuthospora lauri* from *E. grandis* leaves, Stell., W. Cape, Sept. 1989, P.W. Crous, PREM 50459; *E. nitens* litter, Belfast, E. Tvl., Feb. 1990, P.W. Crous, PREM 50527; *Pilidium acerinum* from *E. grandis* leaves, Stell., W. Cape, Sept. 1989, P.W. Crous, PREM 50458.

Cultural studies

The optimum temperature for growth of both *C. lauri* and *P. acerinum* was 20–25 °C (*Figure 2a, b*). Spores of both fungi started to germinate after 8 h at 25 °C. The highest level of germination after 9 h occurred at 25 °C, and was 35 % for *C. lauri* and 85 % for *P. acerinum*. Maximum germination was reached after 24 h, being 80 % for *C. lauri*, and 95 % for *P. acerinum*. At 15 °C only 10 % of the conidia of either fungus germinated. No germination of either fungus occurred below 15 °C or above 25 °C after 9 h of incubation. Longest germ tubes developed at 25 °C (*Figure 2a, b*). The spore suspensions used for inoculations showed 80 % and 95 % viability for *C. lauri* and *P. acerinum* respectively.

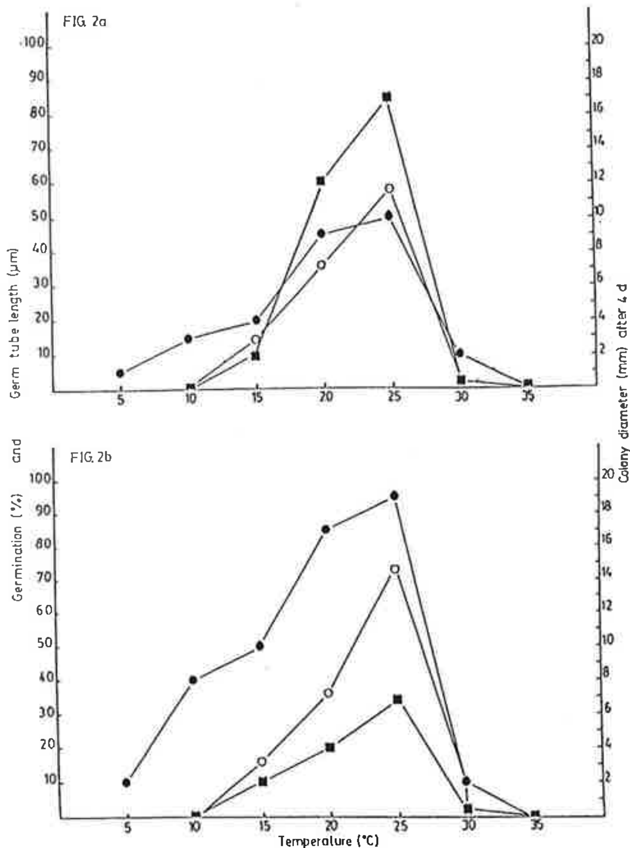


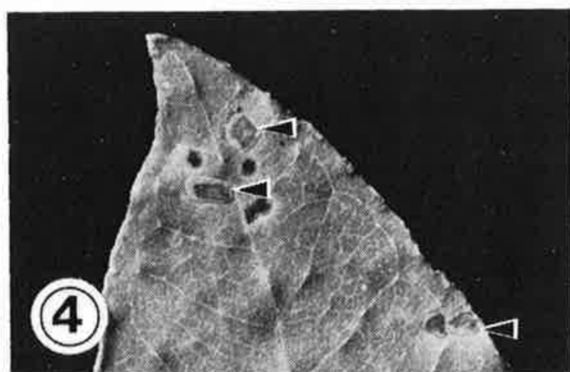
*FIGURE 2a, b. Effect of temperature on spore germination (■), germtube length after 9 h (***O***)* and colony diameter after 4 days (●). a, Pilidium acerinum; *b,* Ceuthospora lauri.

Disease assessment

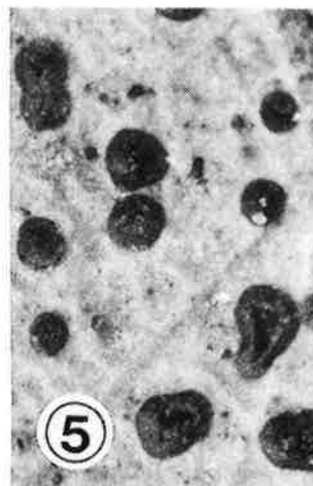
Although leaves inoculated with *P. acerinum* formed water-soaked lesions two weeks after inoculation, disease assessments were made two weeks later. Inoculations of wounded leaves showed typical hypersensitive reactions, with necrotic tissue surrounding the wounded areas. *P. acerinum* and *C. lauri* proved to be not highly virulent, causing leaf spots which covered 0–13 % (*Figure 3*) and 0–3 % (*Figure 4*) of the unwounded leaf area respectively. Both fungi were recovered from the tissue at the edges of the respective lesions. *P. acerinum* sporulated on the symptomatic leaves one month after inoculation *(Figure 5),* whereas lesions with *C. lauri* did not develop further. Leaves in the control treatments remained healthy.

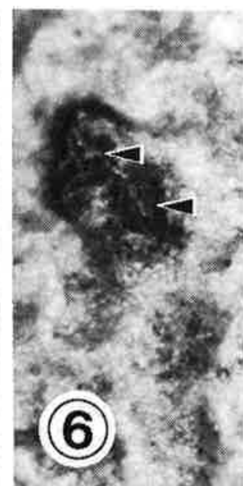
DISCUSSION

Ashton and Macauley (1972) reported a winter leaf spot disease of *E. regnans* F. Muell. in Australia and repeatedly isolated *Ceuthospora innumera* Massee and *Piggotia substellata* Cooke from necrotic tissue. There are some similarities between this disease and that described here. For example, the fungi associated with both diseases occurred on leaves of the lower branches and on leaf litter of *Eucalyptus* spp. In both cases a *Ceuthospora* sp. occurred in association with another more virulent Coelomycete, and both diseases oc-



FIGURES 3, 4. Symptoms on inoculated unwounded leaves of E. grandis. *Figure 3.* Pilidium acerinum. *Figure 4.* Ceuthospora lauri.





FIGURES 5, 6. Conidiomata on leaves of E. grandis. *Figure 5.* P. acerinum *(x 66). Figure 6.* C. lauri *(x 200).*

curred in the cooler, wetter months. Although the Australian disease is regarded as important (Ashton and Macauley, 1972), the South African disease appears to be of limited importance at present.

Pilidium acerinum has been reported from leaves of *Betula*, *Eucalyptus*, *Quercus* and *Acer* in various countries throughout the world (Sutton, 1980). Although *P. acerinum* occurred in association with *C. lauri* on necrotic spots and on leaf litter of *E. grandis* in this study, subsequent collections in the western Cape and Transvaal have shown both fungi to occur individually as colonisers of *Eucalyptus* leaf litter.

Ceuthospora innumera Massee is the only species so far reported from *Eucalyptus* (Ashton and Macauley, 1972), with *C. lauri* and *C. innumera* being quite distinct (Swart, 1988). *C. innumera* does not have conidiophores and its conidiogenous cells are smaller and the conidia larger than those of *C. lauri*. Furthermore, the multilocular conidiomata of the latter are more clearly delimited (*Figure 6*) than those of *C. innumera* (Swart, 1988). No reference could be found to the occurrence of *C. lauri* on *Eucalyptus*. This appears to be the first report of *C. lauri* on this host.

The occurrence of lesions caused by *P. acerinum* and *C. lauri* only towards the end of winter could be explained by rain-splash dispersal. In summer, both fungi can be detected on leaf litter under healthy trees. With the onset of the winter rain, spores are probably splash-dispersed, infecting leaves under favourable conditions. In the western Cape Province (winter rainfall area), symptoms of the disease appear from July until the end of November. Splash-dispersal of spores from leaf litter could explain why lesions occur only on leaves very close to the ground, as inoculation studies showed that leaves of all ages were susceptible to infection. The possibility of synergism between these two fungi was not explored in this investigation, and requires further study.

Although *P. acerinum* and *C. lauri* occur on *E. grandis*, which is the most important *Eucalyptus* species in South Africa (Directorate National Forestry Planning, 1988), their confinement to the lower leaf whorls and relatively low virulence suggest that they are unlikely to have any significant adverse effects on this host.

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