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A WILT DISEASE OF *RHODODENDRON* CAUSED BY *PYTHIUM PROLATUM* AND *CYLINDROCLADIUM SCOPARIUM*

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ABSTRACT

Key words: *Cylindrocladium scoparium, Pythium prolatum, Rhododendron,* wilt disease.

Cylindrocladium scoparium and *Pythium prolatum* were consistently isolated from the roots of wilted *Rhododendron* bushes in the eastern Transvaal. This is the first record of these fungi on *Rhododendron* roots in South Africa. Both pathogens induced wilt and root rot symptoms on inoculated seedlings. When the pathogens were inoculated separately, no significant difference could be detected between the disease severity indices. The morphology of the pathogens and their potential influence on the local *Rhododendron*-nursery industry are discussed.

Uittreksel

'N VERWELKSIEKTE VAN RHODODENDRON *VEROORSAAK DEUR* PYTHIUM PROLATUM *EN* CYLINDROCLADIUM SCOPARIUM

Cylindrocladium scoparium *en* Pythium prolatum *is deurlopend geïsoleer vanuit die wortels van verwelkte* Rhododendron-*struike in die Oos-Transvaal. Dit is die eerste aanmelding van hierdie fungi, geassosieerd met* Rhododendron-*wortels in Suid-Afrika. Beide patogene het verwelking en wortelvrotsimptome by geïnokuleerde saailinge geïnduseer. Die virulensie van die patogene het nie betekenisvol verskil met aparte inokulasies nie. Die morfologie en die invloed wat hierdie patogene op die plaaslike* Rhododendron-*bedryf mag uitoefen, word bespreek.*

INTRODUCTION

In South Africa *Rhododendron* L. hybrids are extensively cultivated in subtropical areas with high rainfall, particularly on the eastern Transvaal escarpment and in parts of the Transvaal lowveld. Recently, wilting azalea bushes were collected from a nursery in the eastern Transvaal. Isolations from roots and root-stocks consistently yielded a *Cylindrocladium* sp. and a *Pythium* sp. Although several species of these genera have been associated with azalea bushes worldwide (Hendrix & Campbell, 1966; Sobers, 1968; Cox, 1969; Hendrix & Campbell 1969; Alfieri *et al.*, 1972; Roos, 1980; 1981), no reference could be found to any species in either genus occurring on this host locally. The aim of the work described here was to identify the fungi and establish their pathogenicity.

MATERIALS AND METHODS

Isolation and identification of fungi

Fine roots, roots and root-stocks of wilted plants were washed with distilled water. Isolations of *Cylindrocladium* were made from root material surface disinfested for 2 min in 1,0 % NaOCl and plated onto malt-extract agar (MEA) (15 g Oxoid malt extract, 20 g Difco agar per litre distilled water) and 1,5 % water agar (WA). To isolate *Pythium* sp., non-surface-disinfested root pieces were plated onto selective medium (P10VP) without hymexazol (Tsao & Guy, 1977). Resulting colonies were subcultured onto P10VP medium and single zoospore isolates were prepared according to the method of Ribeiro (1978). Colonized agar cubes were placed in petri dishes containing non-sterile soil extract and incubated for 16 h under near-ultraviolet illumination at 25 °C. Plates were then chilled at 6–10 °C for 1 h, and returned to room temperature for zoospore release. One millilitre aliquots of the zoospore suspension were pipetted onto petri dishes containing WA supplemented with vancomycin (0,2 g/ℓ), ampicillin (0,25 g/ℓ) and rifampicin (0,01 g/ℓ) and incubated for 16 h at 25 °C in the dark. Single germinating zoospores were subcultured onto P10VP medium. The *Cylindrocladium* isolate was single-spored onto MEA using the method of Hansen & Smith (1932). Cultures of the *Pythium* isolate were incubated at 20 °C and maintained on clarified V-8 juice medium (V8M) (Ribeiro, 1978) supplemented with 0,03 g β-sitosterol/ℓ distilled water. The key of Van der Plaats-Niterink (1981) was used to identify the *Pythium* isolate. Identification of the *Cylindrocladium* isolate was based on comparisons with type cultures and published descriptions (Timonin & Self, 1955; Booth & Gibson, 1973).

Inoculum preparation

A soil-bran mixture consisting of 150 g soil, 30 g bran and 80 mℓ distilled water in 500-mℓ conical flasks was autoclaved for 15 min at 120 °C on three consecutive days. Flasks were left for 1 d, inoculated with agar plugs colonized with the respective fungi, and incubated at 25 °C for 2 wk. To ensure a thorough colonization of the inoculum, flasks were shaken every 2 d. *Cylindrocladium* cultures were incubated under alternating, 12 h light and dark cycles, while *Pythium* cultures were incubated in the dark.

Plant inoculations

The experiment consisted of four treatments, viz. the *Cylindrocladium* isolate alone, the *Pythium* isolate alone, equal proportions of the *Cylindrocladium* and *Pythium* isolates and an untreated control, with five replicates per treatment. The above, colonized soil-bran mixtures were mixed with sterilized vermiculite (1:5 w/w, except in the mixed *Cylindrocladium/Pythium* inoculation, where a ratio of 0,5:0,5:5 was used) and placed in plastic pots (500 mℓ) on saucers. Pots were previously disinfested with 3,5 % NaOCl. Three-month-old *Rhododendron* hybrid plants were carefully uprooted, and repotted in the respective mixtures. Plants were watered regularly and kept at 25 °C in a greenhouse. Treatments were laid out in a completely randomized design.

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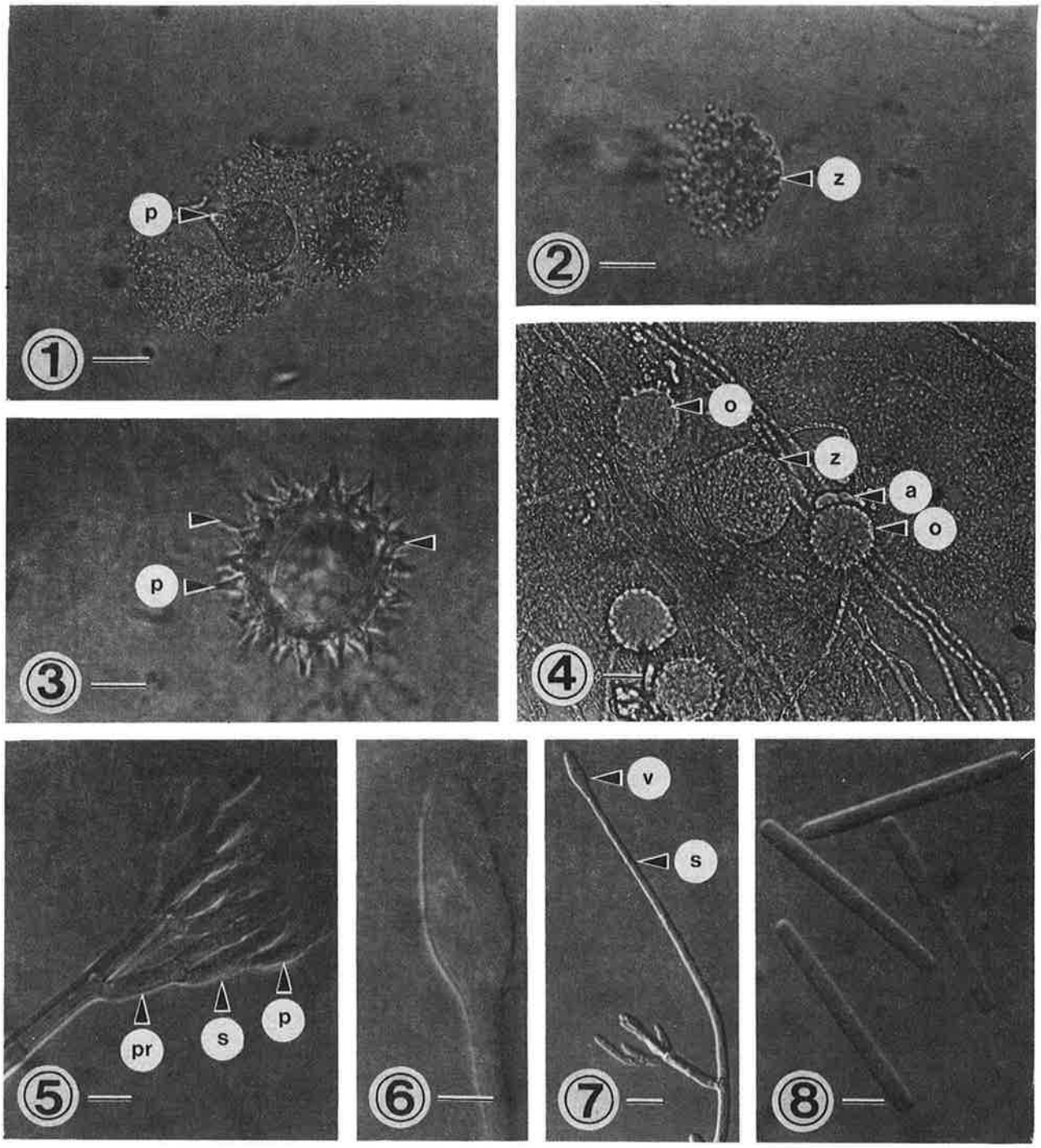

FIG. 1–8. Morphological structures of *Pythium prolatum* (Fig. 1–4) and *Cylindrocladium scoparium* (Fig. 5–8).

FIG. 1. A zoosporangium, showing the beaked, apical protrusion (p). Scale bar = 10 µm.

FIG. 2. A zoosporangium in the process of releasing zoospores (z). Scale bar = 10 µm.

FIG. 3. Mature oogonium, densely covered with conical, mammiform protuberances (p). Scale bar = 10 µm.

FIG. 4. Ornamented oogonia (o), illustrating a diclinous, irregularly shaped antheridium (a) and a zoosporangium (z). Scale bar = 20 µm.

FIG. 5. Penicillate conidiophore, illustrating a primary (pr) and secondary (s) branch, and a phialide (p). Scale bar = 10 µm.

FIG. 6. A terminal, thin-walled, obpyriform vesicle. Scale bar = 5 µm.

FIG. 7. A conidiophore with septate stipe (s) and vesicle (v). Scale bar = 20 µm.

FIG. 8. Hyaline, cylindrical, median septate conidia. Scale bar = 10 µm.

Disease assessment

Plants were assessed for disease symptoms 4 wk after inoculation. Disease was rated according to a scale of 1–3, where 1 = healthy, 2 = wilted and 3 = plants dead. A two-way analysis of variance was performed to determine significant differences between treatment means.

To confirm infection, inoculated plants were uprooted and washed in distilled water. Fine roots, roots and rootstocks were removed, placed in 70 % ethanol for 2 min, rinsed in distilled water, and plated onto the respective media described above.

RESULTS

Description of isolates

The *Pythium* isolate was identified as *P. prolatum* Hendrix & Campbell, and the *Cylindrocladium* isolate as *C. scoparium* Morgan.

Pythium prolatum. Colonies on V8M formed a coarsely radiate pattern, with a raised (2–3 mm) aerial mycelium; optimum temperature for growth was 26 °C. Zoosporangia ovoid to elongate (Fig. 1), with a small beak or hypha-like outgrowth, non-proliferating usually irregularly shaped, 27–42 µm wide, up to 100 µm long; zoospores 4–7 × 9–12 µm (Fig. 2); oogonia terminal (Fig. 3, 4), 22–44 µm diam., densely covered with conical to mammiform protuberances, 4–7 µm long, base broad, 4 µm wide; antheridia diclinous, 1–2 per oogonium, irregularly shaped and inflated, making broad, lengthwise contact with the oogonium; oospores aplerotic 22–30 µm diam., wall 1–2 µm thick; hyphal diameter 4–9 µm. The measurements of the local isolate match those of the original description by Hendrix & Campbell (1969).

Cylindrocladium scoparium colonies on MEA were orange-brown, white-margined, sporulating within 1 wk, forming immersed and superficial microsclerotia; optimum temperature for growth was 28 °C. Conidiophores penicillately branched (Fig. 5), having primary, secondary and sometimes tertiary branches, ending in doliiform phialides, 11–22 × 3–4 µm; most sporogenous heads with a septate, frequently branched stipe, ending in a terminal, thin-walled, elliptical to obpyriform vesicle, 5–7 µm in width (Fig. 6, 7); conidia hyaline, cylindrical, 1-septate, encased in mucous, tapering, becoming swollen at one end with age, 41–57 × 4–4,5 µm (Fig. 8); chlamydospores dark brown, arranged in clusters.

Specimens examined: Pythium prolatum from *Rhododendron* hybrid roots, White River, Tvl., July 1990, W. J. Botha & P. W. Crous, PPRI 4079; *Cylindrocladium scoparium* from *Rhododendron* hybrid roots, White River, Tvl., July 1990, W. J. Botha & P. W. Crous, PPRI 4085. *C. scoparium* from *Leucothoe catesbaei* cv. Rainbow, 1981, D. M. Benson, ATCC 46300; *C. scoparium* from *Medicago truncatula,* 16 Mar. 1986, S. Lamprecht, PPRI 3533.

Disease assessment

The disease severity indices (DSI) of inoculated seedlings are presented in Table 1. Both fungi were pathogenic to *Rhododendron,* but no significant differences in DSI between the two fungi could be detected. The highest DSI occurred in the mixed inoculations, which differed significantly (P = 0,01) from the severity of disease caused by *C. scoparium* alone. The control plants were healthy and no fungus was isolated from surface-disinfested roots. However, the roots from plants inoculated with *C. scoparium* and *P. prolatum* were extensively rotted, with a brown discolouration on the root-collar and lower stems. Only *C. scoparium* or *P. prolatum* were re-isolated from the fine roots, root-collar and stem regions of plants inoculated with *C. scoparium* or *P. prolatum* respectively. In the treatment where mixed inoculum of both fungi was used, both pathogens were re-isolated from these sites.

TABLE 1 Disease severity caused by *Pythium prolatum* and *Cylindrocladium scoparium* on inoculated *Rhododendron* seedlings

Treatment	DSI[a]
Control	1,0 A
Cylindrocladium	2,0 B
Pythium	2,6 CB
Cylindrocladium + *Pythium*	3,0 C

[a] Disease severity index with assessment scale;
1 = healthy, 2 = wilted, 3 = seedlings dead
Each value is the mean of five replicates
Values followed by the same letter do not differ significantly at P = 0,01

DISCUSSION

Although *Rhododendron* is a new local host record for *C. scoparium,* the fungus has previously been found to be a pathogen of *Medicago truncatula* Gaertn. (Lamprecht, 1986), *Acacia longifolia* (Andr.) Willd. (Hagemann & Rose, 1988), *Persea americana* Mill. (Darvas, 1978), *Pinus* spp. (Darvas *et al.,* 1978; Lundquist, 1986) and *Eucalyptus* spp. (Lundquist & Baxter, 1985) in South Africa. *C. scoparium* is recognized worldwide as an important pathogen of *Rhododendron* (Timonin & Self, 1955; Cox, 1969; Engelhard, 1971; Linderman, 1974), and has been associated with wilt (Linderman, 1974) and canker symptoms (Miller & Baxter, 1969) on this host. On other hosts this species is known to cause root rot, damping-off, seedling blight (Booth & Gibson, 1973) and leaf spot (Hagemann & Rose, 1988). Other than *C. scoparium,* several species, such as *C. theae* (Petch) Subramanian (Alfieri *et al.,* 1972), *C. pteridis* Wolf (Sobers, 1968) and *C. floridanum* Sobers & Seymour (Sobers & Seymour, 1967; Peerally, 1974) have been associated with disease symptoms on *Rhododendron.*

This is the first report of the occurrence of *P. prolatum* as a pathogen in South Africa. Unlike *C. scoparium,* which has a wide host range (Bertus, 1976), *P. prolatum* has been associated with only two other host plants in Georgia, U.S.A. (Hendrix & Campbell, 1969). Several *Pythium* spp., other than *P. prolatum,* have also been isolated from diseased azalea roots and from soil (Hendrix & Campbell, 1966). They concluded that these *Pythium* spp. may play a major role in limiting the development of feeder root systems, thus reducing plant growth and vigour. The South African isolate of *P. prolatum,* proved to be highly virulent, as the results in Table 1 indicate.

The occurrence of these two newly reported fungal pathogens from the roots of wilting *Rhododendron* bushes, could have serious consequences for nurseries cultivating this ornamental plant. The nursery where this disease was found is, at present, supplying other nurseries with plants (multiplied by clonal cuttings). There is a possibility that these pathogens may spread to other nurseries. Nurserymen should take the necessary precautionary measures, such as effective chlorination of nursery water (to prevent the spread of fungal propagules), and the use of a sterile potting medium. Roos (1980) found that the treatment of *Rhododendron* mother plants with benomyl, protected against infection by *Cylindrocladium* spp., and did not inhibit the rooting of cuttings. Furthermore, treatment with fenaminosulf gave sufficient protection against Pythiaceous fungi, without having any detrimental effect on the rooting of cuttings.

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