Fungal populations associated with root systems of proteaceous seedlings at a lowland fynbos site in South Africa

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Fungi were isolated from rhizosphere and non-rhizosphere soil from root systems of Leucospermum parile and Hakea sericea seedlings growing in sand plain lowland fynbos. Deuteromycotina were most prevalent; Penicillium, Aspergillus and Trichoderma were the main genera. The root systems supported a more varied mycoflora than the non-rhizosphere soil but were non-mycorrhizal. A number of fungi, including representatives of the Ascomycotina, were found almost exclusively in the root region. The proteoid and non-proteoid regions of the roots of both species contained similar fungal populations. Nine fungal species and the genus Helicon were recorded for the first time in South Africa.

Gröndfungi is van die risoefeer van Hakea sericea en Leucospermum parile wat in laaglandfynbos groei geïsoleer. Soorte behorende tot die Deuteromycotina het die algemeenste voorgekom. Hiervan was die genera Penicillium, Aspergillus en Trichoderma die belangrikste. Die wortelstelself was 'n groter verskil in die mycoflora van die risoefeer dan in die risosfeer terwyl die non-proteoid wortels gevind nie. Nege fungussoorte en die genus Helicon is vir die eerste keer in Suid-Afrika aangetoon.

Keywords: Fynbos, Proteaceae, proteoid roots, soil fungi

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Introduction

Shrubs of the Proteaceae dominate the fynbos vegetation of the south-western Cape Province of South Africa and characteristically produce proteoid roots (Lamont 1983a). Proteoid roots are densely packed clusters of short-lived rootlets arising along the lateral roots, and play a special role in nutrient uptake (Lamont 1983b), but are non-mycorrhizal in some members of the Australian Proteaceae (Malajczuk et al. 1981). In this study, fungal populations were identified in the rhizosphere and root tissue of proteoid and non-proteoid roots of members of the Proteaceae growing in an acidic sandy soil (Mitchell et al. 1984) and were compared with the mycoflora of the non-rhizosphere region. Soil fungi are frequently in a dormant state and the rhizosphere is likely to be one of the few sites in the soil where active microbial growth occurs (Robinson et al. 1968; Barber & Lynch 1977). Rhizosphere and rhizoplane saprotrophic fungi have mainly been studied in economically important plants (Newman 1978) whereas this paper reports on micro-organism/root interactions in a natural vegetation.

Materials and Methods

Plant material and study site

Two species of Proteaceae were selected: Leucospermum parile (Salisb. ex J. Knight) Sweet which is endemic to the sand plain lowland fynbos, south-western Cape and Hakea sericea Schrad. which was introduced to the Cape Province from Australia during the 19th century (Annecke & Neser 1977). Seed of H. sericea was germinated in sterile acid-washed sand. During April 1982 5-week-old seedlings were transplanted to Clovelly sand, at the Pella study site (33°31'S, 18°32'E; 160 - 220 m altitude; 294 ha; 62 km north of Cape Town, South Africa). Seedlings of L. parile were naturally growing at the same study area.

The soil is classified as the Clovelly form, Geelhout series (MacVicar et al. 1977) and is a medium sand aeolian in origin, well drained with pH 4.6 - 4.8 and has an organic matter content of 1.8% (Mitchell et al. 1984; Brown & Mitchell 1986). The vegetation is sand plain lowland fynbos (Moll et al. 1984) dominated by evergreen sclerophyllous shrubs and rush-like plants belonging to the Restionaceae. The climate is Mediterranean with a mean annual rainfall of 400 mm falling mainly between May and October. The site had been burnt during November 1980.

Isolation of fungi

The root systems of seedlings of L. parile (one year old) and H. sericea (five months old) were collected during winter (late May to September) 1982 in soil blocks which were transported to the laboratory in sterile polythene bags. The roots were dissected under aseptic conditions leaving undisturbed soil adhering to them. The fungi were isolated within 24 h of collection.

The rhizosphere fungi were isolated by the serial root washing technique (Harley & Waid 1955). One proteoid root complex or a 5-cm length of non-proteoid root from both H. sericea and L. parile were used for each washing series and ten replicates of each were washed 25 times in 5 cm3 of sterile distilled water. The rhizosphere fungi were isolated by plating aliquots of water from the first and 25th washing in 15 cm3 of cool Rose Bengal chloramphenicol agar (Lab M, Salford, U.K.). To isolate the fungi attached to the root systems after 25 washings, four serially washed proteoid and non-proteoid roots of each species were macerated. A few drops of macerate were spread over Rose Bengal chloramphenicol agar. Plates of the first and 25th washing and macerate were replicated four times.

The non-rhizosphere soil fungi were sampled by collecting soil free of roots and away from the canopy of any plants. Soil cores were collected 5 cm below the surface in sterile test tubes. Soil fungi were isolated using the soil plate technique (Warcup 1950) and five replicates of 10 soil samples were prepared.

Plates were examined after 3 - 5 days in the dark at 25°C. Representatives of all species were transferred to Potato Dextrose agar (PDA) and identified after they had sporulated.
Penicillium spp., Eupenicillium spp., Scopulariopsis spp. and Talaromyces sp. were grown on media described by Pitt (1979). Aspergillus spp. were grown on Czapek Dox agar and Malt Extract agar (Raper & Fennel 1965). Trichoderma spp. were grown on Malt agar (Rifai 1969) and Chaetomium spp. were grown on cellulose-amended PDA or sterile carrot plugs (Ames 1963). The histochemical technique of Phillips & Hayman (1970) was used to detect vesicular-arbuscular (VA) mycorrhizal fungi on the root systems. Live cultures of all fungi were deposited in the fungal collection of the Department of Botany, University of Cape Town.

Results

In the serial washing procedure of Harley & Waid (1955), the first three washings removed most of the rhizosphere fungi and after the fifth washing, a low but constant number of fungi were isolated (Figure 1).

A total of 61 species were identified from 380 isolates of the non-rhizosphere Clovelly sand (Table 1) and rhizospheres of H. sericea (Table 2) and L. parile (Table 3). The rhizospheres of the two plant species supported a more varied fungal flora than the non-rhizosphere region. Members of the Deuteromycotina were commonly isolated and the most frequent genera were Aspergillus, Penicillium and Trichoderma. The common species isolated in descending order of frequency, were: Aspergillus fumigatus, Penicillium restrictum, Scopulariopsis brevicaulis, P. novae-zeelandiae, A. duricaulis, Scopulariopsis sp., Trichoderma viride, P. verruculosum, T. pseudokoningii and Eupenicillium hirayamae. Of these, Scopulariopsis sp. and E. hirayamae were absent from the non-rhizosphere region. Other species only colonizing the root zones were P. restrictum, P. janczewskii, P. funiculans, A. unilaterals, E. pinetorum, and Periconia digitata. Representatives of the Ascomycotina with the exception of three isolates from the soil, were restricted to the rhizospheres of H. sericea and L. parile.

The rhizosphere and macerate of washed roots of both H. sericea (Table 2) and L. parile (Table 3) contained 67 and 62% respectively of the total species isolated. None of the common species were specifically associated with either the root systems of H. sericea and L. parile or the soil. There were no differences in the fungal populations associated with the proteoid and non-proteoid roots. The populations of the first and 25th washing and the macerate were similar. Only five species (A. flavus, A. niger, Apiospora montagnai, Botryotrichum sp. and Drechslera sp.) were restricted to the non-rhizosphere soil but none were common.

No VA mycorrhizal fungi were detected in the roots of either H. sericea or L. parile.

Discussion

The fungal populations of sand plain lowland fynbos resemble those reported from tropical, desert and other warm regions where the genus Aspergillus tends to be abundant (Warcup 1951; Barron 1968). Aspergillus fumigatus is the most frequent species isolated in the present study but is rare or absent in other South African soils, viz. Zululand forest soils (Eicker 1969), Transvaal savanna (Eicker 1974) and a western Transvaal Acacia karoo community (Papendorf 1976). This species is common in Australian heathlands as are A. duricaulis and A. unilaterals (McLennan & Ducker 1954) which occur on the root systems of both H. sericea and L. parile but have not been reported elsewhere in South Africa. pH has been implicated as a major determinant of soil mycological populations (Jensen 1931; Warcup 1951; Singh 1980). The Clovelly sand at Pella and Australian heathlands soils have a pH less than those of the Transvaal savanna which have fungal populations dominated by P. multicolor (= P. slorotium v. Beyma) and Gloeocladium roseum not present at Pella (Eicker 1974).

Members of the Zygomyctotina are less frequent in the Clovelly sand of this study compared with other soils of Australian heathlands (McLennan & Ducker 1954) and Californian chaparral (Cooke 1970). Sandy soils in warm dry regions generally contain low Zygomyctotina populations (Nicot 1960; Wohlrab & Tuveson 1965; Papendorf 1976) and
Table 2 Fungi isolated from the 1st and 25th washing of serially washed roots, and macerate of washed roots of *Hakea sericea*. Figures denote the number of samples in which each species occurred from 10 of each washing and four macerated root systems.

| Species                                      | Proteoid                  | Non-proteoid               |
|----------------------------------------------|---------------------------|---------------------------|
| Mortierella vinacea Dixon-Stewart            | 1                         | 1                         |
| Mucor hiemalis Wehmer                        | 1                         | 1                         |
| Byssoschlamys nivea Westling                  |                           |                           |
| Chaetomium cochlodes Pall.                   | 1                         |                           |
| Chaetomium funicolum Cooke                   |                           |                           |
| Eupenicillium hirayamae Scott & Stolk        | 2                         | 1                         |
| Eupenicillium pinetorum Stolk                |                           |                           |
| Microascus triganosporus Emmons & Dodge      |                           | 1                         |
| Talaromyces wortmannii (Klöcker) C.R. Benjamin|                           | 1                         |
| Unidentified Basidiozymyte 1358              |                           |                           |
| Phoma sp. 1361                               | 1                         | 1                         |
| Dark Coelomycte 1315                         |                           |                           |
| Aspergillus duricaulis Raper & Fennell       | 2                         | 4                         |
| Aspergillus fimigatus Fr.                    | 2                         | 2                         |
| Aspergillus unilateralis Thrower             | 1                         | 7                         |
| Curvularia sp. 1316                          |                           | 2                         |
| Fusarium spp. 1321-23                        | 1                         | 2                         |
| Harzia sp. 1324                              |                           | 1                         |
| Helicoon sp. 1325                            |                           |                           |
| Paecilomyces marquandii (Masssee) Hughes     |                           |                           |
| Paecilomyces variotii Bain                   |                           |                           |
| Penicillus funiculosum Thom                  |                           |                           |
| Penicillus janczewskii Zaleski               |                           |                           |
| Penicillus metini Thom                       |                           |                           |
| Penicillus novae-zeelandiae v. Beyma          | 3                         | 1                         |
| Penicillus rastrickii G. Smith               |                           | 1                         |
| Penicillus restrictum Gilman & Abbott        | 4                         | 5                         |
| Penicillus verruculosum Peyronel             | 2                         | 2                         |
| Periconia digitata (Cooke) Sacc.             |                           |                           |
| Scopulariopsis brevicaulis (Sacc.) Bain.      | 4                         | 1                         |
| Scopulariopsis sp. 1348 S. brumptii series   | 1                         | 1                         |
| Trichoderma harzianum Rifai                  |                           |                           |
| Trichoderma koningii Oudem.                  |                           |                           |
| Trichoderma pseudoconingii Rifahi            |                           |                           |
| Trichoderma viride Pers. ex Gray             | 3                         | 1                         |
| Trichoderma sp. 1355                         |                           | 3                         |
| Talaromyces lagena Delitsch                  | 1                         | 1                         |
| Ulocladium atrum Preuss                      |                           |                           |
| Wardomyces anomalus Brooks & Hansf.          |                           | 1                         |
| Unidentified Porospore 1362                  |                           |                           |
| Black sterile 1359                           | 2                         | 1                         |

The root zone compared with the soil (Thrower 1954; Jalalud-din 1975; Odunfa & Oso 1979). The Clovelly sand at Pella is of a low phosphorus status (Mitchell *et al.* 1984), which would limit fungal growth in the non-rhizosphere region (Warcup 1951; Brian 1960; Robinson *et al.* 1968; Barber & Lynch 1977). Active plant roots exude metabolites which may stimulate spore germination and supply nutrients for fungal growth (Rovira 1979; Barber & Martin 1976). Any similarities between the soil and root zone may be ascribed to the fact that the soil contains a species reservoir which colonizes new substrates e.g. growing roots. However, some fungal species were absent or rare in the non-rhizosphere soil e.g. *A. unilateralis, P. restrictum, Scopulariopsis* sp. and members of the Ascomycotina. Roots may provide a relatively permanent habitat in which fungal metabolism can continue and perhaps provide the specialized nutritional needs of some fungi.
Table 3  Fungi isolated from 1st and 25th washings of serially washed roots, and macerate of washed roots of *Leucospermum parile*. Figures denote the number of samples in which each species occurred from 10 of each washing and four macerated root systems

| Species                                | Proteoid  | Non-proteoid |
|----------------------------------------|-----------|--------------|
|                                        | 1st wash  | 25th wash    | Mace-rate | 1st wash  | 25th wash    | Mace-rate |
| *Mortierella isabellina* Oudem.         | 1         |              |           | 1         |              |           |
| *Mucor hiemalis* Wehmer                 |           |              |           | 1         |              |           |
| *Mucor plumbus* Bonord                  | 1         |              |           |           |              |           |
| *Chaetomium funiculum* Cooke            | 1         |              |           |           |              |           |
| *Chaetomium humicolum* v. Warmelo       | 1         | 1            |           | 1         |              |           |
| *Chaetomium sp.* 3133                   | 1         |              |           |           |              |           |
| *Eupenicillium hirayamae* Scott & Stolk| 1         | 1            |           | 1         |              |           |
| *Eupenicillium pinetorum* Stolk         | 2         | 1            | 2         | 1         | 1            |           |
| *Neosartorya fischeri* (Wehmer) Malloch & Cain | 4 | 2 | 1 | 1         |              |           |
| Pale coelomycete 1314                   |           |              | 1         |           |              |           |
| *Aspergillus duricaulis* Raper & Fennell| 1         | 3            | 1         | 2         | 2            |           |
| *Aspergillus flavipes* (Bain & Sart.) Thom & Church | 1 |           |           |           |              |           |
| *Aspergillus fumigatus* Fr.             | 8         | 2            | 3         | 1         |              | 1         |
| *Aspergillus unilateralis* Thrower      | 2         | 2            | 2         | 1         |              | 1         |
| *Aspergillus versicolor* group (Vuill.) Tiraboschi | 1 |           |           |           |              |           |
| *Cladosporium herbarum* (Pers.) Link ex Gray | 1 |           |           |           |              |           |
| *Fusarium* spp. 1321 – 23               | 1         | 1            |           |           |              |           |
| *Penicillium citrinum* Thom             | 1         |              |           |           |              |           |
| *Penicillium fumiculosum* Thom          | 1         | 1            |           | 1         |              |           |
| *Penicillium glabrum* (Wehmer) Westling | 2         | 1            |           |           |              |           |
| *Penicillium janczewskii* Zaleski       | 1         |              |           | 2         | 2            |           |
| *Penicillium miczynskii* Zaleski        |           |              | 1         | 1         |              |           |
| *Penicillium novae-zeelandiae* v. Beyma | 4         | 2            | 1         | 1         | 1            | 2         |
| *Penicillium purpurogenum* Stoll        | 1         |              |           |           |              |           |
| *Penicillium raistrickii* G. Smith      | 4         | 3            | 3         | 4         | 2            | 1         |
| *Penicillium restrictum* Gilman & Abbott| 7         | 3            | 1         | 2         | 2            | 1         |
| *Penicillium versuculosum* Peyronel     | 1         |              | 2         |           |              |           |
| *Penicillium sp.* 1. 1344               | 1         |              |           |           |              |           |
| *Penicillium sp.* 2. 1345               |           |              | 1         |           |              |           |
| *Periconia digitata* (Cooke) Sacc.      |           |              |           | 2         |              |           |
| *Scopulariopsis brevicaulis* (Sacc.) Bain | 2         | 1            | 3         | 2         | 1            | 2         |
| *Scopulariopsis* sp. 1348 *S. brumptii* series | 3 | 1 | 1 | 1 |           |           |
| *Tolypocladium lagena* Delitsch          | 1         |              | 1         |           |              |           |
| *Trichoderma pseudokoningii* Rifai      | 1         |              | 1         |           |              |           |
| *Trichoderma viride* Pers. ex Gray      | 1         |              |           |           |              |           |
| *Ulocladium atrum* Preuss               | 1         |              |           |           |              |           |
| White sterile                           | 1         | 3            |           |           |              |           |
| Black sterile                           | 1         |              | 1         |           |              |           |

(Robinson et al. 1968; Barber & Lynch 1977).

The roots of *H. sericea* and *L. parile* do not possess VA mycorrhizal fungi, agreeing with the study of Malajczuk et al. (1981). The fungal flora of the proteoid roots of *L. parile* was more diverse than that of the non-proteoid regions. Proteoid roots occur in regions of high organic matter (Purnell 1960; Lamont 1981), where a more diverse fungal population may be present (Bisset & Parkinson 1979). The similarity of the fungal flora of the root systems of *H. sericea* and *L. parile* growing in the same soil agrees with the studies of Thrower (1954), showing that six Australian heathland plant species supported similar rhizosphere fungal populations. *H. sericea* is an aggressive alien in the SW Cape (Fugler 1982) and one possible reason for its success is the ability of its root systems to accommodate the indigenous soil mycoflora.

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