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Biodiversity in the Cape Floral Kingdom: fungi occurring on *Proteaceae**

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The South African Cape Floral Kingdom is the world's smallest and most diverse plant Kingdom, but is greatly under threat from urbanisation and agriculture. It covers only 90000 km² and has a Mediterranean climate. This area has a long history of botanical studies, but little research has been undertaken on the associated fungi. Therefore, a biodiversity study of the fungi has been initiated. The catalyst for this study has been the diversity of unusual fungi encountered whilst studying the fungal pathogens of *Proteaceae*. These pathogens include species, or entire genera of fungi that are specific to genera of *Proteaceae*. The diversity and uniqueness of the *Proteaceae* pathogens may indicate a similar diversity in saprobic fungi in this unusual habitat. Therefore, microfungi associated with genera of *Proteaceae* in various habitats are being systematically sampled over a two-year period. The initial results of this study are presented.

INTRODUCTION

The Western Cape Province of South Africa is a botanically important area as it contains the world's smallest floral Kingdom, known as the Cape Floral Kingdom (CFK). There are five other floral Kingdoms but these are generally much larger such as the majority of the Northern Hemisphere and the whole of Australia, whereas the Cape Floral Kingdom covers only 90000 km² (Bond & Goldblatt 1984). Within this area there are approximately 8600 species of seed plants and pteridophytes. The Cape Floral Kingdom is thought to have unusually high levels of alpha-diversity (number of species in a small area) and appears to be particularly high in differences in species composition from one area to another (delta-diversity) (Cowling 1992). There is no doubt that CFK is one of the richest and most distinctive of any small area of the world. In addition there is a high degree of endemism with approximately 70% of the species and 20% of the genera comprising the Cape flora being endemic. This also includes seven plant families found nowhere else in the world (Bond & Goldblatt 1984).

Geographically the area covers South Africa's southern and western Cape, in a crescent-shaped band from Niewoudtville in the north to Cape Town in the south and extends as far eastwards as Port Elizabeth. The topographic situation is mixed with mountains, valleys and coasts. The climate of the Cape Floral Kingdom is Mediterranean or semi-Mediterranean. The majority of the Cape Floral Kingdom is comprised of fynbos. This vegetation type is characterised by mainly schlerophyllous to microphyllous woody taxa, and the presence of large numbers of taxa from the *Proteaceae*, *Ericaceae*, *Rutaceae* and *Restionaceae* (Bond & Goldblatt 1984). There are approximately seven other vegetation types ranging from renosterveld which also has a high degree of endemism, to Knysna forest vegetation which is species poor and low in endemism. All of these vegetation types are under threat and preservation of the Cape Floral Kingdom is an urgent (inter-) national conservation priority.

Even though the fynbos cannot support large mammals due to the lack of rich protein sources, resulting from nutrient-poor soils, it harbours a large amount of butterfly, reptile, amphibian and fish species which also have high endemic rates and are equally threatened (Cowling & Richardson 1995). The CFK is ecologically very delicately balanced. As a result of urbanisation, the spread of agriculture, the naturally limited range of many species and the spread of alien plants, numerous fynbos inhabitants are now seriously endangered or extinct (Cowling & Richardson 1995).

The *Proteaceae* are the most characteristic and floristically diverse family of plants in the fynbos. This family is a member of the *Proteales* in the *Rosidae*, and dates back about 96 million years, representing one of the most prominent families of the Southern Hemisphere in southern Africa, Asia, Australia and Central and South America, especially in areas with long dry seasons. The majority of genera, however, are found in Australia and South Africa. The family is divided into more than 60 genera and 1400 species (Rebelo 1995). In Africa this family is represented by 16 indigenous and naturalised genera,

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including *Protea*, *Leucospermum* and *Leucadendron* with about 400 species, and in the south-western Cape more than 330 species with a percentage of 96% endemism. They are, however, also one of the main extinction groups, with about 125 southern Africa's proteas listed in the Red Data Book (Rebelo 1995).

The importance of diversity in this part of South Africa is well recognised, and there is a long history of Botanical and Zoological research which still continues to the present day. The issues threatening the Cape Floral Kingdom are now being realised, as the pressure facing certain parts of this Kingdom increases. Thus, there have been several initiatives proposed which will support its conservation. For instance, Table Mountain was proclaimed a Natural and Historic Monument in 1951 and is now applying for recognition as a World Heritage Site. However, despite the obvious diversity in the CFK, the microbial component has largely been ignored, other than some studies on mycorrhizae (mainly vesicular-arbuscular mycorrhizas, but also orchid and ericoid mycorrhizas, with indigenous ectomycorrhizal species lacking) (Allsopp & Stock 1993) and ecological studies on soil microorganisms (Reenen, Visser & Loos 1992).

Currently there is a great interest in biodiversity issues, especially in relation to conservation of biodiversity, not least in the mycological world (Hawksworth 1991). Habitats in tropical regions have been targeted by mycologists as areas where little research has been undertaken (Isaac *et al*. 1993, Hyde 1997). This has been due to a combination of factors, most importantly that most mycological research has taken place in the temperate Northern Hemisphere. In addition, tropical habitats occur mainly in developing countries where there is a lack of necessary resources for mycological biodiversity studies. However, despite the diversity of plant hosts in the Cape Floral Kingdom, mycological biodiversity studies have never been undertaken. This is in spite of the fact that this botanical region is in desperate need of conservation and therefore an immediate priority for study.

In an attempt to redress this imbalance, therefore, the mycota associated with certain components of the CFK is being investigated. This approach is taken in order to determine trends in the preliminary data. As mentioned, one of the major components of the CFK is the fynbos vegetation type of which the *Proteaceae* is characteristic, and this forms the subject matter for the present study.

MATERIALS AND METHODS

The data in this study were compiled from various sources. The search for data was comprehensive, but possibly not exhaustive. Records of fungi reported in the literature on *Protea*, *Leucospermum* and *Leucadendron* were utilised, as well as unpublished data from the saprobic fungal biodiversity study, which is currently in progress. Records were sorted according to substrate and to whether their association was unspecific or specific to the above *Proteaceae* genera. Two graphs were then generated of numbers of species and genera associated with each substrate, with the relationship (unspecific or specific) indicated.

A biodiversity study has recently been initiated, concentrating on *Proteaceae* (specifically *Protea*, *Leucospermum* and *Leucadendron*), which forms part of the fynbos vegetation type in the CFK. The aim of this research is to undertake a systematic study of the fungi associated with certain members of the Cape Floral Kingdom, in order that a fungal biodiversity database can be developed, that can ultimately provide baseline data for ecological and other studies. Overall, the study involves collection and identification of saprobic microfungi from up to 58 species of *Protea*, *Leucospermum*, *Leucadendron* at 8 different sites, over 4 seasons for a two year period. Herbarium specimens are deposited at PREM (National Collection of Fungi, Pretoria), while fungal cultures are maintained at the Department of Plant Pathology at the University of Stellenbosch (STE-U). These data form the basis of a fungal biodiversity database for *Proteaceae*. The experimental design of the latter allows for comparisons of the diversity of fungi from the same hosts at different sites (e.g. coastal vs montane sites); of hosts within a site (within and between host genera); and during different seasons of the year. Endophyte studies are also being carried out (Swart *et al*. 2000) in conjunction with normal field collections.

The current understanding of fungi associated with *Proteaceae* is summarised in Figs 1–2. These present the numbers of genera and species recorded on *Protea*, *Leucospermum* and *Leucadendron* from published sources (van Wyk

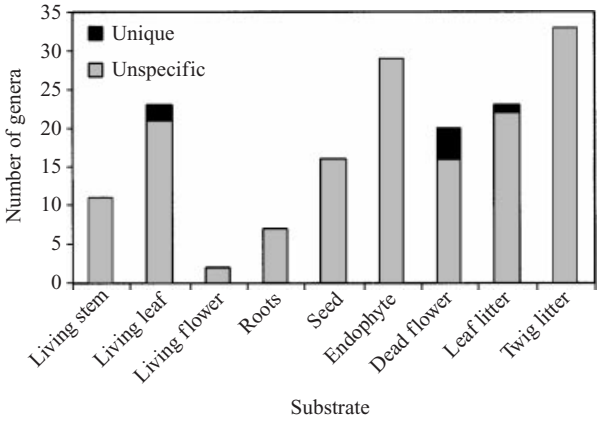

Fig. 1. Number of protea-specific and unspecific genera associated with different substrates of protea hosts.

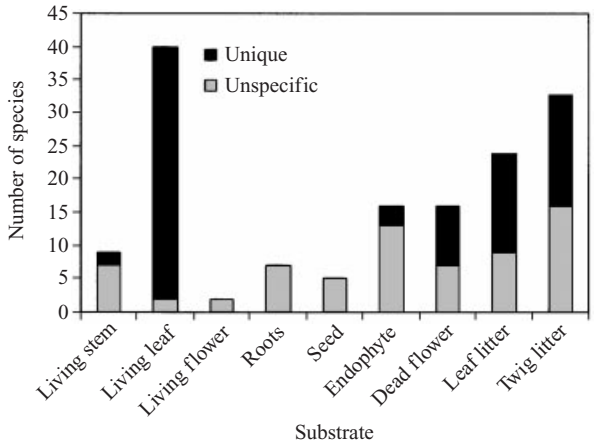

Fig. 2. Number of protea-specific and unspecific species associated with different substrates of protea hosts.

1973a, b, van Wyk & Koeppen 1974, Knox-Davies 1975, 1981, 1987, van Wyk, Marasas & Knox-Davies 1975a, b, 1985, van Wyk, Marasas & Hattingh 1976, von Broembsen 1979a, b, 1981, 1984a, b, 1986, 1989, Knox-Davies *et al*. 1981, Benic & Knox-Davies 1983a, b, van Wyk *et al*. 1985, von Broembsen & Brits 1985, 1986, von Broembsen & Kruger 1985, Benic 1986, Knox-Davies, van Wyk & Marasas 1986, 1987, Serfontein & Knox-Davies 1990a, b, van Wyk & Wingfield 1990, von Broembsen & van der Merwe 1990, Crous & Wingfield 1993, Crous & Braun 1994, Swart *et al*. 1998, Taylor & Crous 1998, Crous & Palm 1999, Swart 1999, Swart *et al*. 1999a, b, Taylor, Crous & Wingfield 1999, Crous, Phillips & Baxter 2000, Swart *et al*. 2000, 2001, Taylor *et al*. 2001, Taylor & Crous 2000, 2001) and from currently unpublished data maintained in our database.

When analysing Figs 1–2, the following must be taken into account. In Fig. 1, any genus that is reported at least once, associated with a given host substrate category is included. This is regardless of how many different species are recorded. In Fig. 2, all species are represented, except members of those genera that were not identified to species level, such as *Alternaria* spp., *Pestalotiopsis* spp., *Phoma* spp. etc., and morphotypes (such as the *Mycelia sterilia* from the endophyte studies), that were not identifiable to species level. Thus, this may effect the outcome for endophyte, seed and saprobe studies where many unspecific genera with largely unidentifiable members are encountered.

RESULTS AND DISCUSSION

The first fungus described from proteas in the Cape was *Cercospora protearum* (Cooke 1883). Approximately 30 fungal species were listed by Doidge (1950), with 50–60 species recognised by Crous *et al*. (2000). The majority of the studies have been carried out since 1970 and this has been in response to the increase in the commercial cultivation and trade in proteas. Much of this work was pioneered by P. S. Knox-Davies and co-workers. Recent interest in protea pathogens has been stimulated in response to new and comprehensive phytosanitary regulations introduced by the World Trade Organisation (WTO) (World Trade Organisation 1994, Taylor 2001, Taylor, Crous & Palm 2001, Taylor, Crous & Swart 2001, Crous *et al*. 2001). Thus there is a basic knowledge of fungi associated with proteas which forms the basis of the current biodiversity study.

A combination of the published information and the results of preliminary studies thus far indicate that there are few genera specific to *Proteaceae* (Fig. 1). Host-specific genera are primarily linked to living leaves, and are then represented by obligate, biotrophic pathogens. In addition, the unique association between ophiostomatoid fungi and serotinous flowers of *Protea* is confirmed (Wingfield, van Wyk & Marasas 1988, Marais & Wingfield 1992, 1993, 1994, Mouton, Wingfield & van Wyk 1993, Wingfield & van Wyk 1993, Marais *et al*. 1998). Thus unlike substrates in tropical habitats such as palms which consistently have host-specific genera and even families of fungi associated with them as pathogens, endophytes and saprobes (Hyde, Fröhlich & Taylor 1997), this does not appear to be the case for proteas from a mediterranean habitat. However, of the genera that are associated with proteas, many of the species appear to be protea-specific (Fig. 2). This is certainly true of those species with more intimate associations such as those from living leaves and dead flowers, but also for many of the saprobic species from leaf and twig litter. Thus a trend is emerging of unspecific, common or widespread genera being represented by protea-specific species.

A further trend to emerge is that these fungi tend to be host-specific at the host genus level. For instance, in South Africa species of *Teratosphaeria* are unique to *Protea*, whereas *Helicosingula leucadendri* is limited to *Leucadendron* hosts. Thus far, there are no fungal species that are restricted to a single host species. However, genera or species tend to be restricted to genera in the *Proteaceae*, or to the family itself in South Africa.

The outcome of this preliminary study would suggest that there are many new species to be recorded in the CFK. There are almost 9000 plant species and many other habitats and substrates which have barely received any attention as well as additional groups such as lichens, aquatic fungi, etc. However, the likelihood of finding genera unique to this environment is speculative. A useful exercise would be to investigate the mycota recorded from unique hosts in other better-studied Mediterranean habitats. For instance, in Europe, there has been a long history of mycological research and there must be many plant hosts in the Mediterranean, which are as well studied as the proteas. Furthermore, in conjunction with the study of the fungi of South African *Proteaceae*, the mycota of the *Proteaceae* in Australia will also be investigated. Preliminary surveys of the literature, and herbarium specimens indicate that this mycota appears to be unique, differing from that in South Africa. For instance, members of *Phyllachoraceae* have not been recorded from *Proteaceae* in South Africa, but fourteen taxa occur on Australian *Proteaceae* (Pearce, Reddell & Hyde 2001). These differences represent an interesting dichotomy, which may mirror the taxonomic divisions in the family *Proteaceae*.

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