Protea infructescences represent a unique fungal niche

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The biodiversity of the saprobic microfungi occurring in *Protea* infructescences (flowerheads) was investigated. A total of 28 fungal species including 14 ascomycetes and 14 anamorphic fungi were collected from 2000-2001. The mycoflora of the infructescences, especially the flowers, were found to differ totally from that of the bracts and other *Protea* tissues. This indicates their uniqueness as fungal micro-habitat. Furthermore, the majority of ascomycete species isolated from these flowers were characterised by having long ostiolar necks. This finding indicates that insects play a major role in the dispersal of the ascomycetes that occur on these infructescences, which is further corroborated by the unusually high number of insects that frequent these flowers. From these data it is clear that the saprobic fungal flora of *Protea* infructescences have a unique ecological role. However, the exact nature of this interaction will only become clear once further studies are conducted monitoring the individual components of this ecosystem.

Key words: flowerheads, fynbos, *Gondwanamyces*, micro-habitat, *Ophiostoma*, *Rhynchostoma*, species diversity.

Introduction

Fynbos is a type of vegetation uniquely characterised by restioids and confined to the nutrient-poor soils of the southwestern and southern Cape of South Africa. The Fynbos biome is defined by moderate to high amounts of winter rain and a predominance of low to medium-height shrubs, and as such includes three vegetation types: fynbos, renosterveld and subtropical thicket (Cowling and Richardson, 1995).

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Plants of the genus *Protea* L. (*Proteaceae*) are considered to be keystone members of the Fynbos biome. Many species are of considerable economic importance to the country, as they produce flowers that attain high prices on international markets, and the industry also provides employment for numerous workers in rural areas (Crous *et al.*, 2004). The genus *Protea* includes some 82 species, 69 of which are found in the biome (Rourke, 1980). The estimated age of the genus *Protea* is about 36 million years (Reeves, 2001).

Protea flowers are arranged in inflorescences surrounded by involucral bracts (Rebelo, 1995). The development of the *Protea* inflorescence starts off with a bud stage, which can last for a couple of months. At this stage the still developing flowers are protected by the closed bracts (Fig. 1). At the flowering stage the bracts curve backwards and expose the mature flowers, making them accessible to pollinators (Figs. 2, 3). Pollination can take a couple of weeks, after which the bracts recurve and enclose the flowers once again (Fig. 4). After some months the mature fruits are formed within this protective infructescence and, in many cases, are retained for several years. The infructescences usually open just after fire or when the water supply between the infructescence and the rest of the plant is severed. The structure and cycle makes serotinous *Protea* infructescences ideal micro-habitats to support a wide range of organisms, including arthropods and fungi.

In the past some attention was given to arthropods associated with these inflorescences (Visser *et al.*, 1999; Wright and Samways, 1999, 2000; Fleming and Nicolson, 2003), as well as the saprobic fungi (Wingfield *et al.*, 1988; Wingfield and Van Wyk, 1993; Marais and Wingfield, 1994, 1997, 2001; Marais *et al.*, 1998; Lee *et al.*, 2003, 2004). The present study was undertaken as part of a fungal biodiversity programme in the Cape Fynbos, aiming to investigate the diversity of saprobic fungi colonizing serotinous *Protea* infructescences.

Materials and methods

Nature reserves, national botanical gardens and undisturbed areas of the Fynbos in the Western Cape province of South Africa were visited to collect specimens. A total of 30 infructescences of 12 *Protea* species from nine different sites were made, of which 27 were made throughout the year 2000 and three in early 2001. Each specimen consisted of at least two senescent infructescences. The specimens were brought into the laboratory and dissected. Individual flowers and bracts within these infructescences were either immediately studied for fungal structures or air-dried for later study. Air-dried specimens were not rehydrated. Morphological characteristics of fungi were observed using a Nikon Eclipse E600 light microscope with differential interference contrast (DIC) and a Nikon SMZ800 dissecting microscope. Photos were taken using a digital camera (Nikon DXM1200) mounted on the microscopes. Measurements for identification were made from specimens mounted in clear lactophenol. Herbarium specimens were deposited at PREM (National Collection of Fungi, Pretoria, South Africa) and reference cultures are maintained in the culture collection of the Centraalbureau voor Schimmelcultures (CBS) in Utrecht, the Netherlands.

Results

A total of 51 fungal collections were made from senescent infructescences, of which 36 (71% of the total collections) were ascomycete fungi and 15 (29%) were anamorphic fungi. From these collections, 28 fungal species (27 genera) were identified including 14 ascomycete (50% of the total species) and 14 anamorphic fungi (50%). All genera were represented by one species only except for the genus *Gondwanamyces*, which contained two species, and also represented the highest number of fungal collections (12 collections). In *Gondwanamyces*, *G. capensis* was more common than *G. proteae*. Based on host substratum tissue types, 21 fungal species were isolated from flowers and seven from the bracts. Fungi associated with *Protea* infructescences recorded from other studies and this study are listed in Table 1.

Discussion

Since the first report of a *Protea*-specific ophiostomatoid fungus, *Gondwanamyces proteae* (M.J. Wingf., P.S. van Wyk & Marasas) G.J. Marais & M.J. Wingf. by Wingfield *et al.* (1988), and the identification of this rather unique ecological niche, special attention was drawn to the fungi associated with these flowers. The hypothesis that some interesting fungi might exist in this unusual niche, led Marais and Wingfield (1994) to survey the fungi associated with *Protea* infructescences in the Western Cape province of South Africa. During this survey they discovered four ascomycetes, including a new species, *Ophiostoma splendens*, and 23 anamorphic fungi. The current study was thus an extension of this original survey. While several additional fungi were recorded in our survey, only three ophiostomatoid fungi, *G. proteae*, *G. capensis* and *O. splendens*, were commonly isolated in both studies. The difference in the fungi recovered between the two studies can be explained by the different ages of material sampled, which also relates to different kinds of insects (vectors) being involved, as well as by changes in environmental

Table 1. List of fungi recorded from *Protea* infructescences.

Fungi	Literature Source*	Host substratum	
		Tissue	Host species
Ascomycetes			
Chaetomium globosum Kunze	9	Flowers	*P. magnifica* (PREM 58634) *P. lepidocarpodendron* (PREM 58636) *P. repens* (PREM 58635)
Chaetomium indicum Corda	3	Flower	*P. repens*
Gibberella sp.	9	Flowers	*P. magnifica* (PREM 58637)
Gloniopsis praelonga (Schwein.) Underw. & Earle	7	Bract	*P. lepidocarpodendron* (PREM 57554) *P. repens* (PREM 57538)
Glonium chambianum A.L. Guyot	7	Bract	*P. susanne* (PREM 57560)
Gondwanamyces proteae (M.J. Wingf., P.S. van Wyk & Marasas) G.J. Marais & M.J. Wingf. (≡ *Ceratocystiopsis proteae* M.J. Wingf., P.S. van Wyk & Marasas)	1, 3, 5, 9	Flowers	*P. repens* (PREM 58638) *P. neriifolia* (PREM 58639) *P. amplexcaulis* (PREM 58640)
G. capensis (M.J. Wingf. & P.S. van Wyk) G.J. Marais & M.J. Wingf. (≡ *Ophiostoma capense* M.J. Wingf. & P.S. van Wyk)	2, 3, 5, 9	Flowers	*P. burchellii* (PREM 58645) *P. lanceolata* (PREM 58647) *P. laurifolia* (PREM 58646) *P. lepidocarpodendron* (PREM 58643) *P. longifolia* (PREM 58641) *P. magnifica* (PREM 58642) *P. neriifolia* (PREM 58644)
Hysterium angustatum Alb. & Schwein.	7	Bract	*P. susanne* (PREM 57581)
Thyridaria macrostomoides (De Not.) M.E. Barr	9	Bract	*P. laurifolia* (PREM 58648)
Lophiostoma fuckelii Sacc.	9	Bract	*P. lepidocarpodendron* (PREM 58650)
Monascostroma sp.	9	Bract	*P. lepidocarpodendron* (PREM 58649)
Ophiostoma africanum G.J. Marais & M.J. Wingf.	6	Flowers	*P. gaguedi*
O. protearum G.J. Marais & M.J. Wingf.	4	Flowers	*P. caffra*
O. splendens G.J. Marais & M.J. Wingf.	3, 9	Flowers	*P. laurifolia* (PREM 58651)
Rhynchostoma proteae S. Lee & Crous	8	Flowers	*P. burchellii* (PREM 57499) *P. laurifolia* (PREM 57498, CBS 112051)
Sordaria sp.	9	Flowers	*P. burchellii* (PREM 58652) *P. laurifolia* (PREM 58653) *P. repens* (PREM 58654)
Unidentified ascomycete	9	Flowers	*P. lepidocarpodendron*
Anamorphic fungi			
Acremonium spp.	3	Flowers	*P. lepidocarpodendron* *P. neriifolia* *P. repens*
Alternaria alternata (Fr.) Keissl.	3	Flowers	*P. neriifolia* *P. repens*
Cephalotrichum stemonitis (Pers.) Nees	3	Flowers	*P. repens*
Cladosporium cladosporioides (Fresen.) de Vries	3	Flowers	*P. neriifolia*

Table 1 continued. List of fungi recorded from *Protea* infructescences.

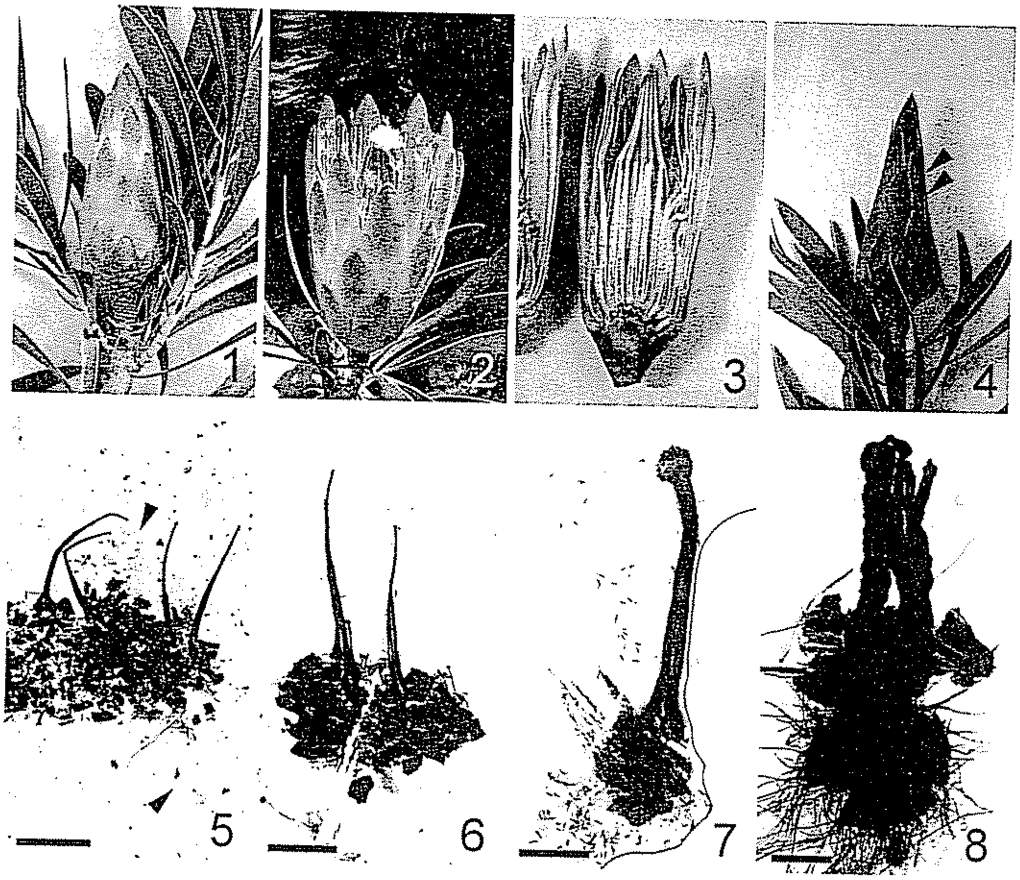
Fungi	Literature Source*	Host substratum	
		Tissue	Host species
C. sphaerospermum Penz.	3	Flowers	*P. nitida*
C. tenuissimum Cooke	3	Flowers	*P. neriifolia*
Cladosporium spp.	3, 9	Flowers	*P. repens* *P. acaulos*
Coniothyrium proteae Crous & Denman	9	Flowers	*P. laurifolia* (PREM 58655)
Drechslera erythrospila (Drechsler) Shoemaker	8	Flowers	*P. burchellii* (PREM 58058)
Fusarium anthophilum (A. Braun) Wollenw.	3	Flowers	*P. burchellii* *P. longifolia* *P. magnifica* *P. neriifolia* *P. nitida* *P. repens*
Fusicoccum sp.	9	Bract	*P. obtusifolia* (PREM 58656)
Gliocladium solani (Harting) Petch	8	Flowers	*P. nitida* (PREM 58067, CBS 113336)
Heteroconium solaninum (Sacc. & Syd.) M.B. Ellis	8	Flowers	*P. laurifolia*
Penicillium canescens Sopp	3	Flowers	*P. longifolia* *P. neriifolia* *P. nitida*
P. chrysogenum Thom	3	Flowers	*P. repens*
P. dendriticum Pitt	3	Flowers	*P. repens*
P. funiculosum Thom	3	Flowers	*P. neriifolia*
P. glabrum (Wehmer) Westling	3	Flowers	*P. longifolia*
P. minioluteum Dierckx	3	Flowers	*P. neriifolia*
P. novae-zelandiae J.F.H. Beyma	3	Flowers	*P. neriifolia*
P. purpurescens (Sopp) Biourge	3	Flowers	*P. repens*
P. rugulosum Thom	3	Flowers	*P. neriifolia*
P. thomii Maire	3	Flowers	*P. longifolia* *P. repens*
Penicillium spp.	8	Flowers	*P. repens* (PREM 58120) *P. laurifolia*
Phaeoisaria clematidis (Fuckel) S. Hughes	8	Flowers	*P. lepidocarpodendron* (PREM 58090) *P. neriifolia*
Phoma sp.	9	Flowers	*P. neriifolia* (PREM 58657)
Pithomyces valparadisiacus (Speg.) P.M. Kirk	8	Flowers	*P. lepidocarpodendron* (PREM 58094, CBS 113339)
Sporidesmium sp.	8	Flowers	*P. lepidocarpodendron*
Stachybotrys albipes (Berk. & Broome) S.C. Jong & Davis	8	Flowers	*P. amplexcaulis* (PREM 58117)
Torula herbarum Sacc.	8	Flowers	*P. lepidocarpodendron* (PREM 58106)
Unidentified species	9	Flowers	*P. neriifolia*

*1. Wingfield *et al.* (1988); 2. Wingfield and Van Wyk (1993); 3. Marais and Wingfield (1994); 4. Marais and Wingfield (1997); 5. Marais *et al.* (1998); 6. Marais and Wingfield (2001); 7. Lee and Crous (2003a); 8. Lee *et al.* (2003); 9. This study.

conditions over the 10-year time gap, site disturbance by increased human activity, urbanization, vegetation rotation, and frequent fires. In an ecological study of ophiostomatoid fungi, Roets *et al.* (2005) observed changes in fungal composition and occurrence ratio during the course of different seasons. Furthermore, they found a high occurrence of ophiostomatoid fungi in July, which is the wet, winter season. Most of *Penicillium* species presented in Marais and Wingfield (1994) were isolated from the infructescences where insects damaged the individual flowers (G.J. Marais, pers. comm.). In our study, however, most of the infructescences collected were more or less free from insect damage. To some extent the degree of insect activity within the infructescences corresponds to the ages of the structures. In consideration of sequential changes of fungi (fungal succession) by season and age, a further study with a wider spectrum of collections incorporating different seasons, infructescence ages and host species is required to get the full understanding of fungal diversity in the *Protea* infructescences.

The prevalence of ophiostomatoid fungi in *Protea* infructescences was confirmed, along with new records of ascomycete fungi having long ostiolar necks, e.g. *Rhynchostoma proteae* (Lee *et al.*, 2003) and a presumed new species (Figs. 7, 8). Ophiostomatoid fungi are well-known insect-associated fungal groups that are specially adapted to insect dispersal. These fungi are characterised by long ostiolar necks and ascospores produced in a sticky mass (Fig. 5). Ophiostomatoid fungi associated with *Protea* infructescences were shown to be phylogenetically distinct from true ophiostomatoid fungi from the Northern Hemisphere, in spite of their common morphological and physiological characteristics (Wingfield *et al.*, 1999). This provides an example of convergent evolution towards insect spore dispersal and the uniqueness of fungi associated with *Protea* infructescences (Wingfield *et al.*, 1999). Five ophiostomatoid fungi are thus far reported from *Protea* infructescences, two *Gondwanamyces* species with *Knoxdavesia* anamorphs, *G. capensis* and *G. proteae*, and three *Ophiostoma* species with *Sporothrix* anamorphs, *O. splendens*, *O. protearum* and *O. africanum* (Wingfield *et al.*, 1988; Wingfield and Van Wyk, 1993; Marais and Wingfield, 1994, 1997, 2001; Marais *et al.*, 1998). In the present study, two species of *Gondwanamyces* and one *Ophiostoma* species were collected, and also recorded on previously unknown *Protea* hosts.

Various flower parts such as styles, pollen presenters, parts of the perianth, and even the protective bracts play host to a diverse range of saprobic fungi. However, a significant difference in fungal taxa was observed between the two tissue types, namely bracts and flowers. In terms of ascomycete fungi, species having bitunicate asci and stromatic ascomata were found on the bracts.



Figs. 1-4. *Protea repens.* **1.** Bud stage. **2.** Flowering stage. **3.** Cross section of inflorescence showing individual flowers. **4.** Tightly closed infructescence by involucral bracts (arrowheads). **Figs. 5-8.** Rostrated ascomycete fungi isolated from senescent infructescence. **5.** *Gondwanamyces proteae* showing sticky mass of ascospores (arrowheads). **6.** *Gondwanamyces capensis.* 7. *Rhynchostoma proteae* showing mass of ascospores at the tip of ostiolar neck. **8.** An unidentified species. Bars = 200 μm.

These were also commonly found on other *Protea* plant parts such as dead twigs and leaves (Lee and Crous, 2003a). Species with unitunicate asci were collected from flowers only. No species was common to both tissue types. The uniqueness of fungi present on flowers can be ascribed to the relatively protected and humid space created within the infructescences. Tightly closed involucral bracts, which minimise airflow and rain-splash effects, protect any immigration of fungal species into the infructescences via these routes. Likewise, fungi in this micro-habitat have limited dispersal mechanisms for their progeny.

Reeves (2001) speculated that the diversity of the *Protea* species in the Cape Floral Kingdom is due to a high coexistence of species that diversified over a long period of time, rather than a recent and rapid radiation of this lineage. Rourke (1998) in reviewing the phylogeny of African *Proteaceae*, mentioned that the major morphological diversity in the Fynbos *Protea* species can be ascribed to selection pressure, especially from fire and pollinators. This long history of *Protea* diversification and selection pressure therefore implies that any organisms that is dependent on, or associated with *Protea* species, could potentially have a long co-evolutionary history, and be unique to the plants. Many authors believe that the Fynbos biome as a whole has a fauna and fungal diversity that not only matches that of the plants, but also may even far exceed it (Coetzee and Giliomee, 1987a,b; Coetzee, 1989; Visser, 1992; Swart *et al.*, 2000; Taylor and Crous, 2000; Wright and Samways, 2000; Taylor *et al.*, 2001). Previous studies on the biodiversity of saprobic fungi in the Fynbos support this notion not only by the existence of *Protea*-specific fungal genera and species, but also by fungi specific to other Fynbos plants (Taylor *et al.*, 2001; Lee and Crous, 2003b,c,d; Lee *et al.*, 2003; Mel'nik *et al.*, 2004).

Zwölfer (1979) perceived inflorescences/infructescences as miniature ecosystems, which accommodate different food chains and trophic levels. Insects and birds, which are attracted to inflorescences by their bright colours, nectar and pollen, usually pollinate the flowers. At the same time, predators feed on these insects and other arthropods contained within the inflorescences. After pollination the inflorescences close and boring-insects and seed-feeders become prominent. These have their own predators and parasites associated with them (Coetzee and Giliomee, 1987a,b; Coetzee, 1989; Visser, 1992). At this stage many fungal species can also be found colonizing the senescent flower parts. The fungi associated with inflorescences and infructescences can be seen as forming the basal trophic level, while the predators and parasites fill the upper most trophic level of this miniature ecosystem. The fungi provide nourishment to fungal feeding insects such as *Psocopterans* and other insects, which in turn fall prey to the many predators such as mites and spiders hunting within the infructescences. We recognise that the saprobic fungi colonizing infructescences may influence other macroscopic organisms inhabiting these structures. This in turn impacts on *Protea* seed dynamics, and ultimately on population dynamics. This has important conservation implications. Roets *et al.* (2005) narrowed the possible candidates for spore-dispersal vectors of ophiostomatoid fungi down to six out of 62 species (*ca.* 45 families). More studies are currently underway to identify insect vectors, and to define their relationships with these fungi, to eventually determine the ecological role that saprobic fungi play within this mini-ecosystem.

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