Endophytes isolated from three species of *Protea* in a nature reserve in the Western Cape, South Africa

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Healthy leaves of three species of *Protea, P. nitida, P. repens* and *P. neriifolia*, were tested for endophytic fungi. Nine plants of each *Protea* species were sampled from a natural site which is ecologically undisturbed, other than from periodic bush fires. Leaves were sampled from the current flush (young leaves) and the previous year's flush (old leaves). After surface sterilisation, tissue was excised from the midrib (vein) and the leaf tissue either side of the midrib (intervein), plated on agar and all fungal colonies that developed were identified. Simple correspondence analysis showed that *Protea repens* and *P. neriifolia* had similar fungal endophyte assemblages, which differed from that of *P. nitida*. Significant differences in the numbers of isolates obtained from the different hosts, tissue types and tissue ages were demonstrated. Fewer fungi were isolated from young tissues by comparison with older tissues except in the case of *P. nitida*. Overall, fewer fungi were obtained from intervein tissues than from vein tissues. A much lower number of *Botryosphaeria proteae* isolates were obtained from *P. nitida* compared with the other two hosts. *Botryosphaeria proteae* is commonly associated with leaf tip dieback symptoms on *P. repens*. As this species proved to be the dominant taxon in the present study, isolates were tested for pathogenicity. No disease symptoms were observed thus suggesting that *B. proteae* could be a true endophyte, or at best a weakly opportunistic pathogen.

Keywords: *Botryosphaeria proteae,* fungal endophytes, *Protea*.

Proteaceae (commonly known as protea) is an important plant family in South Africa. Not only does it constitute a major component of the Cape Floral Kingdom, the smallest and most diverse of all of the Floral Kingdoms (Rebelo, 1995), but it is also an important component of the cut-flower industry. As a result of an increased demand in cut-flower proteas for export, there has been an increase in research on the diseases of this crop (Denman & Crous, 1998; Swart & al., 1998; Taylor & Crous, 1998, 1999, 2000; Crous & Palm, 1999; Denman & al., 1999; Taylor & al., 1999a). This research effort

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has aimed at elucidating the etiology of diseases of Proteaceae, and at ensuring the correct identification of fungal pathogens so that quarantine rejections are scientifically based. Furthermore, investigations into the epidemiology and control of these diseases also ensure that farmers produce high quality blooms, reduce yield loss and initiate effective disease control programmes.

Many of the foliicolous fungi encountered have been observed to be specific to members of the Proteaceae at a generic or family level (Van Wyk & al., 1975a, 1975b, 1976, 1985; Knox-Davies & al., 1987; Von Broembsen, 1989; Taylor & Crous, 1998, 1999; Crous & Palm, 1999; Denman & al., 1999; Taylor & al., 1999a). In contrast root and canker causing pathogens, and those which attack flowers and new shoots tend to be widespread and plurivorous (Van Wyk, 1973; Benic & Knox-Davies, 1983a; Von Broembsen & Brits, 1985; Benic, 1986; Knox-Davies & al., 1986; Von Broembsen, 1986; Von Broembsen & Van der Merwe, 1990). For most of these pathogens, their relationship with proteas is poorly understood, which hinders disease management programmes. Therefore, a concerted effort has been focused on understanding the epidemiology of certain important pathogens.

One such foliicolous pathogen is *Botryosphaeria proteae* (Wakefield) Denman & Crous, more commonly manifested by its unusual anamorph, *Fusicoccum proteae* Denman & Crous (Denman & al., 1999). During a study of the endophytes of cultivated proteas (*Protea cynaroides* (L.) L., pure and hybrid *Leucospermum cordifolium* (Salisb. ex Knight) Fourc. and *Leucadendron salignum* P. J. Bergius x *Lcd. laureolum* (Lam.) Fourc. cv. Safari Sunset, *B. proteae* was isolated, but was not found to be a dominant taxon, and also was not recorded at all from *Leucadendron*. In addition, *B. proteae* was isolated from leaves only and not stems (Swart & al., 2000). The latter is not surprising as it is considered a foliicolous fungus and has rarely been isolated from cankers (Denman & al., 1999), where it is considered to be a secondary or opportunistic invader.

The purpose of the present study is to identify and compare the composition and distribution of endophytic fungi associated with leaves of proteas from an undisturbed habitat. In order to understand the infection process and tissue preferences of endophytic fungi associated with proteas, leaves were sampled from the current flush (young leaves) and the previous years flush (old leaves). After surface sterilisation samples were taken from the midrib (vein) and the leaf tissue either side of the midrib (intervein). By using one site, variables caused by differences in habitat, such as microclimate, can be reduced. It is acknowledged that more than one site would enable a more in depth investigation, however, the increase in sample size would not have been manageable. Wild proteas were studied, as cultivated proteas, although being grown within their natural geo-

graphic range often adjacent to their wild counterparts, are commonly sprayed with fungicides. Lastly, the pathogenicity of the dominant endophyte *B. proteae,* was tested on *P. repens* (L.) L. cv Ember in an attempt to elucidate its relationship with the host.

Methods

Endophyte isolation

Collecting was carried out on 4th March 1999. Three species of *Protea* (*P. neriifolia* R. Br., *P. nitida* Mill. and *P. repens*) were selected. All three species occur in an area of 2500 m^2 at the Jonkershoek Nature Reserve, Western Cape, South Africa. For each host species 9 trees were selected and five leaves each of the current flush (young leaves) and the previous years flush (old leaves) were collected.

Selected material was brought back to the laboratory and processed within 12 hr. A total of 270 leaves were collected and each was labelled with a number (1–270), indicating the tree species and age of tissue. A white 'Chinagraph' pencil was used for labelling the samples as the markings remain during surface sterilisation. Whole leaves were surface sterilised for 1 min in 70% ethanol, 10 min in 3.5% sodium hypochlorite and 30 s in 70% ethanol. Four 25 mm^2 pieces were excised from each of the vein (midrib) and intervein (leaf tissue on either side of the midrib) tissues of each leaf using a scalpel. Tissue pieces were plated on 2% potato dextrose agar (PDA; Biolab, Midrand, South Africa) containing a fungal growth retardant (Rose Bengal 0.033 g/l) and 0.1 g l^{-1} streptomycin sulphate.

Petri dishes were incubated at 25 C and checked for fungal colonies twice a week, up to one month after processing. Isolates were transferred onto bottles, and to split plates containing PDA in one half of the dish, and carnation leaf agar [CLA; Fisher & al. (1982)] in the other. These plates were incubated under near-ultraviolet and cool white light at 25 C for 3 wk. The isolates that produced fruiting structures were identified, and those isolates which did not fruit were separated into groups of morphotypes and designated as *mycelia sterilia.*

Pathogenicity trials

Four isolates of *Botryosphaeria proteae* that were isolated from *P. repens* were selected for pathogenicity trials. Pathogenicity tests were carried out on 50 1-yr-old rooted cuttings of *P. repens* cultivar Ember, using the above isolates. This number included 10 plants used for control inoculations. Plant were potted in 15 cm diam. pots with saucers, containing 100 cm^3 potting mixture and were watered every third day. Half of the plants were wounded immediately prior

to inoculation by pricking the leaves with a cork containing five insect needles. The other half of the plants were unwounded.

Plants were labelled and inoculated with a spore suspension of 1×10^6 spores per ml. A sample of the spore suspension was plated out onto PDA to evaluate the viability of the propagules. Plants were sprayed to run-off and control plants were treated with sterile water only. Plastic bags were immediately placed over the inoculated plants and secured with rubber bands around the pots. Bags were removed after 14 d. Leaves that showed symptoms of disease were recorded, and re-isolations were attempted on PDA. A single leaf was also removed from each plant, and the entire leaf was surface sterilised, dissected into 25 mm^2 pieces and plated out on PDA. The experiment was laid out in a randomised design with three block replications, in a green house with temperatures ranging between 18–26 C.

Statistical analysis

Percentage colonisation was calculated [(No. of samples which yielded ≥ 1 isolates / No. of samples in the trial) $\times 100$] and a Chi-square (χ^2) was carried out to test whether the colonisation rates of the three hosts were significantly different. Isolation frequencies (No. of isolates yielded in the trial / No. of samples in the trial) were calculated to demonstrate the degree of multiple colonisation from the samples taken. Three-way analysis of variance (ANOVA) was carried out on the data with species, age of tissue and tissue type as factors. The data were not normally distributed. Transformation (square root and logarithmic) of the data made no improvement due to the nature of the data set. As ANOVA is a fairly robust technique, however, it was considered acceptable to use it with the untransformed data. A Students t-test using the least significant difference (LSD) test to correct for multiple comparisons was carried out on the means to determine the source of interaction. Results were displayed graphically. In all analyses significant differences are indicated as follows: 95% confidence level ($P < 0.05$)*, 99% confidence level ($P < 0.01$)**, and 99.9% ($P < 0.001$)***. The relative importance values (RI) (Ludwig & Reynolds, 1988) of the isolates for each host were calculated to give a measure of dominance. The raw data for the isolates occurring in at least one host at more than 5% RI were used to illustrate the distribution of the most important fungal endophytes in the different hosts. Ordination by simple correspondence analysis was also performed on this data set using MVSP *Plus* Version 2.2 (Kovach, 1995) to investigate the distribution of fungal taxa among tissues. The data for the correspondence analysis were not normally distributed due to the high abundance of *B. proteae*. However, transformation of the data was considered unnecessary, as this was

regarded as a true reflection of the data set, rather than an over representation of this species.

Results

Colonisation and isolation rates

Five hundred and sixteen fungal isolates were obtained from 2160 pieces of tissue plated out (Tab. 1). Of the 432 samples yielding isolates, 360 samples (84%) yielded one isolate only per piece of excised tissue whereas 72 samples (or 16%) produced multiple isolates (including 10 tissue pieces with 3 isolates and 1 with 4 isolates). Twenty-eight isolates were contaminated before identification was possible and the remaining 488 isolates were identified. A χ^2 test showed that there was no significant difference between the colonisation rates of the three hosts ($P = 0.203$).

Tab. 1. – A comparison of the colonisation and isolation rates.

Host	*P. neriifolia*	*P. nitida*	*P. repens*
No. of samples	720	720	720
No. samples yielding isolates	177	99	156
No. of Isolates	210	115	191
Overall colonisation rate (%)	25	14	22
No. of samples yielding 1 isolate (%)	149 (84)	83 (84)	128 (82)
No. of samples yielding 2 isolates (%)	23 (13)	16 (16)	22 (14)
No. of samples yielding >2 isolates (%)	5 (3)	0 (0)	6 (4)
Overall isolation rate (No. of isolates /sample)	0.29	0.16	0.27

The number of samples with multiple isolates was fairly low at 16%, therefore the raw data for the isolations were used in the analysis. The number of isolates recovered from young and old leaves, and vein and intervein tissues from each host are presented in Tab. 2.

Tab. 2. – Number of isolates recovered from vein and intervein tissues.

	Host		
Plant part	*P. neriifolia*	*P. nitida*	*P. repens*
Young leaves	27	34	25
Old leaves	183	81	166
Vein	117	77	115
Intervein	93	38	76

Quantitative analysis of endophytes from different tissue ages and types

The only significant interaction that was found occurred between species and tissue age (Tab. 3). There were no significant

Tab. 3. – Factorial analysis of variance of the untransformed, raw data with species, age of tissue and tissue type as factors.

Factor	Degrees of freedom	F value	P value
Species (Factor A)	2	3.99	0.0217*
Tissue age (Factor B)	1	62.22	0.0000***
Tissue type (Factor C)	1	5.47	0.0214*
A*B	2	5.51	0.0054*
A*C	2	0.12	0.8885
B*C	1	1.11	0.2942
A*B*C	2	0.23	0.79430

differences in the number of isolates obtained from the young and old tissue of *P. nitida*, whereas significantly more isolates were obtained from the old tissues of the other hosts compared with the young tissues of those same hosts (Fig. 1). No differences in the number of fungi isolated from young tissues of the different hosts were observed (Fig. 1). Significantly fewer fungi, however, were isolated from old tissues of *P. nitida* than from the other two *Protea* species tested (Fig. 1). Significantly higher numbers of isolates were also obtained from the vein tissue compared with the intervein tissues in all three-host species (Tab. 2 and 3).

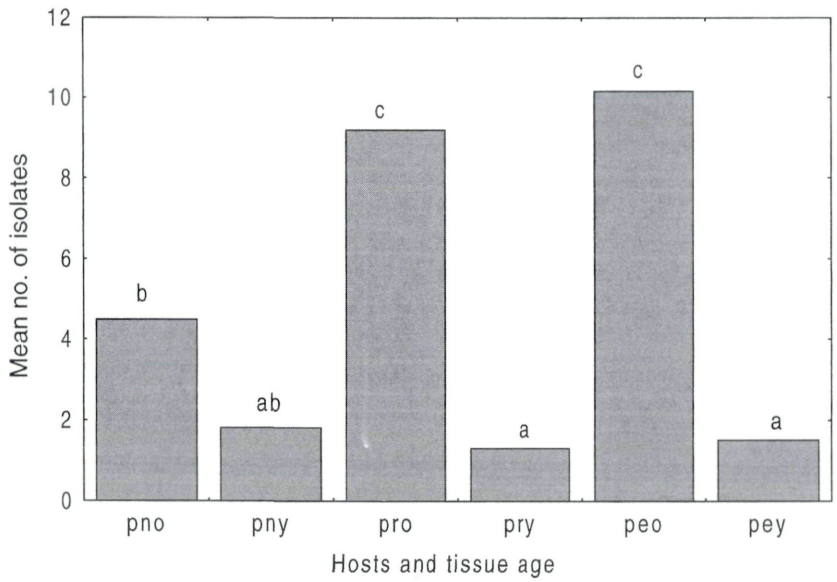

Fig. 1. – Number of isolates obtained from different aged leaves of *Protea nitida*, *P. neriifolia* and *P. repens*. – Abbreviations on the X-axis: pn, *P. nitida;* pr, *P. repens*; pe, *P. neriifolia*; o, old tissue (1-yr-old growth); y, young tissue (current years growth). Bars topped by the same letter do not differ significantly.

Composition of endophyte assemblage

The composition of the endophyte mycota for each host is illustrated in Fig. 2. *Botryosphaeria proteae* dominated the assemblage of *P. repens* and *P. neriifolia,* and represented 70% and 62% of the total isolations, respectively. The remaining taxa were uncommon or rare. In *P. nitida,* there were no dominant taxa, although *Botryosphaeria* sp., *Alternaria* sp. 1 and *Phomopsis* sp. 2 occurred more frequently than other genera.

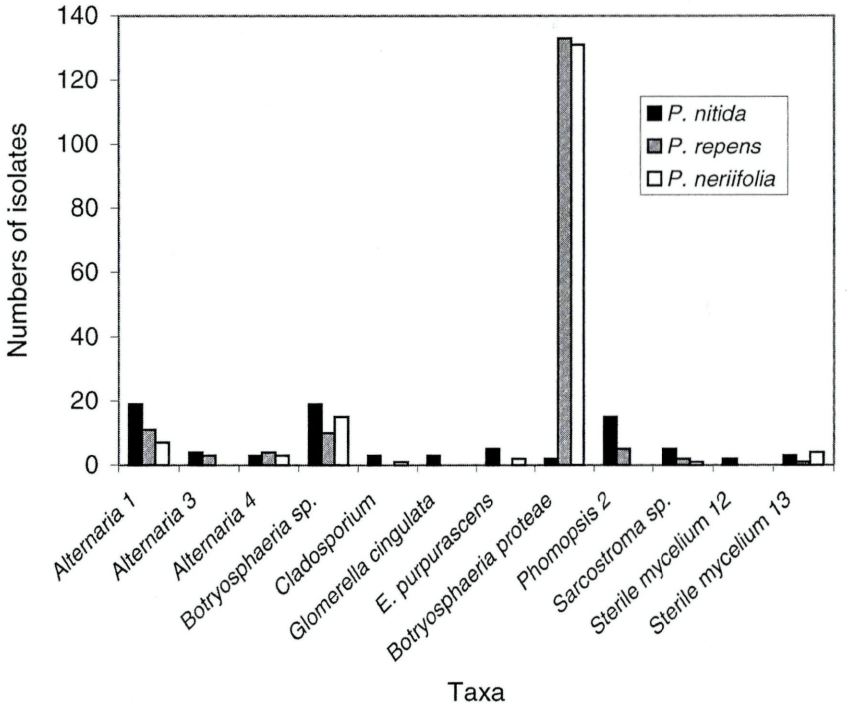

Fig. 2. – Distribution of fungal endophytes in the different *Protea* spp. – Species occurring at RI values of below 5% for each host: *Protea nitida*: *Alternaria* sp. 6, *Gliocladium* sp., *Melanospora* sp., *Nodulosporium* sp., *Phoma* sp., *Phomopsis* sp. 1, *Sporormiella minima* (Auersw.) Ahmed & Cain, *Sporormiella minimoides* Ahmed & Cain, *Stemphylium* sp., Sterile mycelium 3, Sterile mycelium 5, Sterile mycelium 6, Sterile mycelium 7, Sterile mycelium 8, Sterile mycelium 10, Sterile mycelium 14, Sterile mycelium 19, Sterile mycelium 20, Sterile mycelium 21, Sterile mycelium 23, Sterile mycelium 25, Sterile mycelium 28, Sterile mycelium 30. *Protea repens*: *Alternaria* sp. 5, *Botryosphaeria dothidea*, *Pestalotiopsis* sp. 2, *Sporormiella minima, Sporormiella minimoides*, Sterile mycelium 9, Sterile mycelium 11, Sterile mycelium 13, Sterile mycelium 15, Sterile mycelium 17, Sterile mycelium 26, Sterile mycelium 29, *Trimmatostroma macowanii* (Sacc.) M.B. Ellis. *P. neriifolia*: *Alternaria* sp. 5, *Gliocladium* sp., *Melanospora* sp., *Pestalotiopsis* sp. 1, *Pestalotiopsis* sp. 2, *Sporormiella minima, Stemphylium* sp., Sterile mycelium 3, Sterile mycelium 4, Sterile mycelium 16, Sterile mycelium 18, Sterile mycelium 21, Sterile mycelium 24, Sterile mycelium 27, Sterile mycelium 31.

Distribution of fungal taxa

The first three axes of correspondence analysis (Fig. 3) explain 72.7%. of the inertia or variation of the data set, indicating that the model fits reasonably well with the data. The first axis of the correspondence analysis separates all samples from *P. nitida* from those of *P. repens* and *P. neriifolia,* except for PRYV and PEYV. The gradient is determined by the high proportion of *B. proteae* recorded from *P. repens* and *P. neriifolia*. In the young leaves, intervein tissue of *P. repens* and *P. neriifolia* yielded more isolates of *B. proteae,* representing 60% and 77% of isolates recorded, respectively. Whereas the vein tissue of young leaves of *P. repens* and *P. neriifolia* yielded only 33% and 22% isolates of *B. proteae,* respectively, with the total assemblages closely representing those of *P. nitida* tissues. The second axis of the correspondence analysis is not particularly informative as most of the points lay along the axis.

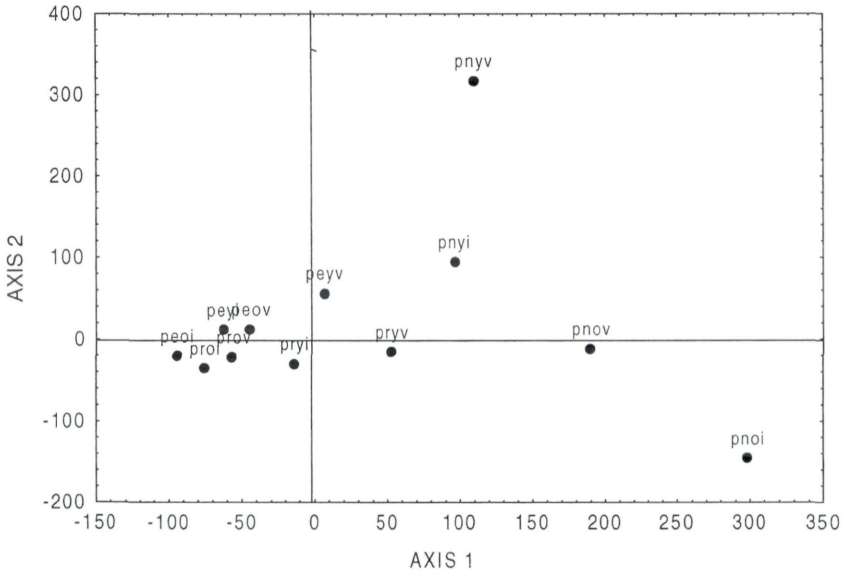

Fig. 3. – Results of ordination by simple correspondence analysis. – The combination of letters on the scatterplot encodes the provenance of the samples (pn, *P. nitida;* pr, *P. repens*; pe, *P. neriifolia*; o, old tissue; y, young tissue; v, vein; i, intervein). Only the positions of the leaf samples are plotted, and not the corresponding position of the fungal taxa. The inertia of the model explained by the first three axes is 72.7%.

There was little evidence of host specificity of the endophytes isolated. Of the isolates that occurred more than once, only Sterile Mycelium 24 (3 isolates) and 27 (2 isolates), were exclusive to *P. neriifolia*; *Glomerella cingulata* (Stoneman) Spauld. & H. Schrenk

was exclusive to *P. nitida* (3 isolates); and *Botryosphaeria dothidea* (Moug.) Ces. & De Not. restricted to *P. repens* (5 isolates). Tissue specificity was similarly lacking, with species occurring singly or very rarely in intervein tissues and several species isolated more frequently and exclusively from vein tissues, such as *Melanospora* sp. (3 isolates), *Sporormiella minima* (4 isolates), *Stemphylium* sp. (6 isolates) and *Sarcostroma* sp. (8 isolates). Only *Glomerella cingulata, Botryosphaeria dothidea* and Sterile Mycelium 24 were exclusive to old leaves, whereas species restricted to young leaves were only recorded once. The distribution of the one dominant taxon, *Botryosphaeria proteae,* is illustrated in Fig. 4.

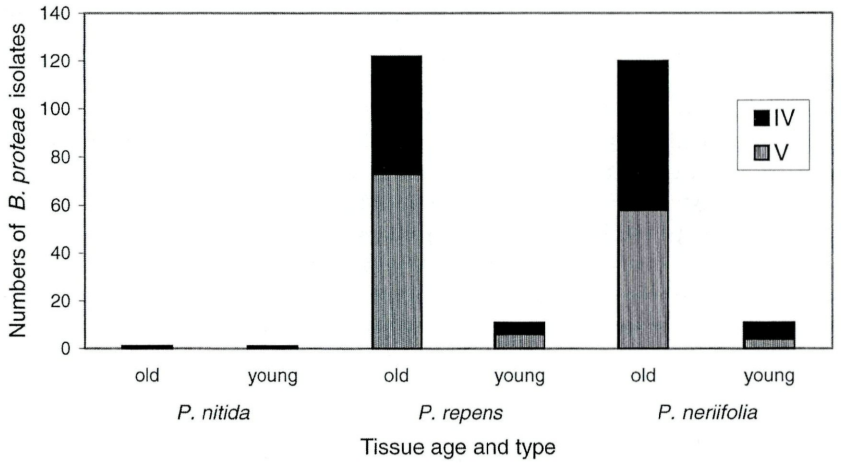

Fig. 4. – Numbers of isolates of *B. proteae* in the different tissue types of the three hosts. – Categories are represented by: old, 1-yr-old growth; young, current years growth; v, vein; iv, intervein.

Pathogenicity trials

The viability of the propagules in the spore suspensions was confirmed with germination and growth of the spores on PDA. No symptoms were recorded on the leaves of the inoculated *P. repens* cultivar Ember plants. Inoculated and control leaves were tested for the fungus 21 d after inoculation as explained above. No isolates of *B. proteae* were recovered, indicating that the attempt to inoculate the leaves had failed.

Discussion

The results of this trial differ, in several respects, from a previous, similar study (Swart & al., 2000). Overall, proportionally

fewer fungi were isolated from leaves than in the previous study, possibly due to the more rigorous surface sterilisation. In the present study, there was no significant difference between the colonisation rates, as the overall proportion of fungi isolated was low. However, in terms of *B. proteae*, the occurrence was much greater in two of the hosts. It is evident, therefore, that levels of *B. proteae* can vary depending on the *Protea* hosts sampled. *Protea cynaroides* [the *Protea* species sampled by Swart & al. (2000)] and *P. nitida* both showed reduced levels of *B. proteae* compared to *P. repens* and *P. neriifolia*. The reasons for this could be due to differential host preferences, as low levels of *B. proteae* have also been isolated from *Protea magnifica* Link (S. Denman, unpubl. data).

Differences in life strategies of the *Protea* spp. may also be influential. For instance, all three species in the present study regenerate after fire, but *P. nitida* is a resprouter, whereas the other species only grow from seeds (reseeder or non-resprouter). The effects of this can only be speculated upon and may be areas for future studies. For instance, non-resprouting proteas might be starting leaf senescence and hence offer less resistance to fungal infection. Alternatively, the resprouters, which per definition have a longer life expectancy, might offer more resistance to fungal infection, while the non-resprouters, with a shorter life expectancy might not develop such strict resistance strategies. The expected leaf 'lifetime' of protea species may also differ and thus affect resistance strategies (Dr. L. Dreyer, pers. comm.).

The age of the host might also have an effect on the results recorded in the present study, although this has not been found to be significant in previous studies on other hosts (Rodrigues, 1994; Taylor & al., 1999b), where tissue age was more important. It was interesting to note that the fungal species isolated from the vein tissues of young leaves of *P. neriifolia* and *P. repens* were more consistent with those of the tissues of *P. nitida*. This similarity, however, changed as the tissues aged.

Protea cynaroides sampled in the previous study (Swart & al., 2000) was under cultivation, another factor that may affect endophyte assemblages. It is likely that the cultivated plants would be treated with fungicides, which would affect not only the pathogens, but the endophytes also. Continued studies on other *Protea* hosts need to be carried out to verify these observations.

Another interesting finding was that the endophytic fungi showed preference for vein tissues rather than intervein tissues. The veins are the main organs of transport of photosynthates in the leaves and are thus a rich supply of nutrients. This is probably why there is a high fungal colonisation level in these tissues.

The endophyte assemblage appeared to increase in numbers and in diversity over time, which is consistent with findings of previous studies (Cabral, 1985; Bertoni & Cabral, 1988; Espinosa-Garcia & Langenheim, 1990). Qualitative or quantitative correlations according to tissue type, however, were not consistently observed.

The status of *B. proteae* as an endophyte or latent pathogen was investigated and the pathogenicity trials suggest that it is the former. Swart & al. (2000) suggested that *B. proteae* was a secondary pathogen of stem cankers, and the present study indicates that it is not an aggressive pathogen and may simply be an opportunist associated with necrotic tissue of leaves, rather than the pathogen and causal organism of the lesions. The inoculated plants in this study were kept under optimum growing conditions (controlled temperature and watering regimes) and such stress free conditions may simply not be conducive to infection and disease development. Alternatively, it is possible that the plants were at a stage in their life cycle where they were not susceptible to infection. The plants used were 1-yr-old rooting cuttings and these were inoculated after the current season's flush had hardened off. Moreover, the results in Fig. 4 suggest that infection by *Botryosphaeria proteae* does not occur in new or immature growth.

In previous studies conducted by Swart (1999), *B. proteae* was observed to cause minor stem lesions on inoculated parts of *P. cynaroides* that were subjected to water stress (–2.0 MPa). No symptoms were observed at water potential levels above –2.0 MPa, suggesting that *B. proteae* is not a virulent pathogen. Furthermore, no symptoms could be induced on *Leucospermum* or *Leucadendron* spp.

There are several published inoculation studies on Proteaceae pathogens. Positive results have been obtained on inoculated stems and new shoots for non-specific pathogens including *Botryosphaeria dothidea* (S. Denman, unpubl. data), *Glomerella cingulata* (anamorph *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc.; Benic & Knox-Davies, 1983a; Forsberg, 1993), and for specific Proteaceae pathogens such as *Elsinoë* sp. (*E. leucospermi* L. Swart & Crous; Swart & al., 2001; Benic & Knox-Davies, 1983b; Ziehrl & al., 2000), and a *Pestalotiopsis* sp. specific to Proteaceae (Swart & al., 1999). No infection, however, was achieved when leaves of *Protea cynaroides* were inoculated with the biotrophic pathogen *Batcheloromyces proteae* Marasas, P.S. Van Wyk & Knox-Dav. (Smit & al., 1983). Unlike *B. proteae,* the former has never been isolated as an endophyte.

Several of the endophytes isolated have previously been reported as plant pathogens including *G. cingulata* and *B. dothidea*, both of which are non host-specific, have a wide host range and are common on Proteaceae world wide. A black sterile fungus producing

cultures which resemble those of *Botryosphaeria* isolates, and named *Botryosphaeria* sp. was also isolated. *Phomopsis* sp. 1 and sp. 2 differ from the protea-specific canker causing pathogen (Orffer & Knox-Davies, 1989), and have never been recorded associated with leaf spots. A species that is commonly found on Proteaceae, and has been the subject of several quarantine rejections, is *Sarcostroma grevilleae* (Loos) Nag Raj. During the course of our studies we have collected specimens of this taxon from numerous host genera in the Proteaceae. In an attempt to resolve the identity of the South African taxon, we re-examined this material, and concluded that the host range of *S. grevilleae* may be much narrower than earlier expected. Collections from *Protea* L., *Leucospermum* R. Br., *Leucadendron* R. Br. and *Telopeae* F. Muell. did not correspond with those from *Grevillea*, which closely followed the description given by Nag Raj (1993). The South African species isolated as an endophyte in the present study can also be distinguished from *S. grevilleae* by its narrower medium brown conidia with short appendages. *Sarcostroma grevilleae* has thus far been confirmed on hosts such as *Grevillea baileyana* McGill., *G. laurifolia* Sieber ex Spreng., *G. robusta* A. Cunn. ex R. Br., *G. rosmarinifolia* A. Cunn. and *G. victoriae* F. Muell. occurring in Australia, New Zealand, Sri Lanka, and U.S.A. (California) (Nag Raj, 1993; J.E. Taylor, unpubl. data).

The aforementioned observations indicate that unlike many other commercial crops, little is known about the relationship between *Protea* spp. and their pathogens and what conditions or circumstances are necessary for the establishment of a pathogenic relationship. This study illustrates that under similar conditions, the endophyte assemblages of different *Protea* species can vary. This reason, and/or the fact that *Protea cynaroides* in the previous study was under cultivation (Swart & al., 2000), may account for the different levels of the most dominant endophyte *B. proteae*. It is possible that true endophytes can occur in high numbers within host tissue, whereas latent pathogens remain at low levels. Most of the endophytes isolated are widespread, plurivorous organisms, and few protea specific leaf pathogens are recorded (Fig. 2). This is possibly due to the poor competitive abilities on artificial media of the slow growing protea pathogens, compared to the aforementioned fungi. However, the occurrence of many Proteaceae-specific pathogens on South African proteas cultivated world-wide suggests that they have in fact, been spread as endophytes on cuttings, or were seed borne.

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