PATHOGENS ASSOCIATED WITH DISEASES OF *PROTEA*, *LEUCOSPERMUM* AND *LEUCADENDRON* SPP.

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DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and has not previously in its entirety or in part been submitted at any university for a degree.

DATE: 2 November 1999

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SUMMARY

The manuscript consists of six chapters that represent research on different diseases and records of new diseases of the Proteaceae world-wide. The fungal descriptions presented in this thesis are not effectively published, and will thus be formally published elsewhere in scientific journals. Chapter one is a review that gives a detailed description of the major fungal pathogens of the genera *Protea*, *Leucospermum* and *Leucadendron*, as reported up to 1996. The pathogens are grouped according to the diseases they cause on roots, leaves, stems and flowers, as well as the canker causing fungi.

In chapter two, several new fungi occurring on leaves of *Protea*, *Leucospermum*, *Telopea* and *Brabejum* collected from South Africa, Australia or New Zealand are described. The following fungi are described: *Cladophialophora proteae*, *Coniothyrium nitidae*, *Coniothyrium proteae*, *Coniothyrium leucospermi*, *Harknessia leucospermi*, *Septoria protearum* and *Mycosphaerella telopeae* spp. nov. Furthermore, two *Phyllosticta* spp., *telopeae* and *owaniana* are also redecribed.

The taxonomy of the *Elsinoë* spp. associated with scab disease of Proteaceae in Australia, California, South Africa and Zimbabwe is elucidated in chapter three. General morphology, symptomatology and phylogenetic analysis based on random amplified polymorphic DNA (RAPD) profiles and DNA sequence of the 5.8S rDNA gene and its flanking ITS1 and ITS2 regions were used. The study provides the first evidence that several distinct *Elsinoë* spp. are associated with scab disease of Proteaceae. The isolates from *Leucospermum*, *Protea* and *Banksia* represent three distinct species. The isolates from *Protea* in Zimbabwe represent an additional species. The isolates from *Leucadendron*, *Leucospermum* and *Serruria* in South Africa and Australia, and the isolates from *Leucospermum* in California and Zimbabwe are representative of the same species.

In chapter four, fungal endophytes occurring in leaves and stems of a species of *Protea*, *Leucospermum* and *Leucadendron* were investigated in three localities in the Western Cape province. The aim of the study was to determine if *Botryosphaeria proteae* was an endophyte of Proteaceae, and is so, how the role of water stress would influence canker development. *B. proteae* was routinely isolated as an endophyte but was not regarded as a dominant taxon. Inoculation studies were done on non-stressed plants, as well as plants with a leaf water potential of –1.0 MPa (moderately stressed) and –2.0 MPa (severely stressed). From the results of the study it was concluded that *Botryosphaeria proteae* is primarily an endophyte and can cause leaf necrosis of *Protea*, and is not a serious stem canker pathogen.

A new disease of cultivated *Protea* in southern Africa, Fusarium wilt, is described in chapter five. The disease is caused by *Fusarium oxysporum*. It occurs on various *Protea* cultivars in the North-Western province and in Zimbabwe. Disease symptoms first become visible as necrotic leaves on infected plants. Subsequently, a dark lesion develops from the roots along the stem, usually visible only on one side of the stem. The vascular tissue is discoloured, leading to branch die-back and plant death. Glasshouse trials were conducted to prove Koch's postulates on six *Protea* cultivars. Forty-five rooted plants of each of six cultivars were inoculated with isolates of *F. oxysporum* derived from the same cultivar. Disease symptoms similar to those occurring in the field, developed 6 weeks after inoculation. This is the first record of Fusarium wilt on *Protea* plants.

In chapter six, Pestalotiopsis leaf spot disease of Proteaceae in Zimbabwe is described. *Pestaloptiopsis* Steyart causes necrotic leaf spots in *Leucospermum* R. Br. and *Protea* L. species in Zimbabwe. Inoculation studies conducted to prove pathogenicity, confirmed the *Pestalotiopsis* sp. as the causal agent of the disease. A description of the fungus is given, and it is compared to other *Pestalotiopsis* spp. associated with Proteaceae.

In conclusion, the present study has shown that several unique species of fungal pathogens are associated with the Proteaceae. Several of these have proven to be new to

science, and are described in this thesis. It is clear, however, that the taxonomy of some of these pathogens, their host range and distribution needs to be further investigated. Furthermore, much more research needs to be done on the biology, epidemiology and control of the diseases of the Proteaceae.

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OPSOMMING

Die tesis bestaan uit ses hoofstukke wat handel oor navorsing op verskillende siektes en nuwe rekords van siektes van die Proteaceae wêreldwyd. Die swambeskrywings wat in hierdie tesis verskyn is nie effektief gepubliseer nie, en sal dus elders in wetenskaplike joernale gepubliseer word. Hoofstuk een is 'n oorsig wat 'n in diepte beskrywing gee van die belangrikste swampatogene van die genera *Protea*, *Leucospermum* en *Leucadendron*, soos aangeteken tot 1996. Die patogene word gegroepeer volgens die siektes wat hulle op die wortels, blare, stamme en blomme veroorsaak, asook die kanker veroorsakende swamme.

In hoofstuk twee word verskeie nuwe swamme wat op blare van *Protea*, *Leucospermum*, *Telopea* en *Brabejum* voorkom, en versamel is in Suid-Afrika, Australië of New Zealand, beskryf. Die volgende swamme word beskryf: *Cladophialophora proteae*, *Coniothyrium nitidae*, *Coniothyrium proteae*, *Coniothyrium leucospermi*, *Harknessia leucospermi*, *Septoria protearum* en *Mycosphaerella telopeae* spp. nov. Verder word twee *Phyllosticta* spp., *telopeae* en *owaniana* herbeskryf.

Die taksonomie van die *Elsinoë* species wat geassosieer word met skurfbassiekte van Proteaceae in Australië, Kalifornië, Suid-Afrika en Zimbabwe, word in hoofstuk drie vasgestel. Algemene morfologie, simptomatologie en filogenetiese analise, gebaseer op RAPD-analise en DNA volgorde bepaling van die 5.8S rDNA geen en sy ITS1 en ITS2 areas is gebruik. Die studie verskaf die eerste bewys dat verskeie species van *Elsinoë* geassosieer word met skurfbassiekte van Proteaceae. Die isolate van *Leucospermum*, *Protea* en *Banksia* verteenwoordig drie verskillende species. Die isolate van *Protea* in Zimbabwe verteenwoordig 'n verdere species. Die isolate van *Leucadendron*, *Leucospermum* en *Serruria* in Suid-Afrika en Australië, en die isolate van *Leucospermum* in Kalifornië en Zimbabwe verteenwoordig dieselfde species.

In hoofstuk vier is die swam endofiete wat in die blare en stamme van 'n species van *Protea*, *Leucospermum* en *Leucadendron* in drie lokaliteite in die Wes-Kaap voorkom, ondersoek. Die doel van die studie was om vas te stel of *Botryosphaeria proteae* 'n endofiet van die Proteaceae is, en indien so, vas te stel hoe waterstres kanker-ontwikkeling sal beïnvloed. *B. proteae* is gereeld as endofiet geïsoleer, maar is nie beskou as 'n dominante taxon nie. Inokulasie studies is op nie gestreste plante, asook plante met 'n blaarwaterpotensiaal van –1.0 MPa (gematig gestres) en –2.0 Mpa (uiters gestres), uitgevoer. Uit die resultate van die studie kan die gevolgtrekking gemaak word dat *Botryosphaeria proteae* hoofsaaklik 'n endofiet van *Protea* is en blaarnekrose kan veroorsaak, en nie 'n belangrike stamkankerpatogeen is nie.

'n Nuwe siekte van verboude *Protea* in suidelike Afrika, Fusarium verwelk, word in hoofstuk vyf beskryf. Die siekte word deur *Fusarium oxysporum* veroorsaak. Dit kom op verskeie *Protea* kultivars in die Noord-westelike provinsie en in Zimbabwe voor. Siekte-simptome word sigbaar as nekrotiese blare op die geïnfekteerde plante. Kort daarna ontwikkel 'n donker letsel vanaf die wortels opwaarts langs die stam, gewoonlik sigbaar slegs aan een kant van die stam. Die vaskulêre weefsel is verkleur en lei tot takterugsterwing en plantafsterwing. Glashuisproewe is uitgevoer ten einde Koch se postulate op ses *Protea* kultivars te bewys. Vyf-en –veertig gewortelde plante van elk van ses kultivars is geïnokuleer met isolate van *F. oxysporum* van dieselfde kultivar. Siektesimptome soortegelyk aan die in die veld, het ses weke na inokulasie ontwikkel. Dit is die eerste rekord van Fusarium verwelk op *Protea* plante.

In hoofstuk ses word Pestalotiopsis blaarvlek op Proteaceae in Zimbabwe beskryf. *Pestalotiopsis* Steyart veroorsaak nekrotiese blaarvlekke in *Leucospermum* R. Br. en *Protea* L. species in Zimbabwe. Inokulasiestudies, uitgevoer ten einde patogenisiteit te bewys, bevestig *Pestalotiopsis* as die veroorsakende agent van die siekte. 'n Beskrywing van die swam word gegee, en dit word vergelyk met ander *Pestalotiopsis* spp. wat al met die Proteaceae geassosieer is.

Ten slotte, die studie het getoon verskeie unieke species van swampatogene met die Proteaceae geassosieer word. Verskeie van die siektes is nuut vir die wetenskap en word in die tesis beskryf. Dit is egter duidelik dat die taksonomie van sommige van die patogene, hul gasheerreeks en verspreiding nog verder nagevors moet word. Verdere navorsing word op die biologie, epidimiologie en beheer van die siektes van die Proteaceae verlang.

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CONTENTS

1. PATHOGENS ASSOCIATED WITH DISEASES OF *PROTEA*, *LEUCOSPERMUM* AND *LEUCADENDRON* SPP.: A REVIEW

ABSTRACT

Cultivation of Proteaceae for fresh-cut-flowers and foliage occurs on a large scale in South Africa, but some products are still harvested in large quantities from naturally vegetated areas. In their natural habitat, the plants are hosts to numerous diseases, which cause damage to the foliage and flower heads. Since many of the Proteaceae species are found naturally in South Africa, most of the pests and diseases are indigenous to this country. A demand for high quality indigenous South African flowers exists on international markets. However, the diseases and disease causing organisms of this crop are poorly researched. The most important diseases of the Proteaceae can be grouped as root diseases, leaf spot diseases, diseases of the shoots, stem and flowers and canker diseases. With the exception of one bacterial disease, all of these diseases are caused by fungi. To date there have been no confirmed reports of Proteaceae infected by viruses. The present review strives to give a summary of the diseases occurring on Proteaceae, their distribution, host range, and possible means for control.

INTRODUCTION

Proteus was the mythological Greek god who, when grasped tightly, changed into numerous animate or inanimate forms. The variability within the Proteaceae may be the reason why this family was named after Proteus (Rebelo, 1995). The word 'protea' is commonly used to refer to any member of the plant family Proteaceae. Proteas are grown for their exotic brightly coloured flowers or bracts that are excellent as cut-flowers, and form part of a huge international cut-flower export industry (Forsberg, 1993).

The Proteaceae, one of the southern hemisphere's most prominent flowering plant families, existed before Gondwana began to break up 140 million years ago (Rebelo, 1995). The family divided into two subfamilies before the break up of the continent. The Proteoideae occurs mainly in southern Africa, but also in Australia and New Zealand. The Grevilleoideae occurs mainly in Australia, to a lesser degree in South America and on the South-Western Pacific Islands, and a single species occurs in Africa. Today, about 1400 species (in more than 60 genera) are identified in the Proteaceae. The majority of species are found in the southern

hemisphere. More than 800 species, representing 45 genera, are found in Australia, of which 550 species are found in the south-western parts. Approximately 400 species occur in Africa, of which 360 species are found in southern Africa (Rebelo, 1995). Three hundred and thirty species (in 14 genera) are confined to the south-western Cape between Nieuwoudtville in the north-west and Grahamstown in the east. About 90 species are found in Central and South America; 80 species occur on the islands east of New Guinea; 45 species in New Caledonia and a few species in Madagascar, South-East Asia, New Guinea and New Zealand (Rebelo, 1995).

South Africa has more than 20 000 plant species which comprise about 10 percent of the world's floral wealth. The proteas are of the 8600 plant species in the Cape Floral Kingdom, most unique to South Africa. The *Flora capensis* or Cape Fynbos, which only covers 0.04% of the world's surface, is utilized for harvesting of floriculture products. These species of Proteaceae are amongst the most endangered species of southern African flora and therefore need to be protected by conservation. Picking from natural habitats needs to be reduced and the cultivation of rare and endangered species must be implemented (Coetzee & Littlejohn, 1995; Rebelo, 1995).

In the Cape Floral Kingdom, most species of the Proteaceae are found only on nutrient-poor soils derived from Table Mountain sandstone. Limestone and calcareous sands are favourable for some species, but very few grow in dry, shale-derived soils. More than two-thirds of the species grow in the area from Cape Agulhas to Ceres. In the Caledon area alone, an astonishing 130 species occur. Some proteas are specific to the mountain ranges in the Western Cape, but no definite distribution exists in the east (Rebelo, 1995).

Most cultivated proteas, *Protea* L. (*P.*), *Leucospermum* R. Br. (*Lsp.*) and *Leucadendron* Berg. (*Lcd.*) species, are indigenous to South Africa. *Banksia* L. f. and the waratah (*Telopea speciosissima* R. Br.) are, however, indigenous to Australia (Forsberg, 1993). Proteas are grown as cut-flower crops in Australia, California, Chile, El Salvador, Hawaii, Israel, Madeira, New Zealand, South Africa and Zimbabwe (Regional reports, 1998). Proteaceae is in high demand as niche product on the world floriculture market (Forsberg, 1993).

The commercial trade in Fynbos in South Africa is composed of the dried flower industry (40%) and the fresh-cut-flower industry (60%), of which the fresh-cut-flower industry is considered the backbone of the industry. Dried flowers are considered by-products of fresh flowers, since discarded fresh flowers are often processed and sold as dried products. The dried

flower industry markets about 64 products, which are mainly harvested from the wild, and the fresh-cut-flower industry markets 137 species or cultivars. About 70% of the fresh-cut-flowers and 80% of the dried flowers are exported (Coetzee & Middelmann, 1997; Wessels *et al.*, 1997). The fresh-cut-flowers are mainly exported to Europe, but the American and Far East markets are also possibilities. The turnover of the dried and fresh-cut-flower industries was about R37.2 million and R64.5 million, respectively in 1996 (Coetzee & Middelmann, 1997). About 700 producers were involved in the wild flower industry in 1994. The majority of these producers still harvested from the field, although 200 producers were in cultivation for the export of fresh-cut-flowers (Coetzee & Littlejohn, 1995). The wild flower industry directly employed 4000 people in 1996 (Coetzee & Middelmann, 1997), but approximately 20,000 people were involved in the industry in 1994 (Coetzee & Littlejohn, 1995). The monetary value of the industry was approximately R81.7 million in 1996 (Coetzee & Middelmann, 1997).

More than 20 indigenous species and several Australian species are cultivated on a large scale. The most important production species are within the two major indigenous genera, *Leucospermum* and *Protea* (Von Broembsen & Brits, 1986). The giant or king *Protea, P. cynaroides* (L.) L., is probably the most well-known member of this genus, while the queen *Protea, P. magnifica* Link, is one of the most attractive ones. Other proteas marketed in great quantities, are *P. compacta* R. Br., *P. eximia* (Salisb. ex Knight) Fourc., *P. grandiceps* Tratt, *P. neriifolia* R. Br., *P. obtusifolia* H. Buek ex Meisn. and *P. repens* (L.) L. Eighty percent of proteas harvested as fresh-cut-flowers, are cultivated. Almost 100% of all pincushions sold, are cultivated. *Leucadendron* spp., marketed as "Cape Greens", are largely picked from natural stands. *Leucadendron platyspermum* R. Br. is sold in the largest quantities, while *Lcd. argenteum* (L.) R. Br., *Lcd. laureolum* (Lam.) Fourc. and *Lcd. discolor* E. Phillips & Hutch. are also sold in large quantities (Coetzee & Littlejohn, 1995). Figures for 1993/1994 indicate that in South Africa, farmers had access to 701084 ha of which 600626 ha were still natural Fynbos vegetation. During the survey, 2507 ha were reported to be cultivated with 40 species. There are about 80 cultivars (selected or hybrid material) available to the South African Fynbos flower producer. Eighty hectares are intensively planted with 25 of these cultivars. Estimates indicated that respectively 30 and 40 ha were planted during 1994 and 1995 (Malan, 1995). According to a survey done in 1997, a total of 285 ha was under intensive cultivation (*Protea* 207 ha; *Leucospermum* 66 ha and *Leucadendron* 12 ha) (Middelmann, 1998). Export of fresh Fynbos from the Western and Eastern Cape for the 1993/1994 season amounted to nearly R9 million

(Malan, 1995). During 1995, 2860 metric tons of fresh flowers were exported, which represented a 15.5% increase over the previous year (Coetzee & Middelmann, 1997). From January to December 1997, a total of 3570 tons of fresh material was exported, an increase of 8.2% over the previous year (Sappex News, 1998).

In the past, proteas in South Africa were picked from the wild by the disadvantaged communities, and sold on the markets and streets of Cape Town. Today, proteas are cultivated on a large scale in pure stands in orchards, although an estimated 45% of all protea cut-flowers were still harvested from the natural habitat in 1996 (Wessels *et al.*, 1997). Disease problems increase under the intensive cultivation of genetically uniform areas of plants. In breeding programmes, disease resistance or tolerance is not taken into account, because species and cultivars are selected primarily for their flower characteristics (Knox-Davies, Van Wyk & Marasas, 1986). Damage caused by the various diseases lowers the quality of the product arriving at international markets, and detracts from their marketability. The cut-flower industry of proteaceous plants has considerable potential, but plant disease is one of the problems which contributes to the high risk factor of this crop (Greenhalgh, 1981).

Cultivated proteas in South Africa are threatened by the unique and diverse native pathogens that evolved with African Proteaceae and exist on proteas growing in the surrounding natural habitat (Greenhalgh, 1981; Knox-Davies, 1981; Benic, 1986; Wright & Saunderson, 1995). Diseases are often a bigger threat to a particular plant in their country of origin than in other countries. South African Proteaceae therefore have the potential to grow well in other protea producing countries such as Australia, Hawaii and New Zealand. In spite of this, however, new disease problems have also appeared in these new environments (Greenhalgh, 1981). Diseases are therefore not only a problem locally, but also threaten the international protea industry (Knox-Davies, 1981; Wright & Saunderson, 1995). Wright and Saunderson (1995) stated that any internationally important new protea pests are likely to originate from South Africa.

The description of *Cercospora protearum* Cooke (1883) represents the first reference of a leaf pathogen on *Protea, Leucospermum* and *Leucadendron*. Since 1883, numerous diseases of the Proteaceae have been recorded and described. However, no records of diseases caused by rusts, powdery or downy mildews, viruses, viroids or nutritionally fastidious prokaryotes have yet been reported (Knox-Davies *et al.*, 1986). The following literature review gives a detailed

description of the major pathogens of the genera *Protea*, *Leucospermum* and *Leucadendron*, as reported up to 1996. The pathogens are grouped according to the diseases they cause on roots, leaves, stems and flowers, as well as the canker causing fungi.

SOIL-BORNE PATHOGENS

It is very difficult, if not impossible, to completely eradicate a soil-borne pathogen from soil in the field. Once the above-ground symptoms become visible, root rot is usually advanced and widespread in the root system, and control impossible. Control must be based on prevention. Disease-free planting material and sanitation form part of an integrated control strategy (Von Broembsen, 1979, 1989).

In the past, all cases of sudden death or poor growth of proteas were ascribed to *Phytophthora cinnamomi* Rands. Today, we know that other pathogens and abiotic and nutritional factors may sometimes also be involved (Knox-Davies *et al.*, 1986). Since the research conducted by Van Wyk (1973a), a number of common root disease fungi have been recovered from damped-off seedlings, blighted young plants, rooting cuttings, plants with root decay, as well as from cuttings showing dieback in mistbeds (Benic, 1986; Knox-Davies *et al.*, 1986). These wide-ranging fungi are not necessarily pathogenic on the plants from which they have been isolated, but some are actually secondary colonisers of injured, stressed or already diseased tissue (Knox-Davies, Van Wyk & Marasas, 1987).

The most important nursery diseases are caused by soil and seed-borne fungi. Pre- and post-emergence damping-off and seedling blights in seed, seedlings and cuttings, are the most typical diseases in nursery beds, particularly in the genus *Protea*. Benic (1986) did not encounter damping-off symptoms on *Leucospermum* or *Leucadendron* spp.

Several soil-borne pathogens cause damping-off and cutting rot of seedlings and cuttings of proteas. Seedlings may fail to emerge. Other typical symptoms include decay at ground level or root rot development after emergence. Sometimes basal stem rot of cuttings, and stem or leaf rot occur. Soil, organic matter and infested water from dams and rivers harbour the causal fungi, which may be seed- or wind-borne (Forsberg, 1993).

Benic (1986) found that protea cuttings in mistbeds often turn brown from the base and from wounds caused when leaves are removed during preparation of cuttings. Many wide host-

range pathogens such as *Rhizoctonia solani* J. G. Kühn, *Botrytis cinerea* Pers.: Fr. and various *Fusarium* Link: Fr. spp. were isolated.

Armillaria luteobubalina Watling & Kile, Transactions of the British Mycological Society 71: 79 (1978).

Symptoms - The fungus infects and kills cambial tissue. Dieback of shoot tips and branches, followed by eventual death of the plant are typical of this disease. The first above-ground symptom is often decline, with wilting and discoloured leaves that drop prematurely. The disease is also characterised by basal trunk rot or stem and root rot. Fan-like mats of white or creamy mycelia can be seen beneath the bark of the stems, particularly at the base of the stems. A distinctive mushroom-like odour is typical of the fungus. Yellow-brown mushrooms sometimes form at the base of affected plants, particularly during cool, wet weather. Rhizomorphs (dark shoestring structures) which allow the fungus to spread, form beneath the bark and along roots in the soil. They can sometimes be seen on affected plant material (Forsberg, 1993; Porter *et al.*, 1996).

Host range and Geographic distribution - **Australia: Victoria, Queensland**, *Leucospermum*, *Leucadendron* and *Protea* spp. (Forsberg, 1993).

Notes - In Australia, Armillaria root rot is only economically important where proteas are grown on land cleared of diseased woody plants such as *Eucalyptus* (Von Broembsen, 1989; Forsberg, 1993). The disease has not been recorded in South Africa. The fungus is favoured by moist conditions and spread takes place from plant to plant via root contact. Infection of a host can take place (via the rhizomorphs growing through the soil) from an original food source such as old tree stumps or root material. Wind dispersed spores are released from the mushroom fruiting bodies at the base of the plant. Infected plants must be removed and in severe cases, infested land must be cleared, because the disease is very difficult to control (Von Broembsen, 1989; Porter *et al.*, 1996).

Fusarium oxysporum Schltdl.: Fr., American Journal of Botany 27: 65 (1940).

Taxonomy - Microconidia are generally abundant, single-celled, ellipsoidal to cylindrical, straight or curved, 5-12 x 2.3-3.5 µm. Borne in false heads on short (often reduced), unbranched

and branched monophialides, never forming chains. Macroconidia are abundant, fusiform, slightly curved, pointed at both ends, thin-walled, and delicate, with an attenuated apical cell and a foot-shaped basal cell, 3(-5)-septate, (20-)27-46(-60) x 3.0-4.5(-5.0) µm. Chlamydospores form readily and profusely in culture, hyaline, smooth-walled or roughened, 5-15 µm diam. Chlamydospores are formed in terminal or intercalary positions, singly or in pairs, often also in conidia. Sclerotial pustules (pale to green or deep violet) and orange sporodochia are present in some strains (Domsch, Gams & Anderson, 1980; Nelson, Toussoun & Marasas, 1983).

Symptoms - Various *Fusarium* spp. cause damping-off of seedlings (Greenhalgh, 1981; Benic, 1986). *Fusarium oxysporum* is often associated with diseased roots of young plants, and dieback of cuttings in mistbeds. Basal and tip dieback and necrosis of leaves are typical symptoms (Benic, 1986). Benic (1986) frequently isolated a *Fusarium* sp. associated with blight symptoms similar to those caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. No root symptoms were, however, present.

Host range and Geographic distribution - **South Africa: North-Western province,** *P. aristata x repens* cv Venus*, *P. compacta x susannae* cv Pink Ice*, *P. eximia x susannae* cvs Sylvia & Cardinal*, *P. magnifica x susannae* cv Susara*, *P. repens**, *P. repens* cvs Sneyd and Sugar Daddy*; **South-Western Cape,** *Leucadendron* spp., *P. magnifica* (Benic, 1986); **Zimbabwe,** *P. compacta x susannae* cv Pink Ice*, *P. cynaroides**, *P. eximia x susannae** (present study*).

Macrophomina phaseolina (Tassi) Goid., Annali della Sperimentazione Agraria n.s. 1: 449 (1947).

Taxonomy - Sclerotia are black, smooth, hard, 100 µm - 1 mm diam. (50-300 µm *in vitro*). Pycnidia are dark brown, 100-200 µm diam., solitary or gregarious on leaves and stems, immersed, becoming erumpent, 100-200 µm diam., opening by apical ostioles; pycnidial wall darkly pigmented, with thick-walled cells on the outside. Conidiophores hyaline, with short obpyriform to cylindrical phialides, 5-13 x 4-6 µm. Conidia hyaline, ellipsoid to obovoid, 14-30 x 5-10 µm (Holliday & Punithalingam, 1970; Sutton, 1980).

Symptoms - The pathogen causes root and collar rot in nursery beds. Benic (1986) isolated the pathogen from drought stressed plants. Plants were slightly chlorotic and lacked vigour (Holliday & Punithalingam, 1970).

Notes - *Macrophomina phaseolina* is a root fungus with a low competitive saprophytic ability (Holliday & Punithalingam, 1970). The fungus invades immature, wounded or senescent roots. Healthy plants are not likely to be infected. Disease is favoured by high temperatures (35-39°C). Transmission is through plant debris in soil. The main source of infection is sclerotia, but infection can also occur through conidia. Sclerotia remain viable for long periods (Holliday & Punithalingam, 1970).

Phytophthora cinnamomi Rands, Mededelingen van het Instituut voor Plantenziekten **54**: 41 (1922).

Taxonomy - Hyphae (on malt agar) coralloid, becoming broad (8 µm) and tough; hyphal swellings spherical (av. 42, max. 60 µm diam.), clustered; wall slightly thickened. Sporangia form only in aqueous solutions (Waterhouse & Waterston, 1966) in sympodial succession or with internal proliferations, mostly 57-67 x 33-39 µm, with an inconspicuous apical thickening, leaving a wide (over 8 µm diam.) opening on dehiscence (Domsch *et al.*, 1980). Sporangiophores thin (3 µm), sometimes branched, more often proliferating through the empty sporangium. Sporangia broadly ellipsoid or ovoid, 57(-100) x 33(-40) µm, no papilla, apical thickening slight; not shed. *Phytophthora cinnamomi* is heterothallic. Oogonia 32-40(-58) µm diam., wall smooth, colourless, oospores nearly plerotic (almost filling the oogonium), wall yellow, 2 µm thick; antheridia typical amphigenous, long, 21-23 x 17 µm (Waterhouse & Waterston, 1966; Domsch *et al.*, 1980). The species is characterised by thin-walled, globose hyphal swellings, about 40 µm diam., abundantly produced in terminal or lateral clusters (Domsch *et al.*, 1980). In Hawaii, all the isolates tested were of the A2 mating type (Kliejunas & Ko, 1976). In Australia, the A2 type is dominant (Forsberg, 1993), while the A1 and A2 type occur in South Africa in a 1:1 ratio on Proteaceae (S. Denman, Dept. Plant Pathology, University of Stellenbosch, pers. comm.).

Symptoms - *Phytophthora cinnamomi* (PC) infects the feeder roots, moves into the main roots, and up into the stem, eventually girdling the stem. The most typical symptom is plant death (Rohrbach, 1983).

Above-ground - Infected plants become chlorotic (Fig. 1). Chlorosis starts on the older leaves at the bottom of the stem and progresses to the younger, fully expanded leaves (Cho, 1981). Gradual decline, chlorosis and stunting, due to inadequate uptake of nutrients, precede wilting and death in the case of more tolerant species (Von Broembsen & Brits, 1985; Von Broembsen, 1989; Forsberg, 1993). More susceptible species rapidly wilt ("sudden death" syndrome) and die with retention of the leaves on the branches (Fig. 2) (Von Broembsen & Brits, 1985). The fungus rapidly invades the root system, cuts of the water supply to the above-ground parts, and causes seemingly healthy plants to suddenly wilt (Brits & Von Broembsen, 1978; Von Broembsen, 1989). The sudden dieback of silver trees (*Lcd. argenteum* (L.) R. Br.) is common throughout the Western Cape of South Africa. Younger plants are killed at a faster rate than older plants (Van Wyk, 1973a). If only some of the main roots of a large plant become infected, above-ground symptoms may be displayed on only a part of the plant (Von Broembsen, 1989; Forsberg, 1993). Premature leaf drop and wilting of the young branches are also observed (Cho, 1981).

Root symptoms - The above-ground symptoms are indirect consequences of extensive root rot caused by PC (Von Broembsen, 1979, 1989; Cho, 1981; Von Broembsen & Brits, 1985; Forsberg, 1993). Proteoid roots (densely clustered rootlets characteristic of the Proteaceae) and fibrous, secondary roots are often absent or destroyed (Cho, 1981; Greenhalgh, 1981; Von Broembsen & Brits, 1985). Those that are present are black and shrivelled (Greenhalgh, 1981; Forsberg, 1993). Plants often fall over in wind or wet conditions due to poor root systems (Von Broembsen, 1989).

Dark brown lesions occur on the roots of diseased plants and sometimes extend above ground on the collars and lower stems. The stele of feeder roots remains intact, but the cortical tissues are decayed. The cortex of dead trees is rotted, extending from the crown into the root. A progressive browning of the cortex and phloem can be seen. Necrosis of the cortex is slower as in the case of the cambium. The pathogen moves intracellularly in the cortex and younger xylem (Van Wyk, 1973a; Brits & Von Broembsen, 1978; Von Broembsen & Brits, 1985; Von Broembsen, 1989).

Figs 1, 2. *Phytophthora cinnamomi*. **1.** Chlorotic *Leucospermum* plant infected with *P. cinnamomi*. **2.** Infected plant displaying typical "sudden death" symptoms.

Nursery - *Phytophthora cinnamomi* is also isolated from seedlings with damping-off symptoms in seedbeds. Root and collar rot of young plants in nursery beds are also common (Benic, 1986; Forsberg, 1993). Symptoms are similar to those on older or mature plants. *Phytophthora cinnamomi* appears in patches amongst densely packed nursery stock, usually killing all plants in the affected areas. In the mistbed, dieback and root rot of rooted and rooting cuttings, especially those of *Lsp. cordifolium* (Salisb. ex Knight) Fourc., are caused by PC. Black basal stem lesions extend from the cut surfaces up into the stem. Girdling and reddening of the stem above the crown lesion in rooted cuttings are typical (Benic, 1986). Benic (1986) also isolated PC from young *P. cynaroides* plants with dieback symptoms from the tip of the stem and from necrotic areas on the leaves. No typical root rot symptoms were, however, observed and roots appeared healthy.

Favourable conditions - Shallow, low-lying, poorly drained areas of a field are most favourable for disease development (Weste & Marks, 1974; Von Broembsen & Brits, 1985). However, diseased plants can also occur in patches at sites with good drainage (Von Broembsen & Brits, 1985). Re-infestation usually results where the site is replanted with the same or closely related species (Robertson, 1970).

Warm, wet conditions, particularly during summer rainfall periods, are favourable for disease development (Forsberg, 1993). Most protea deaths, however, occur during the hot, dry periods (Van Wyk, 1973a; Von Broembsen & Brits, 1985). The effect of high temperatures in enhancing disease development is also noted in Australia where PC is particularly destructive to local Proteaceae during the spring and summer months (Newhook & Podger, 1972). In Victoria, Australia, Weste (1974) found that maximum expression of disease occurs on shallow, poorly drained soils during wet periods at temperatures above 12°C, and most important, during subsequent periods of water stress. In soils with greater fertility and depth, where drainage is adequate and water stress does not normally occur, PC may be present without causing visible damage (Weste & Marks, 1974). The damage caused by PC in Southern Queensland is less devastating than in South-Western Australia. This might be attributed to climatic factors. In coastal South-Eastern Queensland, the annual rainfall mainly occurs at times of high light intensity and temperature, and these climatic factors may favour rapid regeneration following root infection by PC (Pegg & Alcorn, 1972). The environment is therefore of great importance in the manifestation of disease due to PC infection (Weste & Marks, 1974).

In Australia, disease often occurs under conditions favourable for the pathogen, long after full canopy is restored and equilibrium re-established. Dieback and death, associated with root rot and PC, are therefore not related to recent disturbance, but are always related to the presence of the pathogen (Weste & Marks, 1974). Rohrbach (1983) also reported a higher severity of PC with poorly drained soils and high rainfall conditions in Hawaii. Pratt and Heather (1973) stated that disease develops as result of increased soil moisture levels. Reduced water absorption due to root destruction, results in a marked increase in soil moisture on diseased sites, which in turn favours an increase in the pathogen population (Weste, 1974).

Transmission - The pathogen is soil-borne, persisting in the soil as thick-walled resting spores (chlamydospores), enabling it to survive until more favourable conditions arise (Von Broembsen, 1979). Infested soil may stay contaminated for several years after infested plants have been removed (Von Broembsen, 1989). Under optimum conditions of high soil moisture and warm soil temperatures (15-25°C), motile zoospores are produced which can move to healthy plants (Von Broembsen, 1979, 1989). *Phytophthora cinnamomi* is spread naturally by surface run-off water, by underground water seeping downhill through infested soil, by rivers and streams and by root-to-root contact of neighbouring plants. It can be spread artificially by careless transport of infected plant material and contaminated soil particles on contaminated field implements, vehicles, clothes, plant debris and particularly by the planting out of infected nursery material (Brits & Von Broembsen, 1978; Von Broembsen, 1979, 1989). In Australia, PC has been introduced in Victoria through infested soil or gravel used for roadbuilding, this has in turn infested the drainage water, and extended along roads and down gullies or table drains (Podger, 1972; Weste, 1974; Weste & Marks, 1974). In Hawaii, PC was recovered from soil particles on boots, tyres of vehicles and hooves of pigs. Zoospores of PC were dispersed by rain splash or runoff water and streams (Kliejunas & Ko, 1976).

Host range and Geographic distribution - **Australia,** *Lsp. conocarpodendron* (L.) Buek**, *Lsp. cordifolium****, *Lsp. reflexum* Buek ex Meisn.*, *Lcd. argenteum* ***, *Lcd. daphnoides* (Thunb.) Meisn.***, *Lcd. discolor****, *Lcd. eucalyptifolium* (H. Buek ex Meisn.)***, *Lcd. laureolum****, *Lcd. salicifolium* (Salisb.) I. Williams***, *P. compacta***, *P. cynaroides***, *P. magnifica***, *P. neriifolia**, *P. repens**; **South Africa: South-Western Cape,** *Leucospermum catherinae* Compton***, *Lsp. conocarpodendron*$^{**/*}$, *Lsp. cordifolium****; cv Gold Dust; *x tottum* (L.) R. Br. cv Firefly; *x lineare* R. Br. cv Red Sunset, *Lsp. cuneiforme* (Burm. f.) Rourke**, *Lsp. erubescens* Rourke, *Lsp.*

formosum (Andr.) Sweet*, *Lsp. fulgens* Rourke, *Lsp. glabrum* Phill.*, *Lsp. grandiflorum* (Salisb.) R. Br., *Lsp. lineare* R. Br***, *Lsp. muirii* Phill., *Lsp. mundii* Meisn., *Lsp. patersonii* Phill.***, *Lsp. pluridens* Rourke, *Lsp. praecox* Rourke, *Lsp. praemorsum* (Meisn.) Phill.***, *Lsp. prostratum* (Thunb.) Stapf.**, *Lsp. reflexum****/*, *Lsp. tomentosum* (Thunb.) R. Br., *Lsp. tottum* (L.) R. Br.***, *Lsp. truncatum* (Buek ex Meisn.) Rourke, *Lsp. utriculosum* Rourke, *Lsp. vestitum* (Lam.) Rourke, *Lcd. album* (Thunb.) Fourc., *Lcd. argenteum****, *Lcd. comosum* (Thunb.) R. Br., *Lcd. daphnoides*, *Lcd. discolor* ***, *Lcd. galpinii* Phill. & Hutch., *Lcd. gandogeri* Schinz ex Gand., *Lcd. laureolum*, *Lcd. loranthifolium* (Salisb. ex Knight) I. Williams, *Lcd. meridianum* I. Williams, *Lcd. microcephalum* (Gand.) Gand. & Schinz., *Lcd. nervosum* Phill. & Hutch.*, *Lcd. nobile* I. Williams, *Lcd. orientale* I. Williams**, *Lcd. pubescens* R. Br., *Lcd. rubrum* Burm. f., *Lcd. salicifolium***, *Lcd. salignum* Berg***, *Lcd. spissifolium* (Salisb. ex Knight) I. Williams, *Lcd. tinctum* I. Williams***, *Lcd. tradouwense* I. Williams, *Lcd. uliginosum* R. Br.*, *Lcd. xanthoconus* (Kuntze) K. Schum., *P. aurea* (Burm. f.) Rourke**, *P. burchellii* Stapf.*, *P. caffra* Meisn.*, *P. compacta**, *P. cynaroides**, *P. effusa* E. Mey. ex Meisn., *P. eximia***/*, *P. grandiceps***, *P. lanceolata* E. Mey. ex Meisn.*, *P. laurifolia* Thunb.*, *P. lepidocarpodendron* (L.) L., *P. longifolia* Andr. **/*, *P. lorifolia* (Salisb. ex Knight) Fourc.**, *P. magnifica**, *P. mundii* Klotzsch**, *P. nana* (Berg) Thunb., *P. neriifolia**, *P. nitida* Mill.*, *P. obtusifolia**, *P. punctata* Meisn.**, *P. repens***/*, *P. roupelliae* Meisn., *P. susannae* Phill.; **USA: California,** *Lsp. lineare#*, *Lsp. reflexum*, *Lcd. argenteum*, *Lcd. discolor*, *Lcd. rubrum*, *P. neriifolia*, *P. obtusifolia*; **Hawaii,** *Lsp. glabrum x conocarpodendron* cv Veldfire, *Lsp. lineare x glabrum* cv Hybrid 24; **Zimbabwe,** *Lsp. cordifolium x patersonii* cv High Gold#, *Lsp. lineare#* (Van Wyk, 1973a, b; Van Wyk & Koeppen, 1974; Raabe, 1978; Von Broembsen, 1979; Greenhalgh, 1981; Von Broembsen, 1984b; Von Broembsen & Brits, 1985; Benic, 1986; Knox-Davies *et al.*, 1986; Protea Disease Letter, 1991,1993; Forsberg, 1993; present study#).

* tolerant

** susceptible

*** highly susceptible

Species susceptibility - Most species of Proteaceae are sensitive to seasonal drought (Hnatiuk & Hopkins, 1980). Species that are killed by PC die largely as a result of reduced ability to absorb water. It is possible that species that are drought-tolerant may be more able to survive short

periods of exposure to the disease, recovering when fungal activities become limited by temperature and/or moisture availability.

Von Broembsen and Brits (1985) and Van Wyk (1973a) found important differences in disease severity and disease patterns among *Leucospermum*, *Leucadendron* and *Protea* spp. *Leucospermum* and *Leucadendron* spp. appear to be most susceptible to root rot since severe symptoms are found most frequently on these species. Plants of any age can be killed within a short period of time, but most losses occur during the first two years after planting (Von Broembsen, 1989). Symptoms are generally less severe and develop more slowly on *Protea* spp. *Protea magnifica* and *P. grandiceps* appear to be moderately susceptible, while *P. compacta, P. cynaroides, P. neriifolia* and *P. repens* appear to be resistant to PC (Von Broembsen, 1979). Disease of mature *Protea* spp. rarely occurs at sites with good drainage. Growers therefore use *Protea* spp. to replant sites where *Leucospermum* have died due to root rot (Von Broembsen & Brits, 1985).

In Australia, *Protea* spp. appear to be more susceptible than the same species in South Africa (Forsberg, 1993). In Queensland, proteas appear to be more susceptible than in the southern states. This can be attributed to the disease pressure being much higher in the summer rainfall areas (Forsberg, 1993).

An antifungal substance, effective against PC, has been isolated from the roots of *P. cynaroides* (Van Wyk & Koeppen, 1974). The active compound, *p*-hydroxybenzoylcalleryanin, that occurs only in the root bark of the plant, inhibits the mycelial growth of PC. The inhibitory effects of the compound can be ascribed to the presence of p-hydroxybenzoylacid in the molecule (Van Wyk & Koeppen, 1974). Van Wyk (1973a) suggested that this antifungal substance and other phenolic compounds were responsible for the resistance of *P. cynaroides* to PC. Young seedlings of *P. cynaroides* were, however, found to be highly susceptible to Phytophthora root rot in Hawaii (Cho, 1981). Benic (1986) also isolated PC from typical root and collar rot symptoms on *P. cynaroides* in South Africa. Results of this study indicated that young *P. cynaroides* plants are susceptible to root rot caused by PC (Benic, 1986).

Control - The soil is a complex, delicately balanced ecosystem. Management practices can create potential disease hazards by altering drainage and providing soil conditions that favour PC. The interface between agricultural or forestry lands and the natural Fynbos is where the most drastic ecological disturbances and changes are occurring. Instances have been reported where

the fungus has spread to Fynbos areas from adjacent agricultured or forestry lands (Von Broembsen, 1979).

Preventative measures

Prevention is the most effective control measure. The contamination of an area with infested soil or plants must be prevented. Once PC becomes established in a planting, it cannot be eradicated completely. Damage to established proteas can, however, be minimised by keeping the inoculum level in the soil as low as possible. The following preventative control measures can be implemented to confine the fungus to those areas where it is already present and to reduce the risk of it spreading to disease-free areas (Brits & Von Broembsen, 1978).

Exclusion and cultural practices. Prevention in commercial orchards relies on the planting of highly susceptible species on well-drained, suitable soils, or the use of resistant species on less suitable, moderately drained soils or soils previously infested with PC, and on avoiding the use of PC-infested water for irrigation, as well as establishing plantations using PC-free planting material (Von Broembsen, 1979; Von Broembsen & Brits, 1985). In the nursery, PC can be controlled by planting PC-free propagation material in PC-free soil or soil medium, irrigating with PC-free water, and maintaining a programme of sanitation in the nursery to prevent recontamination (Von Broembsen, 1981).

It is extremely difficult to prevent root rot, since PC is widespread in mountain Fynbos vegetation and the rivers of the South-Western Cape province (SWCP) (Von Broembsen & Brits, 1986). Since irrigation water from any river in the SWCP area may be infested with PC, it should be disinfested before irrigating susceptible crops, especially nursery material. This can, however, be very expensive and is only recommended when alternative sources such as quality borehole water or chlorinated municipal water are unavailable (Von Broembsen, 1984a; Von Broembsen & Brits, 1986).

Introduction of the fungus from infested areas to disease-free plantings through surface run-off water or soil carried on equipment, should be prevented. Regular, prompt removal and destruction of plants that have died of root rot reduce build-up of inoculum (Von Broembsen & Brits, 1986). Movement of vehicles, people and animals into the planting area should be restricted (Greenhalgh, 1981). Hosts of PC, other than Proteaceae, should be avoided in the vicinity of protea plantings (Von Broembsen, 1989).

Non-competitive cover crops or natural Fynbos as ground cover can be used to minimise soil disturbance, control weed and erosion, and promote the activity of beneficial soil micro-organisms (Brits & Von Broembsen, 1978). It minimises the spread of the fungus; it prevents root damage and it removes soil moisture (Greenhalgh, 1981). Ground cover also plays a role in reducing sporulation of the fungus (Von Broembsen & Brits, 1986). Greenhalgh (1981), however, stated that weed and organic mulches should be kept away from the base of the stems in order to prevent the build-up of moisture that is favourable for collar rot. Mulching systems should therefore be used with care.

Host resistance. The most promising factor in an integrated control strategy against PC root rot of proteas lies in breeding resistant rootstocks or resistant hybrids (Von Broembsen & Brits, 1986). The variation in susceptibility observed among and within genera by Von Broembsen and Brits (1985) suggested that the use of resistant or tolerant varieties can form part of an integrated control strategy. Breeding and selection for resistance in the more tolerant *Protea* spp. appear promising. Most *Protea* spp. are fairly tolerant when grown under suitable conditions. In South Africa, important commercial *Protea* spp. such as *P. compacta, P. cynaroides, P. neriifolia* and *P. repens,* have strong mature-plant resistance and are planted back onto land where pincushions have died due to PC. Within the genera *Leucospermum* and *Leucadendron*, resistant varieties are less likely, but tolerance can be used in a rootstock development programme. A *Lsp. reflexum* selection is the most tolerant line tested. Certain selections or hybrids of *Lsp. cuneiforme* and *Lsp. conocarpodendron* also have some tolerance (Von Broembsen & Brits, 1985, 1986; Von Broembsen, 1989).

Losses due to PC can be reduced by the use of resistant rootstocks. Research in South Africa regarding the control of PC has been aimed largely at the development of resistant rootstocks (Von Broembsen & Brits, 1985; Turnbull, 1991). *Leucospermum cordifolium x lineare* cv Sue Ellen wedge grafted onto a hybrid rootstock (*Lsp. tottum x formosum*) had a significantly lower death rate in PC infested soil than the ungrafted control of Sue Ellen (Van der Merwe *et al.*, 1991). Cultivar Spider (*Lsp. tottum x formosum*) is an effective rootstock for pincushion cultivars such as Flamespike, Firedance, High Gold and Sunrise to be grafted onto (G. Brits & G. Malan, private Fynbos consultants, Stellenbosch, pers. comm.).

In Australia, *Phytophthora* tolerant rootstocks are being developed for use with *Protea* and *Leucadendron* species (Turnbull, 1991), but may not be commercially available for some

years. These studies found no suitable rootstock for use with *Leucospermum* species. Even limited tolerance in highly susceptible genera, especially in combination with other control strategies, can be useful in the control of PC (Von Broembsen & Brits, 1986).

Biological control. According to Turnbull, biological control of PC on proteas has been attempted with variable success using a bacterium antagonist, *Pseudomonas cepacia* (strain 65). Initial results indicated that there was potential for the use of biological control in the nursery environment (potted plants), but these results were not sustainable in the field (Turnbull & Crees, 1995; Wright & Saunderson, 1995).

Curative measures

Sanitation. Once the disease is present in an orchard, it must be minimised or eliminated. Dead or dying plants should be carefully removed without spreading the infested soil and roots to nearby healthy plants or soil. Diseased material should be burned (Von Broembsen, 1979, 1989). Individual infested planting sites can be disinfested with dazomet (Basamid^{R}), metham-sodium (Herbifume^{R}, Busan^{R}), vapam (Von Broembsen & Brits, 1986; Von Broembsen, 1989) or methyl-bromide (Greenhalgh, 1981). Sites will, however, only stay free of inoculum for a period before re-infestation from the surrounding infested soil will take place again.

According to Marks, a wildfire temporary reduces the population of PC in the soil. The reduction lasts from two to six months and is the result of a change in soil microbial balance and not of pathogen elimination due to heat (Hardison, 1976).

Integrated control. Affected plants use much less water than healthy ones. Less water must therefore be applied to diseased plants to prevent the accumulation of water and thereby creating favourable conditions for disease development (Greenhalgh, 1981). No single method has been found to have universal application for control of *P. cinnamomi* in the Proteaceae. An experiment undertaken by Dixon, Frost and Sivasithamparam (1990) on two *Banksia* spp. in Western Australia, showed that integrated control may hold some promise for the control of PC in banksias and proteaceous plants in general. Root rot was partly suppressed by amending the soil with organic matter, a herbicide and a fungicide not phytotoxic to banksias.

Chemical control

Fungicides can be used to reduce the activity of the fungus and minimise disease, as they are incapable of eliminating the pathogen from diseased areas, especially where disease pressure is high (Marks & Smith, 1990). The application of fungicides in South Africa has been largely unsuccessful due to the lack of information on the disease cycle under South African conditions (Von Broembsen & Brits, 1986). Fungicides should be applied when the fungus is in its active phase. In the winter rainfall regions of South Africa, this is from mid spring (October) to mid summer (December) and again in autumn (April) after the first rains. Treatment should extend from mid spring through the entire summer in summer rainfall regions (Von Broembsen & Brits, 1986; Von Broembsen, 1989).

Disease activity is highly dependent upon soil moisture status and temperature. In Australia, phosphonate (1 g a.i./l) is applied every four to six weeks during the summer months when the disease is most active. This may, however, result in wastage of chemical in a non-irrigated crop when summers are dry and disease activity limited. On the other hand, failure to spray during unseasonable wet periods (e.g. autumn), can result in crop losses as high as 40% (McCredie *et al.*, 1985). For highly susceptible species, the spraying interval may be too great when disease pressure is high. Turnbull and Crees (1995) reached the conclusion that phosphonate can limit rather than prevent disease development. The level of disease control will vary, depending upon disease pressure.

Marks and Smith (1992) also stated that the efficacies of the systemic fungicides are influenced by climate, soil type, disease resistance and cultural practices, such as trickle irrigation. Neither metalaxyl (root drench) nor phosphonate (foliar spray), controlled PC stem infection of *Lcd. laureolum x salignum* when applied 10 days after inoculation. Neither fungicide is able to kill PC within established infections. The fungus survives within the vascular tissues of treated plants. Infection is confined to cortical tissue by wound periderm when the rate of tissue invasion by PC is slowed by fungicide application. The systemic fungicides have poor curative properties, but are most effective when applied prior to inoculation. The timing of fungicidal applications in relation to the infection process of PC and the uptake of the fungicide are important. Systemic fungicides should be used as a prophylactic, in combination with other disease control methods, so that the benefits are not lost due to increased disease levels. The

fungicides should be applied before the onset of seasonal weather conditions that favour disease (Marks & Smith, 1992).

Farmers in South-Eastern Australia find *P. cinnamomi* difficult to control in *Protea, Leucadendron* and *Leucospermum* species, even when using systemic fungicides at the scheduled application time (i.e. spring) (Marks & Smith, 1992). According to Shearer and Fairman, the application of phosphonate ("phosphorous acid") is currently the most practical management technique for control of PC in South-Western Australia. The fungicide has a double action within plants, inhibiting fungal growth and enhancing host resistance, and low mammalian toxicity and cost (Guest & Grant, 1991; Lamont, Wills & Witkowski, 1995; Smith, Shearer & Sivasithamparam, 1997). Soil applications of phosphonate are more effective in controlling PC than foliar sprays (Zahid & Guest, 1998).

Only fosetyl-Al and furalaxyl are registered in South Africa for use on soil-borne diseases (Krause, Nel & Van Zyl, 1996). Fosetyl-Al can be used as a prophylactic on pincushions. Once root rot has become established in a planting, fosetyl-Al can also be used to reduce losses. This is, however, often not cost efficient (Von Broembsen & Brits, 1986). Fosetyl-Al is registered as a foliar spray (375 g/100 l water) at monthly intervals (during the growth phase of the plant), or as a soil-drench (Krause *et al.*, 1996). Fosetyl-Al is less effective than metalaxyl, but it is also much less phytotoxic to pincushions and less expensive. Metalaxyl effectively controls PC infections in the field, but it is unacceptably phytotoxic to *Leucospermum* spp. under field conditions (Rohrbach, 1983; Von Broembsen & Brits, 1985, 1986; Dixon *et al.*, 1990).

Notes - ***Phytophthora cinnamomi*** is a pathogen of numerous hosts world-wide (Thorn & Zentmeyer, 1954), including many proteaceous genera. The pathogen is regarded as highly significant in agricultural and forestry areas, as well as various native florae, because it attacks a wide range of host plants, primarily through their root systems (Robertson, 1970; Newhook & Podger, 1972; Podger, 1972; Weste, 1974; Von Broembsen, 1979, 1984b).

Phytophthora cinnamomi root rot is the most important root disease of cut-flower proteas in Australia (Forsberg, 1993), New Zealand (Greenhalgh, 1981), South Africa (Knox-Davies *et al.*, 1986; Von Broembsen, 1989) and the U.S.A., especially Hawaii (Kliejunas & Ko, 1976; Rohrbach, 1983). In South Africa, Von Broembsen and Brits (1985) recorded a 52% loss in a *Lsp. cordifolium* plantation infested with PC within two years of disease discovery. High plant

mortality, which is normally induced by root diseases, invariably forces premature abandonment of plantings.

In the SWCP, the fungus was first reported as a pathogen of silver trees (*Lcd. argenteum*) (Van Wyk, 1973a, b). It was also associated with mortality of other Proteaceae (Van Wyk, 1973b; Knox-Davies, 1975). Von Broembsen (1984b) recovered PC from 130 hosts in the SWCP. Most were indigenous plants from nurseries, cultivated areas or natural vegetation, as well as native vegetation in mountain Fynbos. *Phytophthora cinnamomi* was well distributed throughout the SWCP on proteas and grapevines, except in the drier areas. All the PC isolates recorded from other parts of South Africa were from exotic hosts such as avocado and *Eucalyptus*, or from cultivated indigenous proteas. There are no records of *Phytophthora cinnamomi* isolated from naturally occurring indigenous flora outside the SWCP (Von Broembsen, 1984b).

Many hosts of PC are distributed among the native vegetation in the mountains of the SWCP (Von Broembsen, 1984b; Von Broembsen & Kruger, 1985). Some of these hosts are highly susceptible to PC (Von Broembsen & Brits, 1985). No patterns of disease occurrence and spread such as deaths in paths, advancing fronts, or spread from possible points of introduction, are, however, observed in the plant community as observed in Australia. The appearance of PC in dead and dying plants, scattered in wet, mountain Fynbos areas of the SWCP without causing appreciable damage to the native vegetation, indicates that PC appears to be indigenous to the mountain Fynbos of this region (Von Broembsen & Kruger, 1985). Knox-Davies *et al.* (1987), however, point to the fact that extremes in susceptibility or resistance of some of the native vegetation indicate that the local vegetation was "in little contact" with PC during evolution. *Phytophthora cinnamomi* may therefore not be "indigenous" to South Africa, but rather endemic. Introduction of PC into South Africa could have taken place in historic or prehistoric times (Knox-Davies *et al.*, 1987). Regardless of its origin, PC is present in the mountain areas without causing extreme damage to the native vegetation (Von Broembsen & Kruger, 1985). In Victoria, Australia, many root rot sensitive species grow in wet gullies where disease should flourish if it was present (Weste & Marks, 1974). Consequently, Davison (1972), Podger (1972), Weste (1974) and Weste and Marks (1974) came to the conclusion that PC is not a native organism in Southern and Eastern Australia.

During 1977 - 1980, PC was recovered from the river systems of the entire SWCP, even small streams serving as drinking water sources. The five major rivers, the Berg River, Eerste River, Breede River, Oliphants River and Palmiet River, arising in these mountain catchments, flow through the SWCP where they are used to irrigate various crops, including proteas and nursery material. The fungus was found in headwaters of these rivers at concentrations of up to 60 propagules/l. Infected mountain vegetation probably provides the sources of inoculum for the rivers. All river water within the SWCP should therefore be considered potentially infested with PC (Von Broembsen, 1984a).

In Australia, PC attacks a wide range of indigenous plants and in some areas had selectively eliminated certain species and changed the composition of the vegetation (Weste & Marks, 1974). Since 1964, PC has caused increasing destruction of forest, woodland and heath communities in Western Australia (Podger, 1972; Von Broembsen, 1979). Many of the same native Australian genera and families comprise a significant proportion of the indigenous flora of the Western Cape (e.g. *Protea*, *Leucadendron* and *Leucospermum*) (Von Broembsen, 1979; Marks & Smith, 1992). In a study carried out during December 1988 - April 1989, the Proteaceae was found to be one of the two largest plant families in Western Australia. Eighty-five percent of the proteaceous species assessed were rated as susceptible to PC. In healthy plant communities, proteaceous plants had a mean projective foliage cover of 40%, but at sites that had a long history of infestation with PC, the mean cover was only 10%. This research highlights the serious ecological impact of PC on native plant communities and indicates that significant components of the flora of the south-western part of Western Australia are endangered by this (Wills, 1993). About 92% of Proteaceae assessed from South-Western Australia, by far the greatest concentration of this family in the world, are considered susceptible to PC. The spread of PC has reached epidemic levels also in the southern sandplains (Lamont *et al.*, 1995).

Phytophthora nicotianae Breda de Haan, Mededelingin Planten Tuin, Batavia 15: 57 (1896).

Symptoms - The fungus causes root and collar rot of young plants, but much less frequently than *P. cinnamomi*. Other symptoms include damping-off and cutting rot (Forsberg, 1993). *Phytophthora nicotianae* has been reported from Proteaceae in Australia, and has also been isolated from roots and trunks of dying *Leucospermum* and *Banksia* spp. in Hawaii (Protea Disease Letter, 1991). Root and collar rot caused by *P. nicotianae* is one of the most important

diseases occurring on proteas in Hawaii. The pathogen is widely distributed in Kula and causes significant losses to Maui growers (Protea Disease Letter, 1993). *Phytophthora cinnamomi* is, however, more aggressive, causing symptoms and plant death more quickly than *P. nicotianae* (Protea Disease Letter, 1991).

Host range and Geographic distribution - **Australia,** *Protea, Leucospermum* and *Leucadendron* spp. (Forsberg, 1993); **Hawaii,** *Leucospermum lineare x glabrum* cv Hybrid 24, *Lcd. salignum x laureolum* cv Safari Sunset (Protea Disease Letter, 1991, 1993).

Rhizoctonia solani J.G. Kühn, Die Krankheiten der Kulturgewächsen, ihre ursachen und ihre Verhütung. Gustav Bosselmann, Berlin (1858).

Taxonomy - Sclerotia develop as a crust, radiating from the point of inoculation, or they are individually scattered over the colony surface. Hyphal cells at the advancing edge of the colony are usually 5-12 µm wide and up to 250 µm long. Branches that arise near the distal ends of the cells are constricted at the point of origin and septate just above. Cells are multinucleate (2-25, mostly 4-8) with conspicuous dolipore septa. Older mycelium greatly variate in hyphal dimensions and shorter cells due to the formation of secondary septa. The angle of branching is almost 90° and mycelia may branch at various points along the cell length. Some hyphae differentiate into swollen monilioid cells, often 30 µm or more in width. Similar cells are derived from repeated branching of one or more hyphae from the homogeneous, prosoplectenchymatous sclerotia (Mordue, 1974).

Symptoms - *Rhizoctonia solani*, which causes damping-off of seedlings, is the fourth biggest problem in the cultivation of proteas in Hawaii (Rohrbach, 1983). In South Africa, Benic (1986) found *R. solani* to be pathogenic to rooting and rooted cuttings. It causes severe losses in nurseries. The pathogen causes cutting dieback in mistbeds. Basal stem necrosis and defoliation are the most typical symptoms. Symptoms develop on the cotyledons, but it is not yet known whether *R. solani* is seed-borne (Benic, 1986).

Host range and Geographic distribution – **USA: Hawaii,** *Lsp. lineare x glabrum* cv Hybrid 24, *P. cynaroides, P. eximia* (Protea Disease Letter, 1991); **South Africa: South-Western Cape,** Hybrid *Leucospermum* cultivars, *P. compacta, P. cynaroides, P. repens* (Benic, 1986).

Rosellinia De Not. sp.

Taxonomy – Ascoma an ostiolate perithecium, borne on a subiculum composed of dark hyphae, single or gregarious, often covering the surface of the subiculum. Perithecia globose or broadly obpyriform, normally more than 500 µm in diam., black glabrous, often situated on a definite hypostroma of angular, thick-walled cells. Ostiolar neck papillate or conical, ostiole lined with periphyses. Perithecial wall composed of several layers of flattened cells, outer cells thick-walled and dark brown, inner cells subhyaline or hyaline. Asci unitunicate, cylindrical, long-stalked, with an amyloid apical apparatus, lining inside or perithecial wall, 8-spored. Ascospores 1-cells, dark brown, ellipsoidal, often laterally compressed, with a longitudinal germ slit (Hanlin, 1990).

Symptoms - The fungus causes a basal stem, crown or collar rot. Root rot can be present or absent. The surface of affected plant parts can be covered with a mass of white fungal mycelium (Forsberg, 1993), hence the common name, white root rot.

Host range and Geographic distribution – **Australia,** *Protea* spp. (Forsberg, 1993).

Notes - A *Rosellinia* sp. causes an infrequent but sometimes serious disease in newly cleared land and land adjacent to forests in Australia. Soil, rich in organic matter, probably favours the fungus (Forsberg, 1993).

Verticillium dahliae Kleb., Mycologisches Centralblatt 3: 66 (1913).

Taxonomy - Conidiophores are more or less erect, hyaline, with several whorls of 3-4 phialides. Phialides subulate, generally 16-35 x 1.0-2.5 µm. Conidia ellipsoidal to short-cylindrical, hyaline, typical 1-celled, but sometimes 1-septate, 2.5-6(-8) x 1.4-3.2 µm. Microsclerotia are the only resting structures produced, dark brown to black, torulose, and composed of nearly globose cells, arising from single or adjacent hyphae by repeated budding and multilateral septation, more or less elongate and 50-200 x 15-50(-100) µm. White areas without microsclerotia are typical (Domsch *et al.*, 1980).

Symptoms - Infected plants show terminal shoot wilting and chlorosis of the foliage. Eventually the entire plant collapses, turns brown and dies. Brown flecking and streaking appear in the stem xylem tissue and roots (Koike *et al.*, 1991; Forsberg, 1993).

Host range and Geographic distribution - **Australia: Queensland,** *P. cynaroides* (Forsberg, 1993); **New Zealand,** *P. compacta* (Forsberg, 1993); **USA: California, Santa Barbara County,** *Leucospermum cordifolium* (Koike *et al.*, 1991).

Notes - The first report of Verticillium wilt on proteaceous species was recorded on *Lsp. cordifolium* in California (Koike *et al.*, 1991). The disease is of minor importance in proteas and has not yet been recorded in South Africa. The fungus is soil-borne, invades the vascular tissues through roots and spreads with soil and in moving surface water (Forsberg, 1993).

Table 1. Root pathogens of minor importance

Pathogen	Geographic Distribution	Hosts	References
Curvularia Boedijn sp.	South Africa: South-Western Cape	Seed of various species of Proteaceae	(Benic, 1986)
Cylindrocarpon Wollenw. sp.	U.S.A.: Hawaii	*Lsp. tottum x vestitum* cv Fire Fly *Lsp. lineare x glabrum* cv Hybrid 24 *Lcd. salignum x laureolum* cv Safari Sunset	(Protea Disease Letter, 1991)
Cylindrocladium Morgan spp.	South Africa: South-Western Cape U.S.A.: Hawaii	*Protea* sp. *Lsp. tottum x vestitum* cv Fire Fly *Lsp. lineare x glabrum* cv Hybrid 24 *Leucospermum* spp. *P. eximia* *P. cynaroides* *P. neriifolia*	(P.W. Crous, pers. comm.) (Protea Disease Letter, 1991)
Fusarium Link: Fr. spp.	South Africa: South-Western Cape U.S.A.: Hawaii	*Protea compacta* *P. cynaroides* *P. eximia* *P. cynaroides*	(Benic, 1986) (Protea Disease Letter, 1991)
Graphium Corda sp.	U.S.A.: Hawaii	*Lcd. laureolum*	(Protea Disease Letter, 1991)
Pestalotia De Not. sp.	South Africa: South-Western Cape	Seed of various species of Proteaceae	(Benic, 1986)
Pythium vexans de Bary	South Africa: South-Western Cape	*P. compacta* *P. cynaroides*	(Benic, 1986)

U.S.A.: Hawaii; California	*Lsp. lineare x glabrum* cv Hybrid 24	(Protea Disease
	P. neriifolia	Letter, 1991)
	Lsp. reflexans	
	Lcd. argenteum	
	Lcd. discolor	
	P. eximia	
	P. neriifolia	
	P. obtusifolia	(Raabe, 1978)

General control strategies

Cultural practices. Prevention is the most effective means of controlling soil-borne diseases. Disease-free propagating material, pathogen-free soil media and irrigation water, and good nursery sanitation will prevent contamination with root pathogens (Benic, 1986). Land must be cleared thoroughly to remove all roots, debris and stumps that may be infested with soil-borne pathogens. Diseased plants must be removed and destroyed. Woody weeds and tree stumps near or in plantings must be removed since they may be alternative hosts of the pathogens. Sites previously infested with soil-borne pathogens must rather not be replanted. Replanted sites must be fumigated if only to delay re-infestation. Any kind of plant damage must be avoided. Organic mulches must not be applied where *Rosellinia* occurs (Forsberg, 1993). Overwatering of cuttings, soggy planting media, wounding and drought stress due to cuttings drying out, all favour infection and must be prevented (Benic, 1986).

Chemical control. In South Africa, only fosetyl-Al and furalaxyl are registered for the control of the soil-borne pathogens, *Phytophthora* and *Pythium* (Krause *et al.*, 1996). Dipping of cuttings before planting, followed by regular sprays with effective fungicides can help to prevent the development of diseases of cuttings in mistbeds. Difolitan, captan and benomyl are a few fungicides that can be sprayed on cutting material in mistbeds (Benic, 1986). Decay of rooting cuttings can be prevented by stripping the cuttings of their lower leaves, dipping them in 0.05-0.15% (m/v) captafol in water and drying them briefly before planting in mistbeds (Jacobs, 1981).

According to Benic, soil-borne fungi can largely be controlled by treating the soil with methyl bromide and seeds with thiram dry seed dressing (Knox-Davies *et al.*, 1986). In

Australia, Greenhalgh (1981) reported that a soil drench with benomyl is effective in controlling damping-off caused by *Fusarium* and *Rhizoctonia*. *Rhizoctonia* can also be controlled by applying a soil drench of Folisan^{R}, quintozene or iprodione (Greenhalgh, 1981). In Hawaii, benomyl and mancozeb plus a spreader or sticker control *Cylindrocladium* (Protea Disease Letter, 1991).

FOLIICOLOUS PATHOGENS

Many different fungi and a single bacteria species have been associated with leaf spot diseases of Proteaceae (Knox-Davies *et al.*, 1986, 1987; Von Broembsen, 1989; Forsberg, 1993). Of these, most fungal pathogens are representative of ascomycetes or their anamorphs (Von Broembsen, 1986; Knox-Davies *et al.*, 1987). The description of *Cercospora protearum* Cooke by Cooke (1883), represents the first reference of a leaf pathogen of the genera *Protea, Leucospermum* and *Leucadendron.*

Symptom expression varies from specks and spots to blotches, depending on host as well as the disease causing organism (Forsberg, 1993). These spots range from superficial, sooty spots to necrotic spots, and in some cases extensive lesions are formed (Knox-Davies *et al.*, 1986). However, several leaf spot diseases that are caused by different pathogens, can have similar disease symptoms. Leaf spot diseases usually do not kill the plant, but severe damage may retard growth and result in the loss of infected shoots. Leaf spots are, however, of economical importance when they occur on the flower bearing shoots. The presence of fungal pathogens on cut-flowers, results in rejection of entire batches of exported flowers for aesthetic and phytosanitary reasons (Von Broembsen, 1989).

Protea species are generally more prone to leaf spot diseases than *Leucospermum* and *Leucadendron* species (Van Wyk, 1973a). Many fungi have been described from proteas in South Africa. The causal organisms of many proteaceous diseases in Australia, have not yet been identified, although symptoms have been observed similar to those occurring on Proteaceae in South Africa (Forsberg, 1993). Furthermore, the leaf diseases develop more prominently when wet and humid conditions prevail. Plants that are in poor health due to other causes, are generally also more susceptible to leaf diseases (Forsberg, 1993).

Alternaria alternata (Fr.: Fr.) Keissl., Beihefte zum Botanischen Centralblatt **29**: 434 (1912).

Taxonomy - Colonies usually black or olivaceous, sometimes grey. Conidiophores arising singly or in small groups, simple or branched, straight or flexuous, sometimes geniculate, pale to mid olivaceous or golden brown, smooth, up to 50 µm long, 3-6 µm thick with 1 or several conidial scars. Conidia in long, often branched chains, obclavate, obpyriform, ovoid or ellipsoidal; often with a short conical or cylindrical beak; sometimes up to, but not more than one third the length of the conidium; pale to mid golden brown, smooth or verruculose, with up to 8 transverse and usually several longitudinal or oblique septa; overall length 20-63 (-37) µm, 9-18 (-13) µm thick in the broadest part; beak pale, 2-5 µm thick (Ellis, 1971).

Host range and Geographic distribution - **Australia,** *Leucospermum* and *Leucadendron* spp. (Von Broembsen, 1989); **Hawaii,** *Leucospermum* and *Leucadendron* spp. (Von Broembsen, 1989); **New Zealand,** *Leucospermum* and *Leucadendron* spp. (Von Broembsen, 1989).

Notes - Alternaria leaf speck has been reported as one of the most important diseases on proteas in Hawaii (Protea Disease Letter, 1993). Numerous small-spored *Alternaria* spp. have been isolated from Proteaceae in South Africa. This suggests that there are probably several distinct *Alternaria* spp. within the *A. alternata*-complex associated with leaf spot diseases of these hosts world-wide. Although not treated here, there are also a whole complex of several *Pleospora* Rabenh. ex Ces. & De Not. spp. with *Stemphylium* Wallr. anamorphs that are also frequently associated with leaf spots of *Protea, Leucospermum* and *Leucadendron* spp. (P.W. Crous, Dept. Plant Pathology, University of Stellenbosch, pers. comm.).

Batcheloromyces leucadendri P.S. van Wyk, Marasas & Knox-Dav., South African Journal of Botany **51**: 33 (1985).

Taxonomy - Conidiophores arise singly as short, simple, erect or ascending lateral branches on the superficial hyphae, effuse but concentrated in the middle of the colonies directly above the stomata, normally consisting of a single terminal conidiogenous cell, but the conidiogenous cell may proliferate to produce a hypha. Annellidic conidiogenous cells are dark brown, discrete, terminal, percurrent with up to four annellations, typically calyciform, 2.8-4.6 µm long, 2.2-3.0 µm diam. at the base and widening towards the apex, 3.1-5.4 µm wide. Conidia arise singly as blown-out ends of the apex of conidiogenous cells, solitary, dry, brown, oblong-ellipsoidal or

bacilliform with both ends rounded, thick-walled, smooth to verrucose, one-celled, 4.6-7.4 x 4.1-6.0 µm, two-celled 6.4-10.3 x 4.0-7.2 µm and three-celled 13.1-13.7 x 3.2-5.4 µm (Van Wyk, Marasas & Knox-Davies, 1985a).

Batcheloromyces leucadendri differs consistently from *B. proteae* in the following respects. The sporodochium-like plates are smaller, normally half the size of those in *B. proteae*; the conidia are frequently one to two-septate and larger than the usually non-septate, rarely one-septate spores of *B. proteae*; and the walls of the conidia are rough, even verrucose in *B. leucadendri*, whereas those in *B. proteae* are finely verruculose (Van Wyk *et al.*, 1985a).

Symptoms - Van Wyk *et al.* (1985a) described the lesions on the leaves as "...amphigenous, circular to irregular, radiating, discrete, but becoming confluent to cover large areas of the leaf surface, dark brown to black and sometimes causing a discoloration of the leaf tissue" (Fig. 3).

Histology - Mycelia in the leaf tissue are composed of thick, dark brown stromatic mycelial plugs in the stomatal cavity and dark brown hyphae in the substomatal cells. The mycelial plugs are erumpent through the stomata and form black, pulvinate, sporodochium-like plates of radiating hyphae closely adhering to the leaf surface. Leaf spots consist of black mycelial plates, 25-105 µm diam. A single plate is composed of a single layer of radiating, septate, brown hyphae, 2.2-4.0 µm diam. (Van Wyk *et al.*, 1985a).

Host range and Geographic distribution - **South Africa: Cape province,** *Leucadendron argenteum, Lcd. coniferum* (L.) Meisn*, *Lcd. coniferum x floridum* R. Br. cv Pisa*, *Lcd. discolor, Lcd. elimense* E. Phillips*, *Lcd. gandogeri, Lcd. laureolum, Lcd. salicifolium, Lcd. salignum, Lcd. uliginosum, Lcd. xanthoconus**, *Leucadendron* spp. (Van Wyk *et al.*, 1985a; present study*).

Batcheloromyces proteae Marasas, P.S. van Wyk & Knox-Dav., Journal of South African Botany **41**: 43 (1975).

Taxonomy - Conidiophores arise solitary as short, simple, erect or ascending lateral branches on the superficial hyphae, effuse, but concentrated in the middle of the colonies, directly above the stromata; generally consisting of a single terminal conidiogenous cell, but sometimes the conidiogenous cell proliferates through the conidial scar to produce a second locus at a higher

level (Marasas, Van Wyk & Knox-Davies, 1975). Conidiogenous cells are brown, integrated, terminal, percurrent, with up to three annellations, typically calyciform, 3.0-5.3 µm long, 2.2-3.1 µm diam. at the base, widening towards the apex, 3.3-4.8 µm wide, can be doliiform, 3.0-4.6 µm high and 2.3-3.8 µm wide. Conidia arise singly as blown-out ends of the apex of the conidiogenous cells, solitary, or produced by successive percurrent proliferations of the conidiogenous cell. Conidia regularly form fragile, false basipetal chains of two to three conidia, dry, brown, oblong-ellipsoidal or bacilliform with both ends rounded or truncate at the base, smooth, thick-walled, non-septate or rarely with a single transverse septum, not constricted at the septum, 3.9-9.1 x 2.8-4.2 µm (Marasas *et al.*, 1975).

Symptoms - The disease is characterised by superficial growth and sporulation of the fungus on its host. Lesions on different variants of *P. cynaroides* show great variation in size, superficial development of the fungus, discoloration of host tissue and surface disruption. Some lesions are restricted and consist only of groups of sporodochia. The underlying host tissue shows little disruption or discoloration (little development of mycelium or sporodochia suggests host resistance to the pathogen). Some lesions are erumpent and restricted or spreading (Smit, Engelbrecht & Knox-Davies, 1983). Marasas *et al.* (1975) described the symptoms as "...amphigenous, circular, radiating, up to 2 cm diam., discrete but becoming confluent and covering large areas of leaf surface". The most typical lesions are black, with a red-brown to purple-black discoloration of the leaf tissue which may be visible in the corresponding areas of the opposite leaf surface (Fig. 4). Only in rare cases is the leaf discoloured to the same extent on both sides (Marasas *et al.*, 1975; Smit *et al.*, 1983). In cases of severe infection, the lesions become necrotic and infected leaves fall prematurely. Lesions also occur on the petioles, but no lesions are observed on the stem. Some *P. cynaroides* variants show no disease symptoms. Jacobs (Dept. Horticulture, University of Stellenbosch, pers. comm.) observed that the Western Cape variants of *P. cynaroides* are particularly susceptible to *B. proteae.*

Histology - In the leaf, mycelia are composed of thick, dark brown, stromatic mycelial plugs in the stomata, but no subcuticular or intra-epidermal mycelia are observed. The mycelial plugs are erumpent through the stomata and form black, pulvinate, sporodochium-like plates of radiating hyphae above the stomata, with individual or many hyphae extending from the stomata, adhering to the leaf surface, or into the stomata (Marasas *et al.*, 1975; Smit *et al.*, 1983). Each colony consists of many black, superficial mycelial plates, 110-250 µm diam., composed of a single

layer of radiating, septate, branched, brown hyphae, 2.2-4.2 µm diam. (Marasas *et al.*, 1975). Hyphae and spores are observed in the cavities over the stomata, under the cuticle (Smit *et al.*, 1983).

Host range and Geographic distribution - **South Africa: Cape province,** *Protea cynaroides, P. grandiceps, P. magnifica*, P. neriifolia*, P. punctata, P. repens** (Marasas *et al.*, 1975; Smit *et al.*, 1983; Van Wyk *et al.*, 1985a; Knox-Davies *et al.*, 1986; present study*).

Control - Von Broembsen (1989) suggested that the fungus is seed-borne and therefore advised seed treatment.

Notes - This pathogen was first described in 1975 (Marasas *et al.*, 1975). It is specific to *Protea* spp. and of economical importance in commercial plantings of *P. cynaroides.* The leaf spots are not destructive, but lower the quality of the bloom (Von Broembsen, 1989).

Cercostigmina protearum **var.** ***leucadendri*** (Cooke) U. Braun & Crous, Sydowia **46**: 206 (1994).

Taxonomy - The fungus has 1-3 septate conidia, 25-50 µm long, 5-7 µm thick and 3-4 µm wide at the base (Ellis, 1976; Crous & Braun, 1996). Cooke (1883) described the conidia as having only three septa, measuring 35 x 7 µm.

Host range and Geographic distribution - **South Africa: Cape province,** *Leucadendron argenteum, Lcd. discolor, Lcd. salignum x laureolum* cv Safari Sunset* (Fig. 5) (Chupp & Doidge, 1948; Ellis, 1976; Knox-Davies *et al.*, 1987; Crous & Braun, 1996; present study*).

Cercostigmina protearum (Cooke) U. Braun & Crous **var.** ***protearum***, Sydowia **46**: 206 (1994).

Taxonomy - Sporodochia amphigenous, numerous, black, punctiform, covering the leaf spots. Stromata erumpent, up to 350 µm wide, 250 µm high. Conidiophores in dense fascicles, brown, up to 70 µm long, 4-9 µm thick, with 0-8 annellations. Conidia cylindrical, obclavate or fusiform, pale brown, slightly verruculose, 2-6-septate, 50-82 (-68) µm long, 6-9 (-8) µm thick in the broadest section, 5-7 µm wide at the truncate base (Ellis, 1976). Crous & Braun (1996) described the conidia as 2-6 septate, measuring 50-80 x 5-9 µm.

Symptoms - Lesions vary depending on the host. On *Lsp. conocarpodendron*, the lesion appears as a dead, parchment-like spot with black, raised structures (Fig. 6), while no dead tissue is found on *Lsp. reflexum*. On the latter host, the few black fruiting structures are surrounded by a pink-red margin. The lesion diam. on *Lsp. reflexum* is also smaller (1-3 mm) to those on *Lsp. conocarpodendron* (5-20 mm) (Van Wyk, 1973a). The areas surrounding the blotches turn chlorotic and die. Young shoot tips can also be severely damaged (Von Broembsen, 1989).

Host range and Geographic distribution - **South Africa: Cape province,** *Leucospermum conocarpodendron, Lsp. conocarpum, Lsp. cordifolium*, Lsp. reflexum, P. repens*, Protea sp.* (Saccardo, 1886; Chupp & Doidge, 1948; Doidge, 1950; Chupp, 1953; Ellis, 1972, 1976; Van Wyk, 1973a; Crous & Braun, 1996; present study*).

Clasterosporium proteae M.B. Ellis, More Dematiaceous Hyphomycetes: 101 (1976).

Taxonomy - Colonies are effuse, dark black-brown to black. Mycelium consists of a dense network of repent, hyphopodiate, sinuate, branched, septate, olivaceous brown, 3-5 µm thick hyphae. Hyphopodia alternate or unilateral, subglobose, dark brown, 5-6 µm diam. Conidiophores laterally on the hyphae, erect, straight or slightly curved, cylindrical, septate, dark brown, smooth, up to 35 µm long, 4-6 µm thick. Conidia obclavate, truncate at the base, mid to dark brown, wrinkled, 3-5 septate, 30-50 µm long, 6-9 µm thick in the broadest section (Ellis, 1976). Van Wyk (1973a) observed dark coloured phragmoconidia with six septa, borne terminally on single conidiophores.

Symptoms - Lesions on the leaves are oily and sooty, 4-10 mm diam. The fungus frequently occurs with *Coleroa senniana* (Sacc.) (Van Wyk, 1973a).

Host range and Geographic distribution - **South Africa: Cape province,** *Protea acaulis* (L.) Reich., *P. amplexicaulis* (Salisb.) R. Br. (Van Wyk, 1973a; Ellis, 1976).

Coleroa senniana (Sacc.) Müller & Arx, Kryptogamenflora Schweiz **11**: 418 (1962).

Taxonomy - Individual fruiting bodies occur in groups which are remote from each other or rather close together, subcuticular (Van der Byl, 1929; Doidge, 1941; Van Wyk, 1973a), seldomly circular, angular or irregular in outline, 100-170 µm in diam. (Sydow, 1926), 36-72 µm

high (Van der Byl, 1929). Asci clavate, cylindrical, sometimes slightly distended at the base, tapering slightly to the broadly rounded apex, sessile or with a short, thick, knob-like foot, 8-spored, thick-walled, 35-45 x 10-12.5 µm (Doidge, 1941). Van der Byl (1929) stated that the asci are thin-walled. Ascospores more or less distichous, oblong to sub-clavate, obtusely rounded at both ends, tapering slightly to the lower end, straight or slightly asymmetrical, rarely slightly bent, 1-septate, cells same length or upper somewhat shorter, more or less constricted, pellucid, comparatively light olive-brown, 10-13 x 5-6 µm (Sydow, 1926; Doidge, 1941; Van Wyk, 1973a). Ascospores are dark brown according to Van der Byl (1929). Paraphyses numerous, hyaline or subhyaline, forming indefinite erect masses between the asci, which converge towards the middle of the covering membrane (Doidge, 1941). Van der Byl (1929) stated that paraphyses are absent. Van Wyk (1973a) found great variation in the size of the lesions and in the size and shape of the ascospores in collections of *C. senniana* obtained from a wide range of *Protea* spp.

Symptoms - The fungus causes various types of lesions. On *P. caffra* the lesions are small (< 2 mm), yellow-brown and only a few small, black ascocarps are widely separated in the lesion. On the same host, the fruiting structures can be clustered in groups of 3-5 mm diam. as described by Doidge (1941), with no leaf discoloration. Opposed to this, leaf spots vary on *P. magnifica* from a more or less concentric grouping of ascocarps, embedded in apparently healthy leaf tissue, to raised, light brown zones up to 1 cm diam. (Van Wyk, 1973a; Van Wyk, Marasas & Knox-Davies, 1975a).

Coleroa senniana produces tiny, black leaf specks (pseudothecia of the fungus) and yellow to brown discoloured spots on *P. magnifica* leaves. The pseudothecia are found on the ab- or adaxial surfaces of leaves, depending on which surface is facing upwards (Serfontein & Knox-Davies, 1990a). Pseudothecia are much more abundant on the upper surface and frequently scattered over a large area of the leaf (Van der Byl, 1929). Doidge (1941) described the symptoms caused by the fungus as "...amphigenous, not producing true leaf spots, but often causing an indefinite light brown discolouration." Fruiting bodies occur in groups, which are more or less sharply defined and often coalescent (Figs 7, 8) (Doidge, 1941).

The underlying leaf tissue is apparently healthy - there is no killing of the tissue (Knox-Davies *et al.*, 1987; Serfontein & Knox-Davies, 1990a). However, widespread and destructive, necrotic lesions were seen to be associated with *Coleroa*. They were caused by secondary pathogens, *Fusicoccum aesculi* Corda, invasing the *Coleroa* lesions. *Coleroa senniana* is also

associated with oily, yellow confluent spotting (so-called "copper leaf") of *Lsp. cordifolium* (Serfontein & Knox-Davies, 1990a).

Histology - *Coleroa senniana* is largely confined to the cuticle. The hyphae colonise the cuticle, but also occur subcuticularly, causing the discoloration seen in the underlying tissue. The pseudothecia are also formed within the cuticle, which rupture as they enlarge (Serfontein & Knox-Davies, 1990a).

The basal layer of the pseudothecia is flat, growing out of the epidermis, up to 8 µm thick, filamentous, composed of small cells, sub-hyaline or pale yellow-brown. The covering membrane is flattened-conical, opening in the centre, or often more or less excentrically, by a round or irregular pore, about 25 µm diam. The covering membrane is about 5 µm thick, composed of 1-2 layers of rounded polyhedral cells; thin-walled at the margin, pellucid olive-brown and about 5-7.5 µm diam.; smaller, rather thick-walled, about 3-5 µm diam. in the centre near the pore, black-brown and more or less completely opaque. The covering membrane is not sharply defined at the margin, where it unites at an acute angle with the basal layer, often extending beyond the edges of the basal layer (Van der Byl, 1929; Doidge, 1941).

Host range and Geographic distribution - **North Africa: Addi Nefas,** *P. gaguedi* J. F. Gmel. (Saccardo, 1910); **South Africa: Cape province,** *Leucospermum cordifolium, Leucadendron* sp., *P. acaulis, P. amplexicaulis, P. burchellii, P. caffra, P. compacta*, P. grandiceps*, P. lepidocarpodendron, P. longifolia* Andr., *P. magnifica, P. nitida, P. punctata, P. repens, P. scabra* R. Br., *Protea* spp., **Natal,** *P. simplex* Phill., *Protea* spp., **Pretoria,** *P. caffra* (Doidge, 1920, 1941, 1942, 1950; Sydow, 1926; Van der Byl, 1929; Van Wyk 1973a; Van Wyk *et al.*, 1975a; Knox-Davies *et al.*, 1987; Serfontein & Knox- Davies, 1990a); **USA: California,** *P. compacta x susannae* cv Pink Ice*, *P. laurifolia*, P. laurifolia x neriifolia*, P. magnifica*, P. magnifica x compacta* cv May Day*, *P. neriifolia x magnifica*, P. neriifolia* cv White Owl*, *P. obtusifolia*, P. pudens**; **Zimbabwe,** *P. compacta x burchelli* cv Brenda*, *P. magnifica x susannae* cv Susara*, *P. neriifolia* cv Moonshine*, *P. obtusifolia x compacta* cv Red Baron* (present study*).

Control - Since *C. senniana* grows very slowly in culture, it can be assumed that the incubation period is relatively long. Systemic fungicides, such as benomyl, fenarimol, nuarimol, penconazole and triadimefon are considered to control *C. senniana* (Serfontein & Knox-Davies,

1990a). Von Broembsen (1989) suggested that regular spray programmes that include a benzimidazole fungicide, could be used to control the fungus.

Notes - *Coleroa senniana* was first described by Saccardo (1910) on dying leaves of *P. abyssinica* from North Africa. The fungus commonly occurs on leaves of *Protea* spp. in South Africa (Doidge, 1941) and is, except for the presence of *Mycosphaerella proteae* (Syd.) Arx on cultivated plants, probably the most widespread pathogen of *Protea* spp. (Van Wyk, 1973a). Coleroa leaf spot occurs on foliage of the previous season's flush (Von Broembsen, 1989).

Coniothyrium Corda emend. Sacc. spp.

Symptoms - *Coniothyrium* spp. are usually found in parchment-like and dead tissue, and lesions tend to vary in shape and size. Lesions normally appear on the leaf tip or margin, with pycnidia scattered unevenly throughout (Van Wyk, 1973a).

Host range and Geographic distribution - **South Africa: Cape province,** *Leucospermum conocarpodendron**, *Lcd. laureolum, P. nitida, Protea* sp.* (Van Wyk, 1973a; present study*).

Notes - Preliminary collections of leaf spots on South African Proteaceae have found several *Coniothyrium* spp. to be present. Van Wyk (1973a) referred to a *Coniothyrium* sp. associated with spots on *Protea, Leucospermum* and *Leucadendron* in South Africa. Further studies would, however, be required to characterise these species.

Helicosingula leucadendri P.S. van Wyk, Marasas, Baard & Knox-Dav., Transactions of the British Mycological Society **85**: 183 (1985).

Taxonomy - Conidiogenous cells are borne laterally on superficial vegetative hyphae, discrete, globose, hyaline, later brown, giving rise to a single terminal conidium through a series of holoblastically produced cells; the basal part of the conidiogenous cell stays behind as a cup-shaped cell with ruptured walls after conidial secession (rhexolytic) by circumscissile rupture of the cell wall below the basal septum of the conidium. Conidia arise solitarily as blown-out ends of the apex of the conidiogenous cell, dry, dark brown, smooth, thick-walled, multiseptate, not constricted at the septa, helicoid, very tightly coiled in three dimensions, looks like dictyospores under the light microscope, 12.5-16.5 µm wide x 11.0-16.5 µm high (Van Wyk *et al.*, 1985b).

Symptoms - Van Wyk *et al.* (1985b) described the colonies on the leaf surface as "...amphigenous, circular, radiating, up to 3 mm diam., discrete, but becoming confluent and covering large areas of the leaf surface, black with a sooty appearance".

Histology - Mycelium on the leaf surface is composed of dark brown, smooth hyphae, 3.0-5.0 µm diam., branched, intertwined and anastomosed to form a dense, crust-like network. The superficial mycelial crust is attached to the subcuticular mycelium by narrow, cylindrical, brown infection pegs, penetrating the cuticle. Hyphae in the leaf tissue are subcuticular, hyaline, later thick-walled, dark brown and stroma-like (Van Wyk *et al.*, 1985b).

Host range and Geographic distribution - **South Africa: Cape province,** *Leucadendron tinctum, Leucadendron* sp.* (Van Wyk *et al.*, 1985b; present study*).

Leptosphaeria protearum Syd. & P. Syd., Annals of Mycology **10**: 441 (1912).

Taxonomy - Perithecia are amphigenous, substomatal, scattered, subepidermal, later slightly protruding at the apex, lenticular, 175-275 µm diam., black, with a minute, inconspicuous papilla, parenchymatous in structure, opaque, composed of cells 7-10 µm diam. Asci fasciculate, aparaphysate, eight-spored, frequently curved, rounded at the apex, usually clavate, 80-100 µm x 13-17 µm, with distichous spores, seldomly long cylindrical, up to 200 µm long, 10-12 µm wide, with monostichous spores (Doidge, 1927). Ascospores oblong, obtuse, at first 1-septate and hyaline, later 3-septate and pale brown, slightly constricted at the medial septum, 18-26 µm x 5-9 µm (Doidge, 1927; Van Wyk, 1973a; Van Wyk *et al.*, 1975a).

Symptoms - Leaf spots are necrotic and sunken with a raised, dark brown margin. Black ascocarps are visible in the dead tissue (Fig. 9) (Van Wyk, 1973a; Van Wyk *et al.*, 1975a; Von Broembsen, 1989). Spots are round-irregular and vary in size on different hosts, from 4-25 mm diam. These frequently coalesce to form irregular blotches of larger dimensions (Doidge, 1927; Van Wyk, 1973a). Under favourable conditions, the disease becomes more like a blight than a leaf spot. Young plants can be severely infected and killed (Von Broembsen, 1989).

Host range and Geographic distribution - **South Africa: Cape province,** *Protea caffra, P. compacta*, P. cynaroides, P. grandiceps*, P. lacticolor*, P. lepidocarpodendron, P. lorifolia, P. magnifica, P. punctata, P. repens, Protea* spp. (Sydow & Sydow, 1912; Doidge, 1927, 1950; Nel,

1942; Van Wyk, 1973a; Van Wyk *et al.*, 1975a; Knox-Davies *et al.*, 1986; Knox-Davies *et al.*, 1987; present study*); **South Zimbabwe: Inyanga,** *P. gaguedi* (Doidge, 1950).

Notes - *Leptosphaeria protearum* is apparently specific to proteas (Von Broembsen, 1989). *Protea magnifica* is particularly susceptible to Leptosphaeria leaf spot, as well as the natural hybrid *P. magnifica x burchellii* cv Sheila (Knox-Davies *et al.*, 1986).

Mycosphaerella bellula Crous & M.J. Wingf., Mycotaxon **46**: 20 (1993).

Taxonomy - Ascocarps are amphigenous, black, obpyriform to subglobose, subepidermal and substomatal, 90-150 µm wide, 80-130 µm high; walls composed of medium brown cells; 4-5 layers of *textura angularis*, base composed of 3-4 layers of hyaline cells; developing in a substomatal chamber, becoming erumpent through stomatal pore. Asci bitunicate, aparaphysate, subsessile, 8-spored, cylindrical to obpyriform, 30-58 x 7-10 µm, up to 20 per ascocarp. Ascospores bi- to triseriate or irregularly arranged, oblique, overlapping, straight, ellipsoidal, obtuse at each end, hyaline, smooth, median septate, guttulate, constricted at septum, widest in middle of upper cell as arranged in ascus, 7-11 x 2-3 µm (Crous & Wingfield, 1993).

Symptoms - Lesions are amphigenous, circular, light brown and sunken with a raised, dark brown margin (Crous & Wingfield, 1993).

Host range and Geographic distribution - **South Africa: Cape province,** *Protea repens* (Crous & Wingfield, 1993).

Notes - The leaf spots caused by *M. bellula* are similar to the leaf spots caused by *M. jonkershoekensis*. *Mycosphaerella bellula* can, however, easily be distinguished from *M. proteae* and *M. jonkershoekensis* when comparing their ascospores, which are significantly smaller than those of the other two species (Crous & Wingfield, 1993). Single ascospores germinate and grow readily on 2% MEA in culture. To date, however, no anamorph has been observed for *M. bellula*.

Mycosphaerella jonkershoekensis P.S. van Wyk, Marasas & Knox-Dav., Journal of South African Botany **41**: 234 (1975).

Taxonomy - Ascostromata amphigenous, unilocular, solitary, black, pyriform or subglobose, subepidermal and immersed in the mesophyll below the stomata, apex penetrating the stomata and opening by means of a papilla-like, protruding ostiole, 90-122 µm high, 88-136 µm wide, locular wall 8.2-29.8 µm diam., consisting of several layers of dark brown plectenchymatous cells (Van Wyk, Marasas & Knox-Davies, 1975b). Asci aparaphysate, sessile or very briefly stipitate, obclavate or pyriform, bitunicate with the wall prominently thickened at the rounded apex, eight-spored, 32.0-57.2 x 10.2-15.9 µm. Ascospores obliquely mono- to triseriate, hyaline or subhyaline, ellipsoidal and tapering towards the rounded ends, one-septate, not constricted, 11.2-20.7 x 3.9-7.6 µm; upper cell broadly rounded at the apex 6.9-9.9 x 2.0-4.9 µm, lower cell equal in length or slightly longer than the upper cell and narrowing slightly towards the rounded base 7.3-9.9 x 2.0-4.6 µm (Van Wyk, 1973a; Van Wyk *et al.*, 1975a, b). Crous and Wingfield (1993) described the ascospores widest in the middle of the upper cell as arranged in the ascus, measuring 11.0-23.0 x 4.0-6.0 µm. The ascospores are tri- or multiseriate in the ascus, becoming light brown, somewhat spirally twisted and elongated with age, ranging from not constricted to conspicuously constricted at the median septum (Crous & Wingfield, 1993).

Symptoms - Leaf spots are amphigenous, circular, sunken, necrotic, parchment-like, greyish to light brown with a raised, dark brown margin (Fig. 10). The lesions are up to 5 mm diam. and visible on both sides of the leaf. Leaf spots are discrete and regular in outline, except when the lesions coalesce to form larger lesions (Van Wyk *et al.*, 1975b).

Host range and Geographic distribution - **South Africa: Cape province,** *Protea magnifica, P. repens* (Van Wyk, 1973a; Van Wyk *et al.*, 1975a, b).

Notes - The asci and ascospores of *M. jonkershoekensis* are considerably smaller than those reported for *M. proteae*. The ascospores of *M. jonkershoekensis* are also not as unequally one-septate as those of *M. proteae*. The lower cells of the ascospores of *M. jonkershoekensis* are only very slightly longer and narrower than the upper cells, while this difference is conspicuous in the ascospores of *M. proteae* (Van Wyk *et al.*, 1975b).

Mycosphaerella proteae (Syd.) Arx, Beiträge zur Kryptogamenflora der Schweiz **11**: 357 (1962).

Taxonomy - Ascocarps are subepidermal, substomatal, 100-200 µm diam. and are formed singly (Sydow & Sydow, 1914; Van Wyk, 1973a). Asci sessile, cylindrical-clavate or clavate, 55-144 x 11-25 µm, rounded at the apex, aparaphysate, eight-spored. Spores bi- to triseriate in the ascus, oblong-cuneate, unequally one-septate, not constricted or slightly constricted, 17-33 µm long; upper cell shorter, but more broadly rounded or ovate, 8-10 µm long, 7-9 µm broad, lower cell longer and narrower, 15-19 x 6-8 µm, hyaline or sub-hyaline (Sydow & Sydow, 1914; Doidge, 1921; Petrak, 1924; Müller & Von Arx, 1962; Van Wyk, 1973a; Van Wyk *et al.*, 1975b; Crous & Wingfield, 1993).

Symptoms - The leaf spots caused by *M. proteae* on the different hosts are quite variable in appearance. Typically they develop centrifugally, they are amphigenous, bright red-purple to red-brown, becoming grey or black, roughened, irregular in outline, but clearly delimited by a red-brown margin (Fig. 11). Normally no necrotic areas are associated with the lesions. The lesions are raised and frequently become dome-shaped with a corresponding concave retraction of the axial leaf surface. Lesions are up to 10 mm diam. or larger by confluence (Doidge, 1921; Van Wyk, 1973a; Van Wyk *et al.*, 1975a, b). Discoloured, roughened areas form on both sides of the leaf (Doidge, 1921).

Histology - The stroma is reduced to an epidermal clypeus, 150-250 µm diam. over each loculus and the short, scattered hyphal strands or knots under it. The loculi can become closely crowded resulting in the clypeus over adjoining loculi becoming confluent, forming a more or less continuous stromal plate, or the loculi may be more scattered, solitary, or in groups of two or three. Loculi immersed, globose or ovate globose, 100-150 µm diam., locular wall consisting of small brown cells and fused at the apex with the epidermal clypeus (Doidge, 1921).

Host range and Geographic distribution - **South Africa: Cape province,** *Protea amplexicaulis, P. burchelli, P. compacta, P. compacta x magnifica* cv Pink Duke*, *P. cordata* Thunb., *P. cynaroides, P. eximia, P. grandiceps, P. laurifolia* Salisb. ex Knight, *P. magnifica, P. magnifica x susannae* cv Susara*, *P. neriifolia, P. nitida, P. obtusifolia, P. obtusifolia x magnifica* cv Sheila*, *P. punctata, P. repens**, *P. speciosa* (L.) L., *P. stokoei* Phillips, *P. susannae x eximia* cv Sylvia*, *Protea* spp., **Eastern Cape/KwaZulu-Natal; Kentani,** *P. roupelliae, P. simplex*;

Gauteng, *P. caffra, P. gaguedi* (Saccardo, 1891, 1926; Sydow & Sydow, 1914; Theissen & Sydow, 1915; Doidge, 1921, 1950; Van Wyk, 1973a; Van Wyk *et al.*, 1975a, b; present study*).

Notes - *Mycosphaerella proteae* is the most common pathogen on *Protea* spp. in South Africa (Van Wyk, 1973a). This leaf spot is not as destructive to young plants as Leptosphaeria leaf spot (Von Broembsen, 1989). The fungus grows extremely slowly in culture (5 mm diam. after 6 mo on 2% MEA at 25°C), and to date no anamorph has been observed.

Phyllachora proteae Wakef., Fungi Exotici 26. Kew Bulletin Miscellaneous Information: 164 (1922).

Taxonomy - Perithecia solitary, immersed in the mesophyll, sub-epidermal, flattened, visible as a black spot with a lighter centre (Van Wyk, 1973a), globose or flattened globose, 180-200 µm diam., 150-180 µm high; ostiole flat, papilliform, completely immersed in the clypeus; perithecial wall pale, concentric fibrose, not sharply differentiated outwardly from the stroma. Asci paraphysate, cylindrical, briefly stipitate, 120-150 x 12-15 µm. Spores obliquely monostichous, ovate, tapering slightly to rounded ends, uniformly hyaline, 19-20 x 8-9 µm (Wakefield, 1922; Van Wyk, 1973a; Van Wyk *et al.*, 1975a). Paraphyses branched, filiform, exceeding the asci (Doidge, 1942). Van Wyk (1973a) found that the ascospores were somewhat narrower and showed greater variation in length (13-22 x 6-8 µm) than those measured by Doidge (1942). Unilocular stromata epiphyllous, scattered, minute, 200-300 µm diam., round, discrete, very seldomly coalesce, flat at the periphery, raised and somewhat convex in the centre, dull black (Wakefield, 1922). Stromata formed under the stomata of the leaf (Doidge, 1922). Distinct, though very small epidermal clypeus (Wakefield, 1922). Clypeus covered by the thick cuticle, epidermal, 20-30 µm thick, limited, not extending beyond the perithecium or barely so, opaque, black, composed of dark olive-brown, parenchematous cells ca. 5 µm diam.; the clypeus is continuous with a zone 30-50 µm deep, consisting of opaque, black or dark olive-brown stromatic tissue, surrounding the single perithecium, similar in structure to the clypeus, parenchymatous, but composed of slightly larger, round to angular cells, ca. 7-8 µm diam. Everywhere else, the stroma consists of light or dark brown, branching hyphae, 2.5 – 3 µm thick, which penetrate between the cells of the mesophyll (Doidge, 1942).

Symptoms - The lesions are typically necrotic with a raised margin. Black ascocarps are conspicuous in the dead tissue. Lesions frequently move from the leaf tip inwards and finally cover the entire leaf surface, especially in the case of older and fallen leaves (Van Wyk, 1973a; Van Wyk *et al.*, 1975a). Stromata remain as small solitary spots, except for the centre where the ostiole occurs (Wakefield, 1922).

Host range and Geographic distribution - **South Africa: Cape province,** *Leucadendron laureolum, P. acaulis, P. magnifica, P. neriifolia, P. repens* (Wakefield, 1922; Doidge, 1942, 1950; Van Wyk, 1973a; Van Wyk *et al.*, 1975a).

Notes - Van Wyk (1973a) stated that the fungus must be reclassified as a species of *Botryosphaeria* or *Guignardia.*

Pseudomonas syringae **pv.** ***proteae*** Moffatt, Fahy and Persley, Plant Bacterial Diseases – A Diagnostic Guide: 327 (1983).

Taxonomy - The causal bacteria species is a Gram negative rod, 2.7-3.4 x 0.7-0.9 µm with 2-7 polar flagella (Wimalajeewa, Hayward & Greenhalgh, 1983). Paine and Stansfield (1919) described the bacteria in culture as being small, oval rods, 0.8-1.6 x 0.6-0.8 µm. In the leaf spots it is of the same diameter, but almost coccoid in form. One to three flagella in number, 10-12 µm in length and unipolar, are observed.

The bacteria species produces yellow colonies with green centres on glucose yeast-extract chalk agar and on yeast dextrose carbonate agar, but the colours disappear after a few days. Two types of colonies are produced on King's B Medium. One type of colony is raised, convex, mucoid and coalescing, while the other is low, convex, butyrous, translucent and spreading. Both produce a diffusible green-yellow pigment, that fluoresces blue-green under u.v. light (354 nm) (Wimalajeewa *et al.*, 1983).

Symptoms - The disease begins as distinct, dark green, water-soaked spots on the leaves. These enlarge (5-8 mm diam.), turn a black-brown colour, become sunken and surrounded by a bright crimson halo (Wimalajeewa *et al.*, 1983). Every spot is surrounded by a narrow green band, 1-2 mm, that separates the sunken spot from the halo (Paine & Stansfield, 1919; Forsberg, 1993). The spot slowly enlarges and after several weeks almost the entire leaf surface is covered. In

severe cases where several spots enlarge and coalesce, large portions of the leaves wither. Symptoms observed in Australia resembled those described by Paine and Stansfield (1919) for bacterial leaf spot of *P. cynaroides* in England, except that raised blisters were not observed (Wimalajeewa *et al.*, 1983).

Paine and Stansfield (1919) observed numerous dome-shaped blisters of a reddish brown colour scattered over the lamina of the leaf, mainly upon the upper surface. They varied in size (1-3 mm diam.) and the surface of the blisters was raised (0.5-1 mm) above the general level of the leaf. In stead of the brown blisters, wider areas occurred on the younger leaves, which surfaces were frequently depressed by shrinkage of the underlying cells. It was not known whether one type of spot changed into the other or whether they were the result of difference in age of the leaf at the time of infection. Both types of spots occurred on the same leaves.

Bacteria gain entrance to the leaf through the stomata. The host prevents the lesion, and the spread and development of the parasite by the formation of wound cork and the production of wound gum. The ultimate death of the organism is the result of desiccation within the area enclosed by the cork (Paine & Stansfield, 1919; Paine & Berridge, 1922).

Host range and Geographic distribution - **Australia: Victoria,** *Protea cynaroides* (Wimalajeewa *et al.*, 1983); **England,** *P. cynaroides* (Paine & Stansfield, 1919; Paine & Berridge, 1922).

Control - Copper sprays will protect foliage against infection (Von Broembsen, 1989).

Notes - In England, the bacterial disease occurred on leaves of older plants as well as seedlings (Paine & Stansfield, 1919). In Australia, it was first observed in 1980 in nurseries and field plantings in Southern Victoria. The spotting retards seedling growth and lowers the quality of foliage on stems of cut-flowers (Wimalajeewa, *et al.*, 1983). Bacterial leaf spot has not yet been recorded in South Africa (Knox-Davies *et al.*, 1986). Paine and Stansfield (1919) suggested the name *Pseudomonas proteamaculans* for the organism. The report of Wimalajeewa *et al.* (1983) constituted the first report of a bacterial leaf disease on a *Protea* species in Australia. On the basis of cultural, biochemical and physiological characters, the *Protea* pathogen was identified as a separate pathovar of *Pseudomonas syringae* van Hall (Wimalajeewa *et al.*, 1983). Forsberg (1993) reported the causal organism as *Pseudomonas syringae* pv. *syringae*. The bacteria spread by rain splash and infected nursery stock. Prolonged wet, humid and cool conditions favour disease spread and development (Forsberg, 1993).

Teratosphaeria fibrillosa Syd. & P. Syd., Annals of Mycology **10**: 40 (1912).

Taxonomy - Ascomata ellipsoid or pyriform, perithecium-like, 150-220 µm diam. (Sydow & Sydow, 1912; Doidge, 1922; Van Wyk, 1973a), sunken under the epidermis, arranged in centrifugal, irregularly branched, radiating lines, to a diam. of 1 cm. Ascomatal wall consisting of concentric layers of delicate stromatic hyphae. Apex of the loculus penetrating the epidermis but not protruding, with no true ostiole. Vegetative stroma meagre, penetrating into the intercellular spaces of the mesophyll below the epidermis in the form of perpendicular hyphal strands. At the apex the hyphae also penetrate the adjacent epidermal cells, but without forming a clypeus (Doidge, 1922). Asci basal, sessile, club-shaped, thickened with a rounded apex, 70-110 x 20-36 µm (Sydow & Sydow, 1912), aparaphysate, eight-spored. Ascospores distichous or tristichous, cylindrical, straight, medianly septate, somewhat constricted at the septum, light brown, rounded at both ends, 42-52 x 8-9 µm (Doidge, 1922). Van Wyk (1973a) and Sydow and Sydow (1912) recorded measurements of 35-46 x 9-12 µm.

Symptoms - Leaf spots are characterised by ascocarps sunken under the epidermis, arranged densely together in centrifugal, irregularly branched, radiating lines, to a diam. of 1 cm (Doidge, 1922). No necrotic tissue is noticed around the fruiting structures, but the plant tissue in direct contact with the fruiting bodies frequently shows a raised border (Van Wyk, 1973a).

Host range and Geographic distribution - **South Africa: Cape province,** *Protea nitida, Protea* sp., **Gauteng, Pretoria,** *P. caffra, P. gaguedi* (Sydow & Sydow, 1912; Theissen & Sydow, 1915; Doidge, 1922, 1950; Saccardo, 1926; Van Wyk, 1973a; Van Wyk *et al.*, 1975a, b).

Notes - This pathogen is of no economic importance because it is restricted to only a few hosts of no commercial value (Van Wyk, 1973a).

Teratosphaeria proteae-arboreae P.S. van Wyk, Marasas & Knox-Dav., Journal of South African Botany **41**: 232 (1975).

Taxonomy - Ascostromata aggregated, dark brown to black, unilocular, pyriform or ellipsoidal to subglobose and flattened above and on the sides, 120-194 µm high and 113-234 µm wide, sunken in the leaf tissue below the epidermis with the apex penetrating the stomata and opening by means of an ostiole. Ascomatal wall 10-20 µm wide, composed of several layers of dark brown

plectenchymatous cells. No superficial mycelium present, but strands of brown, septate, vegetative hyphae extend from the ascostromata into the intercellular spaces of the mesophyll. Asci basal, surrounded by delicate paraphysoids that gelatinise early, sessile, obclavate or lageniform, bitunicate with the wall conspicuously thickened towards the apex, eight-spored, 70-96 x 18-35 µm. Ascospores obliquely monostichous or distichous, cylindrical with rounded ends, straight, medianly 1-septate, slightly constricted at the septum, surface finely granular, hyaline, light brown at maturity, 18-32 x 5-9 µm. Spermagonia have the same general structure as the ascocarps and occur intermingled with the latter, however, they contain a hymenium of conidiophores and minute, non-septate, hyaline, bacillar spermatia, 1.6-3.1 x 1.0 µm (Van Wyk *et al.*, 1975b).

Symptoms - The ascostromata are irregularly crowded together in the centre of the leaf spots. The leaf spots are amphigenous, circular, raised, 1-3 mm diam. and black in the centre due to the development of ascocarps. The lesions are surrounded by a narrow, pinkish margin which may enlarge to form a necrotic zone (Van Wyk, 1973a; Van Wyk *et al.*, 1975a, b).

Host range and Geographic distribution - **South Africa: Cape province,** *Protea nitida* (Van Wyk, 1973a; Van Wyk *et al.*, 1975a, b).

Notes - The *Teratosphaeria* spp. on *Protea* are distinguished by means of the appearance of the leaf spots, the arrangements of the ascostromata and the ascospore size. The ascospores of *T. proteae-arboreae* are considerably smaller (23-32 x 5-7 µm) than those of *T. fibrillosa* (Van Wyk, 1973a; Van Wyk *et al.*, 1975b).

Trimmatostroma macowanii (Sacc.) M.B. Ellis, More Dematiaceous Hyphomycetes: 29 (1976).

Taxonomy - Colonies are amphigenous, large, circular, black or grey, frequently confluent. Sporodochia pulvinate, punctiform, black. Stromata in substomatal cavaties, hyaline or pale brown. Conidiophores emerging in fascicles through stomata, straw-coloured or pale brown, smooth to verruculose. Conidia are in simple and branched chains, have no definite shape, with one or several septa, brown or olivaceous brown, verrucose, 8-20 µm long, 5-9 µm thick (Ellis, 1976). Van Wyk (1973a) described the conidia as dark coloured, normally multicellular, lacking any definite shape.

Symptoms - On *P. nitida* the lesion appears as a circular, black, sooty leaf spot, up to 1 cm diam. Spots are often so densely grouped that the entire leaf appears sooty (Van Wyk, 1973a).

Host range and Geographic distribution - **Malawi,** *P. petiolaris* (Hiern) Baker & Wright (Sutton, 1993); **South Africa: Cape province,** *Leucospermum conocarpodendron, Leucadendron sp., P. nitida, Protea* sp. (Saccardo, 1886; Doidge, 1950); **South Zimbabwe: Harare,** *P. angolensis* Welw. (Doidge, 1950).

Notes - Although the fungus has not yet been observed on *P. cynaroides* in nature, it has been isolated on several occasions from healthy leaves, where it lives as an endophyte. Colonies sporulate readily in culture on 2% MEA.

Vizella interrupta (G.Winter) S. Hughes, Mycological Papers **50**: 99 (1953).

Taxonomy - Asci are thick-walled, stipitate, clavate, eight-spored, 60-67 x 20-30 µm. Paraphyses thread-like, indistinct, evanescent. Ascospores are distichous, broadly ellipsoid, always 1-celled, 16-17 x 9-9.5 µm (Doidge, 1927). Van Wyk (1973a) reported that the size of the ascospores were much smaller. Ascospores from which the hyaline band was absent, measured 12.7-16.5 x 5.3-9.3 µm, while those with a hyaline band were the same size as those measured by Doidge (1927). Ascospores are violet-brown with a hyaline, transverse band, 2-3 µm broad (Doidge, 1927; Van Wyk, 1973a; Van Wyk, Marasas & Hattingh, 1976). The ascospores are very characteristic and because the asci dissolve very quickly, they are seldomly noticed in the asci and can easily be confused with conidia (Van Wyk, 1973a). Hughes (1953) found the ascospores of *Entopeltis* Höhn. to be continuous with a well-marked transverse hyaline band, while those of *Vizella* Sacc. are composed of a large upper cell with a hyaline transverse band and a small hyaline to brown appendaged cell. Some 1-celled ascospores, however, lack the appendaged cell. Pycnidia are similar to the ascocarps. Conidia are borne on short conidiogenous cells arising form the upper wall of the pycnidium. Conidia are hyaline when inside the pycnidium but become brown after release, ellipsoidal, 1-celled, 12.5-16.5 x 5.5-9.5 µm, without a hyaline transverse band. Spermagonia are similar in structure to the pycnidia and contain numerous small, hyaline, cylindrical spermatia. The generic position of the pycnidial state can be linked to *Manginula* G. Arnaud, but uncertainty exists if this is the real anamorph state (Van Wyk *et al.*, 1976).

Symptoms - The lesions on the leaf are brown spots, 1-6 mm diam., which often coalesce, and on which the black fruiting bodies appear in groups. These are embedded in the cuticle (Fig. 12) (Doidge, 1927). Leaf spots vary on different hosts. Ascocarps are observed as black spots on slightly discoloured leaf tissue, without any definite pattern on *Protea* spp. On *Leucadendron* and *Leucospermum* spp., leaf spots are light to dark brown, round, coalescing to become irregular with ascocarps concentrically arranged in the discoloured tissue (Van Wyk, 1973a; Van Wyk *et al.*, 1975a).

Histology - Intracuticular hyphae are ribbon-like, 4-10 µm wide, and consist of regularly alternating long, light brown, thin-walled cells, 10-20 µm long, and short, dark brown, thick-walled cells, 2-6 µm long (Hughes, 1953; Van Wyk *et al.*, 1976). The single hyphae normally go along the outlines of the epidermal cells, but a few go out from each fruiting body and also invade the stomata, filling the substomatal cavity with brown hyphae and invading the neighbouring palisade cells. The fruiting bodies are embedded in the cuticle and the upper wall only is developed; they are therefore dimidiate, round, irregular, flat-conical with a flat base, 160-225 µm diam. and about 35 µm high, unilocular. The hyaline base gives rise to a few asci, of which the outer are slanting or almost horizontal and the inner more upright. The outer wall of the fruiting body consists of only a single layer of cells and has in the centre a flat, round, slightly ragged, false ostiole, 16-22 µm in diam. The cells are in radiating lines, thin-walled, 4-6 angled and 6-9 µm diam. (Doidge, 1927). The upper wall of the ascocarp is composed of one to several layers. A central column of hyaline, sterile paraphysoid tissue is usually absent from the ascocarps, but can be present (Van Wyk *et al.*, 1976).

Host range and Geographic distribution - **South Africa: Cape province,** *Leucospermum conocarpodendron, Lsp. cordifolium, Lcd. discolor, Lcd. gandogerii, Lcd. laureolum, Leucadendron* spp., *P. cynaroides, P. grandiceps*, P. lepidocarpodendron, P. lepidocarpon, P. magnifica*, P. neriifolia, P. nitida* (Saccardo, 1891; Theissen & Sydow, 1915; Doidge, 1927, 1950; Hughes, 1953; Van Wyk, 1973a; Van Wyk *et al.*, 1975a, 1976; present study*).

Notes - This pathogen was first described as *Asterina interrupta* G. Winter (1884) from *Leucospermum* and *Leucadendron* material collected in the Cape province, South Africa. Von Höhnel (1910) created the new genus *Entopeltis* with *E. interrupta* (G. Winter) Höhn. as type species. Hughes (1953) treated *Entopeltis* and *Vizella* as synonyms and made the new combination, *Vizella interrupta* (G. Winter) S. Hughes. Von Arx and Müller (1975), however,

considered *Entopeltis* and *Vizella* as separate genera and retained the name *E. interrupta*. Van Wyk *et al.* (1976) presented additional evidence that the genera *Entopeltis* and *Vizella* should not be separated, and that the correct name of the fungus on South African Proteaceae is *Vizella interrupta* (Van Wyk *et al.*, 1976).

The generic position of the pycnidial state is uncertain. In the past, authors have made the mistake of interpreting ascospores of *Vizella* as conidia. This conclusion was reached because in their examination of the isotype of *Asterina interrupta* on *Lsp. conocarpodendron*, Van Wyk *et al.* (1976) did not observe a pycnidial state with conidia morphologically identical to its ascospores. A pycnidial state with 1-celled, non-branched conidia, associated with banded ascospores on *Lcd. laureolum*, was found. These conidia are identical to those described for *V. banksiae* Swart and *V. grevilleae* Swart (Swart, 1975). It is not likely that *E. interrupta* produces two distinct pycnidial states, one with banded conidia, identical to the ascospores, and one with non-banded conidia. The newly described pycnidial state of *V. interrupta* therefore cannot be assigned to a form-genus for now, because of taxonomic problems associated with the genus *Manginula* (Van Wyk *et al.*, 1976).

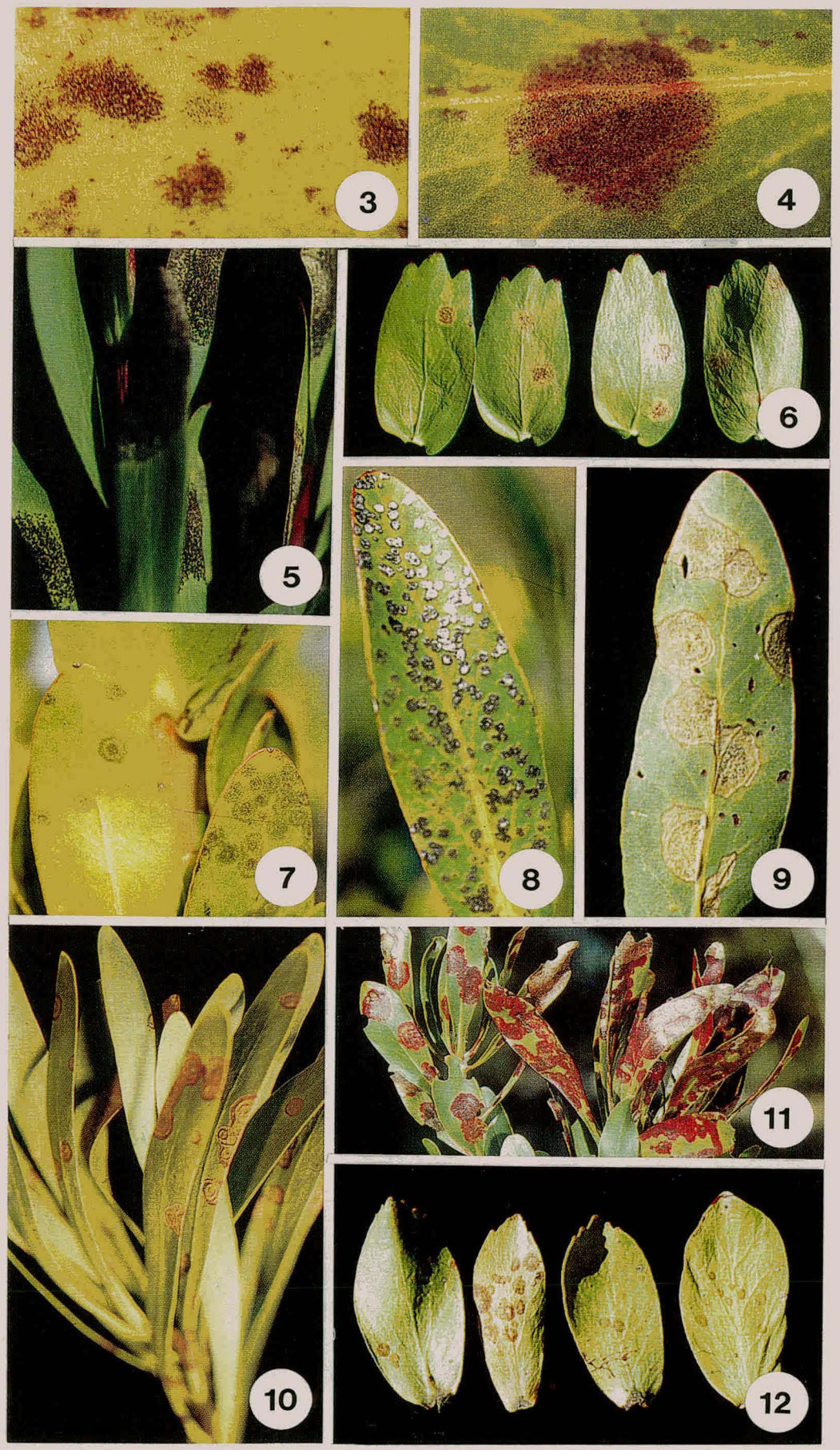
At present three species of *Vizella* are known to occur on Proteaceae: *V. interrupta* (on *Protea, Leucospermum* and *Leucadendron*, South Africa), *V. banksiae* (on *Banksia* and *Hakea* Schrad., Australia) (Swart, 1971, 1975) and *V. grevilleae* (on *Grevillea* R. Br., Australia) (Swart, 1975). *Vizella banksiae* is similar in all essential respects to *V. interrupta*, except that the hyphae of *V. banksiae* are consistently wider (10-15 µm) than those of *V. interrupta* (4-10 µm) and the conidia of *V. interrupta* are somewhat larger than those of *V. banksiae* (Swart, 1975). Consequently, *V. interrupta* and *V. banksiae* are considered to be two closely related but separate species (Van Wyk *et al.*, 1976).

Table 2. Leaf pathogens of minor importance

Pathogen	Geographic Distribution	Hosts	References
Ascochyta Lib. sp.	Hawaii	*Lsp. reflexum*	(Protea Disease Letter, 1991)
Bipolaris Shoemaker sp.	Hawaii	*Lsp. cordifolium* *Lsp. loliofolium* *Lsp. lineare x glabrum* cv Hybrid 24 *Leucospermum* spp. *Lcd. salignum x laureolum* cv Safari Sunset	(Protea Disease Letter, 1991)
Calonectria colhounii Peerally	Hawaii	*Leucospermum* spp.	(Uchida *et al.*, 1996)
Didymosporium congestum Syd.	South Africa	*P. caffra*	(Doidge, 1950)
Dothiorella Sacc. sp.	Hawaii	*Lcd. laureolum* *P. cynaroides*	(Protea Disease Letter, 1991)
Macrophoma Sacc. sp.	Hawaii	*Leucospermum* sp.	(Protea Disease Letter, 1991)

General control strategies

No fungicides are registered in South Africa for control of these diseases on Proteaceae (Krause *et al.*, 1996). Leaf spot diseases can generally be controlled, prophylactically and curatively, with a regular spraying programme using contact fungicides registered on ornamental plants. Sometimes, however, additional applications may be necessary. Mancozeb gives good control when sprayed in two-weekly intervals, alternated with chlorothalonil.



Figs 3-12. Leaf spot diseases caused by various pathogens. **3.** *Batcheloromyces leucadendri*. **4.** *B. proteae*. **5.** *Cercostigmina protearum* var. *leucadendri*. **6.** *C. protearum* var. *protearum*. **7, 8.** *Coleroa senniana*. **9.** *Leptosphaeria protearum*. **10.** *Mycosphaerella jonkershoekensis*. **11.** *M. proteae*. **12.** *Vizella interrupta*.

PATHOGENS OF SHOOTS, STEMS AND FLOWERS

Botrytis cinerea Pers.: Fr. Synopsis Methodica Fungorum: 690; Mycologia Europaea **1**: 32 (1822).

Taxonomy - Sclerotia black. Conidiophores usually 2 mm or more in length, 16-30 µm thick, branched, frequently with a stipe and an open head of branches, smooth, clear brown below, paler near the apex, with the ends of the branches often quite colourless. Conidia ellipsoidal or obovoid, often with a somewhat protuberant hilum, colourless to pale brown, smooth, 6-18 x 4-11 µm (usually 8-14 x 6-9 µm) (Ellis, 1971).

Symptoms - Symptoms are similar to Drechslera blight (Forsberg, 1993). The pathogen causes blight of the flowering branches and flower heads. Brown spots develop on the leaves, flowers and buds. The lesions expand and flower heads can be killed, with necrosis extending down the flower head stalks, causing death of affected parts and new shoots (Serfontein & Knox-Davies, 1990b; Forsberg, 1993). Infected shoot tips collapse, darken and die (Fig. 13). Bending of affected shoots is more typical of Botrytis blight than Drechslera blight. Masses of grey-brown spores are seen on affected tissue under wet conditions, therefore the common name "grey mould" (Von Broembsen, 1989; Forsberg, 1993).

In Hawaii, *B. cinerea* is associated with young shoots of *Lsp. cordifolium* growing in the field, and rooted cuttings growing in propagating benches. The initial water-soaked, round lesion on a young succulent leaf, progresses to the collapse of the leaf and girdling of the young stem, resulting in shoot death (Cho, 1977).

All three stages of inflorescence development are susceptible. Symptoms initially occur on the styles and perianths as small, water-soaked lesions, which normally advance into the flower head (Cho, 1977). *Botrytis* on proteas differs from *Botrytis* on many other flowers in that the pathogen on proteas does not seem to stop with the death of the inflorescence, but will move down into the shoot tip itself and into the leaves. In proteas, it seems to be a strong, active pathogen, in that it can invade actively growing tissues and inflorescences that are not yet in the senescent stage (Rohrbach, 1983).

Botrytis, a wide-ranging soil-borne pathogen, also causes seedling damping-off (Forsberg, 1993), and has been recorded on cuttings showing dieback symptoms, as well as on blighted

seedlings of different Proteaceae (Benic, 1986; Knox-Davies *et al.*, 1986). It is not known whether the fungus, in the case of decayed rooted cuttings, is only an opportunistic coloniser of injured, stressed or already diseased tissue (Knox-Davies *et al.*, 1987).

Host range and Geographic distribution - **Hawaii,** *Lsp. cordifolium, Lsp. cordifolium* cv Pink Star, *Lsp. lineare x glabrum* cv Hybrid 24, *Leucospermum* sp., *P. cynaroides, P. repens,* diseased cuttings (Cho, 1977; Greenhalgh, 1981; Protea Disease Letter, 1991); **South Africa: Cape province,** *Leucospermum cordifolium, Lcd. discolor, Lcd. laxum x lanigerum* cv Jubilee Crown*, *P. cynaroides**, *P. repens,* diseased cuttings (Knox-Davies *et al.*, 1986; Serfontein & Knox-Davies, 1990b); **Zimbabwe,** *Lsp. lineare x cordifolium* cv Succession, *Lsp. reflexum* cv Luteum (present study*).

Control - Botrytis diseases are controlled with benomyl and iprodione sprays (Greenhalgh, 1981). Flowers or young shoots can be protected during moist, cool to moderate weather with two- to three-weekly sprays of iprodione (Von Broembsen, 1989). Blighted flower heads can be removed and the plants sprayed at three-weekly intervals with a mixture of benomyl and iprodione, alternated with procymidone and iprodione (Serfontein & Knox-Davies, 1990b). Jacobs (1981) recommended dipping *Lsp. cordifolium* flower heads in benomyl and dicloran, and drying them thoroughly before packing. Pre-planting dipping of cuttings and spraying with difolitan, captan and benomyl help controlling cutting dieback and blight in mistbeds. Cuttings must also only be taken from disease-free mother plants (Benic, 1986).

Cho (1977) recovered strains of *B. cinerea* resistant to benomyl, methyl thiophanate and thiabendazole. In mycelial growth tests, mancozeb reduced the growth of benomyl-sensitive and benomyl-resistant isolates at a high concentration (500 µg/ml), while low concentrations (10 µg/ml and 25 µg/ml) of chlorothalonil inhibited the growth of all isolates. The benzimidazole fungicides were quite effective in inhibiting the growth of sensitive or wild-type *B. cinerea* strains. Benzimidazole fungicides, when used in combination or alternated with other fungicides which exhibit another mechanism for inhibition of growth, such as mancozeb or chlorothalonil, should reduce the chances of development of resistant strains (Cho, 1977). Sanitation pruning should be implemented to remove diseased plant parts (Von Broembsen, 1989). Crops must also be kept well ventilated to reduce humidity (Porter *et al.*, 1996).

Notes - Botrytis disease occurs on the flower heads and young shoot tips of *Banksia, Leucospermum, Leucadendron, Protea* and *Serruria* Salisb. spp. (Von Broembsen, 1989). When young flowers are infected under wet conditions, they develop abnormally and die off. If damp flowers are packed, they are likely to develop Botrytis rot (G. Malan, Independent Fynbos Consultant, South Africa, pers. comm.). Flower blight can also develop after packing if infection has already taken place in the field (Knox-Davies *et al.*, 1986; Von Broembsen, 1989).

The disease is not a serious problem in Australia, but it could become one in coastal plantings (Forsberg, 1993). Serfontein and Knox-Davies (1990b) reported the pathogen as the cause of flowering branch and flower head blight in the SWCP. *Botrytis cinerea* is also a leaf, shoot and flower blight pathogen of Proteaceae in Hawaii and the second biggest disease problem in the cultivation of Proteaceae (Cho, 1977; Greenhalgh, 1981; Rohrbach, 1983).

In Hawaii, the disease is most severe during the winter months, but has also been recorded in late spring and the early summer months (Cho, 1977). Serfontein and Knox-Davies (1990b) speculated that either wounding is necessary for infection, or flower heads are susceptible to conidial infection only at an early stage of development. Spores spread by wind and water-splash. The disease can rapidly develop under prolonged wet weather, high humidity and cool conditions (Forsberg, 1993).

Colletotrichum gloeosporioides (Penz.) Penz. & Sacc., Fungi Agrumicoli **2**: 6 (1882).

Teleomorph - *Glomerella cingulata* (Stonem.) Spauld. & H. Schrenk, Science series 2 **17**: 751 (1903).

Taxonomy - Cultures of *C. gloeosporioides* from different hosts are highly variable, and may represent several biological species. Conidia are straight, obtuse at the apex, 9-24 x 3-4.5 µm, and appressoria are 6-20 x 4-12 µm, clavate or irregular, sometimes complex (Sutton, 1980). Acervuli, sometimes with setae, are produced on the host. Conidia are produced profusely both on the host and in culture. Conidia from Proteaceae measure 13-14.5 x 6-8 µm (Benic & Knox-Davies, 1983b).

Symptoms - Symptoms include shoot dieback, necrotic stem and leaf lesions, stem rot, sunken stem cankers on old stems, seedling damping-off, seedling blight and cutting dieback. Young growth is most susceptible to infection (Forsberg, 1993). The dieback of young shoot tips is the

most characteristic symptom (Fig. 14). Affected plant tissue is usually dark to black and a definite boundary is evident between healthy and diseased tissue. During moist conditions, orange-pink spore masses are visible on the diseased tissue (Von Broembsen, 1989; Forsberg, 1993). Stem lesions on one side of the stem may cause the new growth to bend, referred to as sheperd's crook symptom (Forsberg, 1993).

Anthracnose of *P. compacta* is characterised by leaf necrosis, development of dark-brown to black, sunken cankers on stems around the bases of leaves, and directly below flower buds and flower heads, stem girdling and eventual death of the whole plant. Terminal leaves die from the tips. Infections can extend into the stems where cankers develop. Orange-pink acervuli develop on the necrotic tissue (Benic & Knox-Davies, 1983b). This dieback disease is prominent during spring and summer. The immature, actively growing shoot tips of proteas are most susceptible. Lesions can then subsequently extend into the older woody parts of the stem (Coetzee, Von Broembsen & Brits, 1988).

In SWCP, pre- and post-emergence damping-off and seedling blights in nursery beds, particularly on *Protea* spp., and dieback of cuttings in mistbeds occur. Dieback of cuttings is characterised by cankers developing from cut tips, extending up into the stem, girdling the stem as it progresses. Necrosis of the leaves and even defoliation can follow (Benic, 1986). Seeds become shrunken and discoloured (Benic & Knox-Davies, 1983b; Benic, 1986).

Host range and Geographic distribution **- South Africa: Cape province,** *Protea compacta, P. compacta x neriifolia, P. cynaroides, P. eximia, P. grandiceps, P. lacticolor* Salisb., *P. laurifolia, P. lepidocarpodendron, P. longifolia, P. magnifica, P. mundii, P. nana, P. neriifolia, P. obtusifolia, P. pudens* Rourke, *P. repens, P. scolymocephala* (L.) Reich., *P. speciosa, P. stokoei, P. susannae* (Benic & Knox-Davies, 1983b; Benic, 1986; Knox-Davies *et al.*, 1986).

Colletotrichum sp.: **Australia,** *Protea compacta, P. coronata* Lam., *P. cynaroides, P. longifolia, P. magnifica, P. neriifolia, P. obtusifolia, P. repens, P. stokoei* (Greenhalgh, 1981); **Hawaii,** *Lsp. cordifolium* cv Coral, *Leucospermum* sp., *Lcd. laureolum, P. cynaroides, Protea* sp. (Protea Disease Letter, 1991).

Leucadendron spp. are reported to be resistant (Benic & Knox-Davies, 1983b). Benic (1986) suggested that *Leucadendron* as well as *Leucospermum* spp. are resistant. According to Coetzee *et al.* (1988), Colletotrichum dieback is the most important disease of *Protea* spp., but *Leucadendron* and *Serruria* spp. are also susceptible.

Control - Seed treatment is advised to control damping-off and seedling blight. A 24 h, warm (30°C) thiram seed soak, or a thiram dusting applied to seed after a 30 min hot water (50°C) treatment, gives good control of seedling diseases. Benomyl dusting, applied after a hot water treatment at 50°C for 30 min, is also effective (Benic, 1986). Seed treatment also enhances seed germination (Benic & Knox-Davies, 1983b).

Regular foliage applications of captafol, captab and mancozeb control dieback diseases to some extent. Captab is, however, phytotoxic to developing seedlings (Benic & Knox-Davies, 1983b). G. Malan (pers. comm.) suggested two- to three-weekly applications of mancozeb, mixed with benomyl or prochloraz, during shoot flushing and wet weather to protect the shoots against infection. Prochloraz applied at 0.3 kg/100 l water causes leaf damage on *P. magnifica*. The concentration should be reduced to 0.1 kg/100 l water.

Pre-plant dipping of cuttings can be used to control cutting dieback. Disease-free cuttings should be dipped into a fungicide solution (difolitan – no longer available in South Africa; captan or benomyl). A combination of difolitan and benomyl can also be used. After planting, the cuttings can be sprayed regularly with difolitan or difolitan combined with benomyl (Benic, 1986). In Australia, difolatan and prochloraz are most effective in reducing severity of Colletotrichum dieback of seedlings (Greenhalgh, 1981; Knox-Davies *et al.*, 1986). Benic also found that it reduced the occurrence of seedling blight in protea nurseries (Benic, 1986).

Sanitation pruning should be implemented as soon as shoot tip dieback is observed and before the lesions can move into the older, woody parts of the stems (Coetzee *et al.*, 1988). Overhead irrigation should be avoided and enough water must be given to plants with severe dieback in order to prevent the development of extensive stem cankers (Von Broembsen, 1989). Some plant diseases are controlled by burning affected lands (Hardison, 1976). *Colletotrichum* inoculum is probably reduced by the practice of burning plantations of Proteaceae as they become unproductive (Benic & Knox-Davies, 1983b).

Notes - Colletotrichum tip dieback is one of the most important diseases of *Protea* spp. in South Africa (Von Broembsen, 1989). The pathogen is the cause of a destructive disease of *P. compacta* in the Betty's Bay, Kleinmond, Bot River area of the SWCP, both in wild and commercial plantings (Benic & Knox-Davies, 1983b). The fungus is also a very important pathogen of protea nurseries in South Africa (Benic, 1986), and is seed-borne (Benic & Knox-

Davies, 1983b). In nurseries in Australia, a *Colletotrichum* sp. is the cause of an economically important dieback disease of terminal shoots of *Protea* spp. (Greenhalgh, 1981).

Prolonged wet periods and moderate to warm temperatures (20°C-25°C) and high humidity favour the development of Colletotrichum dieback. This disease is particularly important in areas where rainfall occurs during summer or warm periods, and in humid coastal climates. Drought stress may induce the stem canker phase, involving other secondary fungi in the disease. The fungus spreads rapidly by splashing rain or irrigation water (Von Broembsen, 1989; Forsberg, 1993). *C. gloeosporioides* is also frequently associated with canker fungi such as *Botryosphaeria* Ces. & De Not. spp. Lesions infected by *Botryosphaeria* can develop into extensive stem cankers (Benic & Knox-Davies, 1983b; Coetzee *et al.*, 1988).

Drechslera blight

Drechslera biseptata (Sacc. & Roum.) M.J. Richardson & E.M. Fraser, Transactions of the British Mycological Society **51**: 148 (1968).

Taxonomy - Conidiophores arising singly from hyphae or in large fascicles from dark brown pulvinate stromata. Conidiophores can be one of two types, flexuous or geniculate, pale to medium brown, thin-walled ones up to about 80 µm long and 3-8 µm thick, and subulate, straight or flexuous, dark brown, thick-walled ones up to 800 µm long, 10-14 µm thick at the base tapering to 6-8 µm at the paler apex. Conidia straight, typically obovoid or broadly clavate, sometimes ellipsoidal, pale to medium brown, smooth or verrucose, with nearly always 2-3 pseudosepta, 20-42 µm long, 11-19 µm thick in the broadest section, generally 23-33 x 14-17 µm (Ellis, 1971).

Drechslera dematioidea (Bubák & Wróbl.) Subram. & P.C. Jain, Current Science **35**: 354 (1966).

Taxonomy - Conidiophores arising either solitary or in small groups, frequently from medium to dark brown cells which form loose stromata, straight or flexuous, sometimes geniculate, brown, 350 x 5-9 (usually 60-150 x 5-6) µm. Conidia straight, cylindrical to clavate, rounded at the ends, golden brown to dark brown, smooth, thick-walled, 2-7 (usually 3-4) pseudosepta, 20-

70(36) x 10-16(14.3) µm; hilum 2.5-3.5 µm wide (Ellis, 1971). Boesewinkel (1986) recorded conidia to be 2-7 septate, measuring 25-70 x 12-16 µm, while Von Broembsen (1986) recorded them as 3-5 septate.

Histology - Non-lateral, bipolar germination is predominant, while distinctive networks of dark brown stromatal initials and anastomising hyphae develop (Von Broembsen, 1986).

Symptoms - The disease results in a severe blight of current-season leaves, stem, and flower heads, particularly during growth flushes. Blight is characterised by discrete leaf lesions and slowly expanding stem cankers that result in death of young shoot tips, or as moderately expanding lesions that are restricted to a few leaves or to leaf tips only. The leaf lesions are yellow, grey or brown and irregularly shaped with distinct red margins, which rapidly enlarge, often covering the entire leaf (Fig. 15). The lesions are at first light brown, then a darker grey-brown as they become necrotic. Spots can develop with or without purple margins (Von Broembsen, 1986, 1989; Forsberg, 1993).

The leaf lesions can spread to the stems causing death of new shoots, stems and flower heads (Forsberg, 1993). Bright red, superficial cankers develop on the stems where infected leaves are attached (Fig. 16). The shoot tips do not collapse and leaves remain aligned with the stem as before infection. Extensive canker development results in shoot death (Fig. 17). Young growth of the current season is more severely affected and young plants sometimes die (Von Broembsen, 1989). On developing flower heads, light brown lesions initiating on a single bract, enlarge rapidly, resulting in wilting and death of the flower heads (Von Broembsen, 1986). Blight can disfigure or kill the flowers, depending on the stage of development (Von Broembsen, 1989). The presence of *Botryosphaeria dothidea* (Moug.: Fr.) Ces & De Not., an important secondary pathogen of the stem cankers caused by *D. dematioidea*, results in significantly larger lesions than with either alone. Entire branches can die back (Von Broembsen, 1986, 1989).

In Hawaii, symptoms with *D. biseptata* are more severe than with *D. dematioidea.* Initially the symptoms on the leaves are light brown, which then enlarge, coalesce and finally cause blighting of the leaves. The lesions and blighted areas on the leaves are surrounded by a yellow band with an outer red border. *Drechslera dematioidea* is regarded as a weak pathogen in Hawaii (Protea Disease Letter, 1993). In South Africa, however, the fungus is a virulent pathogen on pincushions (Von Broembsen, 1986).

Host range and Geographic distribution - **Australia,** *Leucospermum* spp. (Greenhalgh, 1981; Forsberg, 1993); **Hawaii,** *Lsp. cordifolium*, *Lsp. cordifolium* cv Pink Star, *Lsp. cordifolium x lineare* cv Red Sunset, *Lsp. lineare x glabrum* cv Hybrid 24; *Leucospermum* spp., *Lcd. loliofolium*, *Lcd. salignum x laureolum* cv Safari Sunset, *Leucadendron* spp., *P. cynaroides*, *P. repens*, *Protea* spp. (Protea Disease Letter, 1991, 1993); **New Zealand: Auckland,** *Lsp. cordifolium* (Boesewinkel, 1986); **South Africa,** *Leucospermum conocarpodendron*, *Lsp. conocarpodendron x cuneiforme*, *Lsp. cordifolium*, *Lsp. cordifolium* cvs Gold Dust, Yellow Bird, Fire Dance, Vlam, Flame Spike, *Lsp. cordifolium x lineare* cv Red Sunset, *Lsp. cordifolium x tottum* cv Caroline, *Lsp. cuneiforme*, *Lsp. erubescens*, *Lsp. glabrum*, *Lsp. glabrum* cv Helderfontein, *Lsp. grandiflorum*, *Lsp. patersonii*, *Lsp. pluridens*, *Lsp. rodolentum* (Salisb. ex Knight) Rourke, *Lsp. tottum*, *Lsp. tottum x vestitum*, *Lsp. vestitum* (Von Broembsen, 1986).

Leucospermum cordifolium and cultivars derived from *Lsp. cordifolium* (Gold Dust, Yellow Bird and Fire Dance) are the most severely affected (Von Broembsen, 1986; Forsberg, 1993; Protea Disease Letter, 1993). Benic (1986) stated that hybrid *Leucospermum* cultivars are most susceptible.

Control - The susceptibility of the cultivar Gold Dust indicates that major differences in susceptibility exist among cultivars and that selection for resistance is possible (Von Broembsen, 1986). According to Von Broembsen (1986), the cultivars Luteum, Scarlet Ribbon, Vlam, Caroline, Sunrise and Goldie are highly tolerant to Drechslera blight in South Africa.

Control of pincushion blight by fungicides is effective during the early phases of the disease. Iprodione reduces disease severity in the field (Von Broembsen, 1986). Von Broembsen (1989) recommended applications of iprodione or chlorothalonil plus a benzimidazole fungicide every 2-3 weeks during growth periods and flower formation, and under warm, wet conditions. Shoots and flowers will be protected against infection. In Hawaii, mancozeb gives the best control of *D. biseptata*, followed by chlorothalonil, flusilazole and iprodione. Chlorothalonil and flusilazole, however, cause phytotoxicity in the form of leaf reddening and necrotic flecking on the leaves (Protea Disease Letter, 1993). Only propiconazole and prochloraz are effective against the disease in New Zealand. Two-weekly spraying of the fungicides is advised. Seeds are treated with imazalil or carboxin and thiram (Soteros, 1986).

Pre-planting dipping of the cuttings, followed by an effective spraying programme, can prevent disease development. Cuttings must always only be taken from disease-free mother

plants (Benic, 1986). Sanitation pruning should be carried out on all shoot cankers which have become dark and sunken (Coetzee *et al.*, 1988). Adequate air circulation around and through plants should be employed (Von Broembsen, 1989). Seed treatment is advisable since *D. dematioidea* can be seed-borne (Von Broembsen, 1986).

Notes - Drechslera blight is the most important disease of pincushions (*Leucospermum* spp.), particularly the cultivars developed from *Lsp. cordifolium*. Some *Leucadendron* and *Mimetes* Salisb. spp. are also susceptible to blight (Von Broembsen, 1989). The disease is caused by different *Drechslera* spp. in different protea producing regions. In South Africa and New Zealand, *D. dematioidea* is the causal pathogen. *Drechslera biseptata* causes a major disease of pincushions in the southern states of Australia and Queensland, while both species cause blight on pincushions in Hawaii (Boesewinkel, 1986; Von Broembsen, 1986, 1989; Protea Disease Letter, 1991, 1993; Forsberg, 1993). A *Drechslera* sp., distinct from the species recorded on Proteaceae in Australia and South Africa, was recorded from the North Island of New Zealand. The pathogen causes a leaf spot and blight disease (Soteros, 1986).

Drechslera blight occurs in the pincushion production areas of the SWCP, as well as on pincushions in nurseries and in their natural habitat. The presence of the pathogen on pincushions in their natural habitat suggests that it is indigenous to this region. Significant crop losses can result when blight occurs on flower heads or flower-bearing shoots (Von Broembsen, 1986).

Epidemic outbreaks of blight on pincushions occur primarily during the late winter to mid-spring. Under weather conditions characterised by frequent rainfall or heavy dew, morning fog, and moderate midday temperatures that can reach 20-25°C, symptoms develop rapidly and most plants in the field become diseased. Blight infection is most severe at sites that are low-lying, along rivers or the sea, on the shady sides of windbreaks, or in the morning shadows of mountains. Overhead irrigation, crowding and weed growth around the base of the plant also favour disease development (Von Broembsen, 1986, 1989; Forsberg, 1993).

Commercially cultivated pincushions seem to be more severely affected by Drechslera blight than pincushions in their natural habitat. Monoculture results in the crowding together of susceptible plants, compared to what would occur in nature. The use of vegetatively produced cultivars, which provides more uniform growth of susceptible shoots than with seed-grown plants, further enhances this effect. Harvesting of flowers causes pincushions to begin shoot

growth earlier than would occur in nature (G. Jacobs, pers. comm.), therefore shoot growth occurs during the period of optimum weather conditions for disease development. The practice of pruning one-year-old plants in August to force long shoot growth (Jacobs, 1983), also results in shoot growth during the period optimum for disease (Von Broembsen, 1986).

The fungus can be spread with seed, seedlings, cuttings and nursery plants, or from certain grasses and native plants (Von Broembsen, 1989). Spores are spread by wind and water-splash, while warm temperatures of 19-25°C and wet conditions favour spread and development of blight disease (Forsberg, 1993).

Elsinoë Racib., Parasit. Algen und Pilze Javas **1**: 14 (1900).

Anamorph - *Sphaceloma* De Bary, Annals of Oenologie **4**: 165 (1874).

Taxonomy - Ascocarps with asci and ascospores are seldom found in host tissue (Benic & Knox-Davies, 1983a). Ascocarps are dark brown to black, scattered, pulvinate, generally uniloculate, embedded in the cortical tissue, or superficially under the cuticle. Ascocarps are 38.6-129.6 x 68.3-77.8 mm. The ascocarps contain several subglobose to ellipsoidal asci, 20.4-26.6 x 15.5 mm. There are up to eight ascospores per ascus, hyaline, oblong-ellipsoidal, mostly muriform with three transverse septa, but sometimes dictyosporous with a single longitudinal septum, 11.7-14.5 x 5.0-6.2 µm (Benic & Knox-Davies, 1983a).

Hyaline or light brown acervular stromata are generally separate, sometimes confluent. Stromata are subcuticularly immersed in the host tissue and rupture the cuticle irregularly as they develop. Hyaline, setose structures are sometimes seen projecting from the stromata. No distinct conidiophores are observed, but hyaline, aseptate, ovoid-ellipsoid, smooth, thin-walled conidia may be present on material after wet weather (Benic & Knox-Davies, 1983a).

Clusters of uniform, spherical, hyaline cells are present in stromata in host tissue. They are frequently associated with microsclerotia, and it appears that they are developing microsclerotia. Dark chlamydospores and microsclerotia, consisting of clusters of two or more cells with dark, thickened walls, are frequently found on the surface of lesions, under the host cuticle or epidermis, and in acervuli. Hyaline conidia develop abundantly in Fries's medium. The conidia measure 8.1-10.5 x 5.1-6.8 mm (mean 9.5 x 5.9 mm) (Benic & Knox-Davies, 1983a). Conidiophores and conidia are rarely seen, but that is apparently not uncommon in

Sphaceloma (Sutton, 1980). Spermatia are common, ellipsoidal to rod-shaped, measure 2.8 x 1.7 mm, and are produced within stromata as though by dissolution of the tissue, or in distinct spermagonia. Protopseudothecia, sometimes with trichogyne-like structures, also occur (Benic & Knox-Davies, 1983a).

Symptoms - Scab symptoms vary considerably between the different genera. Distinctive white, scab-like lesions appear on the older leaves of *P. cynaroides*, while only restricted lesions are observed on the petioles. These are in direct contrast to the symptoms caused by pincushion scab. Symptoms on *Leucospermum* spp. only appear on the shoots, and leaf lesions are less prominent than the extensive stem lesions. Symptoms also vary between species and cultivars. In Zimbabwe and California, symptoms on *Protea* cultivars appear on the leaves, as well as on the stem. The stem lesions are similar to the scab lesions found on pincushions, but the lesions on the leaves differ considerably. Leaf lesions are small, circular, red-brown, eventually coalescing and killing the entire leaf. Scab disease on *Protea* in Zimbabwe is much more severe than on *P. cynaroides*, as well as on *Leucospermum* spp. in South Africa.

Elsinoë scab is characterised by scab-like lesions on stems, leaves and flower heads, and in many cases, twisting of the stems, rendering the cut-flower unmarketable or marketable at reduced prices (Ziehrl, Pascoe & Porter, 1995). Symptoms on pincushions appear on the young shoots of the current season. The first symptoms appear as small, elliptical, sunken, whitish lesions, normally accompanied by reddening of the surrounding tissue (Fig. 18) (Benic & Knox-Davies, 1983a; Von Broembsen, 1989; Forsberg, 1993). Lesions enlarge and coalesce to form the typical irregular, red-brown, raised, scab-like lesions which often lose the reddish colouring as they progress (Fig. 19) (Benic & Knox-Davies, 1983a; Ziehrl *et al.*, 1995). Dark fruiting bodies are sometimes scattered in these lesions.

The disease progresses to form large, dark, raised, roughened, often cracked areas with a corky appearance, which may eventually girdle the stem. Older lesions often cause localised splitting of the stem and heavy infection may cause abnormal growth and twisting of the stem (Fig. 20). Symptoms take between 3-5 wks to develop after infection has taken place. Flowering is reduced and in extreme cases shoot tips and leaves on infected shoots can be killed (Benic & Knox-Davies, 1983a; Forsberg, 1993; Ziehrl *et al.*, 1995). In South Africa, affected tissue is attacked by chewing insects (Benic & Knox-Davies, 1983a). The insect damage causes the

surrounding tissues to proliferate and take on a corky appearance, therefore the common name "corky bark" (Von Broembsen, 1989).

Leaf lesions are less prominent than the extensive stem lesions. They are initially transluscent, then dull, tan-coloured, raised, hard, rough and corky (Benic & Knox-Davies, 1983a). Benic (1986) also noticed scab on cuttings in a mistbed. The disease results when cutting material is collected from scab-infected mother plants and brought into the mistbed.

Host range and Geographic distribution - **Australia,** *Leucospermum* spp., *Leucadendron* spp., *P. compacta x susannae* cv Pink Ice (Forsberg, 1993; Pascoe, Ziehrl & Porter, 1995; Ziehrl *et al.*, 1995); **South Africa: Cape province,** *Leucospermum conocarpodendron, Lsp. cordifolium, Lsp. cordifolium x tottum* hybrid, *Lsp. lineare, Lsp. oleifolium* (Berg.) R. Br., *Lsp. reflexum, Lcd. gandogeri, Lcd. laureolum, Lcd. laureolum x salignum* cv Silvan Red, *Lcd. linifolium*, Lcd. macowanii, Lcd. orientale* I. Williams, *Lcd. platyspermum* R. Br., *Lcd. pubebracteolatum x chamalea*, Lcd. salicifolium, Lcd. salignum, Lcd. tinctum, P. cynaroides*, Serruria florida** (Benic & Knox-Davies, 1983a; Knox-Davies *et al.*, 1986; present study*); **USA: California,** *Lsp. cordifolium*, Lsp. cordifolium* cv Vlam*, *P. mundii**, **Hawaii,** *Lsp. conocarpodendron x cuneiforme* cv Hawaii Gold; *Lsp. cordifolium, Lsp. cordifolium* cv Coral, *Lsp. lineare x glabrum* cv Hybrid 24, *Lcd. salignum x laureolum* cv Safari Sunset, (Protea Disease Letter, 1991, 1993; present study*); **Zimbabwe,** *Lsp. glabrum x tottum* cv Scarlet Ribbon*, *Lsp. reflexum* cv Luteum*, *P. compacta x susannae* cv Pink Ice*, *P. eximia x susannae* cv Sylvia*, *P. laurifolia* cv Regal Mink*, *P. magnifica x susannae* cv Susara*, *P. neriifolia* cvs Moonshine and Silvertips*, *P. neriifolia* sp.* (present study*).

Species susceptibility - Different cultivars and seedlings differ greatly in susceptibility to scab. In Hawaii, cultivar Hawaii Gold appears to be moderately resistant to scab, while cultivar Coral is highly susceptible (Protea Disease Letter, 1991). A selection of *Lsp. saxosum,* identified as highly resistant to scab, has produced hybrid seedlings which appear to have inherited its resistance (Leonhardt, 1998.). In South Africa, *Lsp. cordifolium* cv Red Sunset, cv Gold Dust (Knox-Davies *et al.*, 1986) and cv Vlam are extremely susceptible. The occurrence of scab on *Protea* cv Pink Ice is the first record of Elsinoë scab on *Protea* (Ziehrl *et al.*, 1995). This, together with the record of scab on *P. cynaroides* in South Africa, and the record of scab on various *Protea* cultivars in Zimbabwe, indicate that all Proteaceae are potentially at some risk from this disease.

Economic importance - In a survey conducted on scab disease of Proteaceae in Australia, scab disease was shown to be the most important disease problem to both plantation and nursery growers, especially those in Victoria, where the disease is very severe (Ziehrl *et al.*, 1995). Furthermore, the disease has also been found in a number of wholesale nurseries that provide the majority of their plants to plantation growers, frequently interstate. The disease thus has the potential to continue to spread throughout the industry (Pascoe *et al.*, 1995). According to a survey held by the Institute for Horticultural Development in Australia in 1994, the disease affected 31% of the area used for the cultivation of South African Proteaceae in Australia. This represented an average loss of 52% of potential revenue in affected crops, namely $ 602 416 and $ 52 1006 per annum for plantation and nursery growers, respectively (Ziehrl *et al.*, 1995). As no similar surveys have, however, been undertaken in California, Hawaii, South Africa or Zimbabwe, the wider impact of this disease complex remains to be determined.

Favourable conditions for infection - Clusters of infection loci, separated by healthy, uninfected tissue, suggest that infection only takes place at certain times in a growing season when spore production, spore dispersal and infection of host tissue are favoured. It seems that only young, actively growing tissue is susceptible. Infection therefore only occurs during periods of active growth (Ziehrl *et al.*, 1995). Young *Lsp. cordifolium* plants grow vigorously during spring and early summer and will therefore be most susceptible to scab during this period (Benic & Knox-Davies, 1983a). Older plants flower under natural conditions from August to January in South Africa (G. Malan, pers. comm). According to Jacobs (1983), vigorous shoot development occurs after abscission of the flower heads (October to March), with a shorter flush period (till December) in older and stressed plants. Plants that have reached the flowering stage will therefore be most susceptible to scab during the summer months (Benic & Knox-Davies, 1983a).

Although moisture favours infection, optimum conditions of humidity, temperature or duration of moist conditions are not known (Benic & Knox-Davies, 1983a; Ziehrl *et al.*, 1995). Shaded plants are more subject to scab than those in exposed sites. This is also prominent on the same plant, where the shaded side is usually more severely affected (Benic & Knox-Davies, 1983a).

Survival - Little is known of the survival strategies of the pincushion scab fungus, however, other species of *Elsinoë/Sphaceloma* survive as mycelium and spores in old scab lesions (Peltier & Frederich, 1926; Bitancourt & Jenkins, 1937). It is possible that this fungus adopts the same

survival strategies. Furthermore, Benic & Knox-Davies (1983a) also found *E. leucospermi* to form microsclerotia within and on the surface of scab lesions from late summer until the following spring, suggesting that it survives mainly in scab lesions as resting mycelium, stromatic tissue, and as chlamydospores and microsclerotia outside the tissue. The possible role of conidia and ascospores as survival propagules is probably of less importance due to the extremely hot and dry summer climate (Benic & Knox-Davies, 1983a). Finally, Von Broembsen (1989) also noted that some shoot infections were latent and only developed further once plants were stressed.

Transmission - The disease spreads relatively slowly in the field (Von Broembsen, 1989). *Elsinoë* spp. have been reported to spread by wind, rain-splash, dew, overhead sprinkler irrigation and other spraying operations (Fawcett, 1936; Bitancourt & Jenkins, 1937; Whiteside, 1975; Von Broembsen, 1989; Forsberg, 1993). Insect dissemination is also possible (Jenkins, 1930; Forsberg, 1993) since scab lesions and fungal structures are very often attacked by chewing insects. Various small insects, their larvae and mites also commonly occur on scab lesions. These insects and the larger chewing insects possible play a role in dissemination of the fungus (Benic & Knox-Davies, 1983a). In accordance with Jenkins (1930) who isolated *E. fawcetti* Bitancourt & Jenkins from insect faeces, cultures of *E. leucospermi* have also been revived from insect faeces on *Leucospermum* spp. (L. Benic, ARC-Fruit, Wine and Vine Research Institute, Stellenbosch, pers. comm.). Ziehrl *et al.* (1995) stated that infected plant material often serves as an inoculum source for new infections. It is often the smaller shoots that were not harvested the previous season, that harbour the fungus.

Control - Benic & Knox-Davies (1983a) recommended that young plants must be sprayed while they are actively growing, and moisture and temperature conditions favour disease development. Prochloraz sprayed during growth flushes gives good protection (Von Broembsen, 1989). Mature plants must be sprayed immediately after flowering or after flowers are cut. It may be necessary to spray plants more than once or twice if the flowers are harvested intensively over an extended period. Contact fungicides (e.g. captafol, mancozeb) or systemics (e.g. benomyl) can be used, although systemics will probably be more effective, particularly in plantations of non-clonal material in which flowering and growth flushes are not uniform (Benic & Knox-Davies, 1983a).

Benic (1986) reported that difolitan, captan and benomyl can be used to spray cutting material in mistbeds, with difolitan being particularly effective for the control of scab. Several different fungicides and various control methods are used by growers in Australia in an attempt to control Elsinoë scab. Chemical control of the disease has, however, proven to be difficult due to the lack of registered fungicides for use on Proteaceae for Elsinoë scab, and lack of knowledge of efficacy of fungicides. Several fungicides have been listed by growers in Australia for the control of scab, namely prochloraz, chlorothalonil, propiconazole, phosphorous acid and benomyl. There is, however, some controversy regarding the efficacy of these chemicals (Ziehrl *et al.*, 1995), suggesting that further screening would be required to solve this problem. The Protea Disease Management Group (Protea Disease Letter, 1993) conducted fungicide experiments on potted *Lsp. cordifolium x lineare* cv Red Sunset in Hawaii. They reported that at maximum recommended rates, chlorothalonil (2.5 ml/l) gave excellent control, followed by benomyl, flusilazole, prochloraz, mancozeb and iprodione. Phytotoxicity, seen by the reddening of the leaves was, however, observed with chlorothalonil and flusilazole. Fungicidal control of Elsinoë scab is poor, and sanitation pruning is essential if scab is to be controlled. In spring and early summer, lesions are often inconspicuous and difficult to detect, but typical scab lesions should be easily seen by late summer. Sanitation pruning should be carried out at this stage (Coetzee *et al.*, 1988).

Cultivars should be selected for tolerance against scab in control programmes. Nursery quarantine, and sanitation in the nursery and orchard must be implemented, especially when working with diseased plant material. Minimal overhead irrigation must be given since the fungus can possibly spread with splashing water. Plants must be established on sites with good air ventilation. Growers introducing new planting stock from any source should quarantine that stock for six months. Since it is not possible to visually detect latent infections, cuttings should be taken from mother plants which have no record of scab disease (Benic & Knox-Davies, 1983a; Von Broembsen, 1989; Ziehrl *et al.*, 1995).

Notes - *Elsinoë* spp. have been associated with scab disease symptoms of South African Proteaceae, and are common on genera such as *Leucospermum*, *Leucadendron*, *Serruria* and *Mimetes cucullata* R. Br. (Benic & Knox-Davies, 1983a; Pascoe *et al.*, 1995; Ziehrl *et al.*, 1995). Furthermore, the disease has also been observed on a species of *Protea* in Australia (Ziehrl *et al.*,

1995), as well as on *P. cynaroides* in South Africa, on *P. mundii* in California and on various *Protea* cultivars in Zimbabwe.

Scab disease of Proteaceae in South Africa was first discovered in 1981. The disease, locally known as corky bark or scab, is associated with severe losses to commercial pincushion plantings (*Leucospermum* spp.). The causal agent, which was identified as a species of *Elsinoë*, was never formally described (Benic & Knox-Davies, 1983a). Scab is also regarded as one of the most important diseases occurring on *Leucospermum* spp. in Hawaii (Protea Disease Letter, 1991) and has also been observed on pincushions in California and Zimbabwe. In Australia, scab disease of Proteaceae was initially observed in Victoria in 1985, and has subsequently also been recorded from all the eastern states (Ziehrl *et al.*, 1995). Although similar reports have been made from Western Australia, these still await confirmation (Pascoe *et al.*, 1995).

Rhizopus Ehrenb. sp.

Host range and Geographic distribution - **South Africa: Cape Province,** *Leucospermum cordifolium*, *Leucospermum* spp. (Jacobs, 1981; Knox-Davies *et al.*, 1986).

Control - Disease development can be prevented by allowing flowers to dry off and harden either in the field in full sun or if cut, with their stems in the water. Flowers can be sprayed with or dipped in a solution of dicloran and benomyl and dried thoroughly before being packed (Jacobs, 1981; Knox-Davies *et al.*, 1986).

Notes - A *Rhizopus* sp., in association with *B. cinerea*, causes postharvest infection of flower heads when the flowers are picked after a rainy period (Jacobs, 1981; Knox-Davies *et al.*, 1986).

Table 3. Pathogens of minor importance

Pathogen	Geographic Distribution	Hosts	References
Chondrostereum purpureum (Pers.: Fr.) Pouzar (silver leaf)	New Zealand	*Leucadendron* spp.	(Von Broembsen, 1989;
		Protea spp.	Forsberg, 1993)
Pestalotia De Not. sp. (dieback)	South Africa	Diseased cuttings	(Benic, 1986)
Schizophyllum commune Fr.: Fr. (trunk rot)	South Africa	*Lcd. argenteum*	(Doidge, 1950; Doidge *et al.*, 1953; Knox-Davies *et al.*, 1987)
		P. repens	(S. Denman, pers. comm.)
Sclerotinia Fuckel sp. (dieback)	South Africa	Diseased cuttings	(Benic, 1986)

PATHOGENS OF WOODY STEMS

Cankers and dieback from injured tissue are a common problem on all Proteaceae wherever they are cultivated. Stem cankers are typically sunken and darker in colour than the surrounding tissue. A distinct margin can always be seen between the healthy and diseased tissue. The underlying woody tissues are discoloured and can spread some distance beyond the injury site. The disease develops slowly, or extremely rapidly, depending on the stress conditions, eventually killing the entire plant. The canker fungi enter through wounds, leaf scars and lesions of other stem diseases. Wet conditions and wind are favourable for the spread of these fungi, but they can also be spread by contaminated pruning and harvesting tools, in seeds, in soil or plant debris, or by insects (Von Broembsen, 1989; Forsberg, 1993).

Botryosphaeria dothidea (Moug.: Fr.) Ces. & De Not., Commentario della Societa Crittogamologica Italiana **1**: 211 (1863).

Botryosphaeria ribis (Tode: Fr.) Grossenb. & Duggar, New York Agricultural Experiment Station Geneva Technical Bulletin **18**: 128 (1911).

Anamorph – Fusicoccum aesculi Corda, Deutschlands Flora **2**: 111 (1829).

Taxonomy - Although the name of the anamorph state of this pathogen is still debatable, *Fusicoccum* Corda is considered to be the anamorph genus of *B. dothidea* (Moug.: Fr.) Ces. & De Not. by some workers (Punithalingam & Holliday, 1973; Sutton, 1980; Morgan-Jones & White, 1987; Rumbos, 1987). Sutton (1980) and Pennycook and Samuels (1985) considered the fungus called *F. aesculi* Corda by Saccardo to be the anamorph of *B. dothidea*.

Some workers considered *Dothiorella* Sacc. as the imperfect stage of *Botryosphaeria dothidea* (Wolf & Wolf, 1939; Von Arx & Müller, 1954; English, Davis & De Vay, 1975; Shanin & Claflin, 1980; Latorre & Toledo, 1984). In many cases, the conidial state has been described as *Dothiorella gregaria* Sacc. (Wiehe, 1952). This is very similar to *Fusicoccum tingens* Goid., the conidial state of *B. ribis*, causing a dieback of pines in East Africa (Ivory, 1967). Sutton (1980) was of the opinion that *Dothiorella* Sacc. can be taken up in the genus *Fusicoccum*. Grossenbacher and Duggar (1911) recognised two anamorphs, a *Macrophoma* (Sacc.) Berl. & Voglino form and a *Dothiorella* form, as well as a strongly parasitic and saprophytic strain of *B. ribis*.

Typically, only the anamorph is found in the field and in culture, and the fungus is identified on this basis (Satour, Webster & Hewitt, 1969; Latorre & Toledo, 1984). The anamorph-teleomorph connection has been demonstrated on hosts where the teleomorph does occur, e.g. apple (Shear, Stevens & Wilcox, 1924) and willow (Wolf & Wolf, 1939), but this has not been possible in most cases. Correct identification of a pathogen as *B. dothidea* in the absence of the teleomorph can be problematic (Worrall, Correll & McCain, 1986).

Descriptions of conidia and conidiomata of *B. dothidea* vary considerably (Wolf & Wolf, 1939; Punithalingam & Holliday, 1973; Sutton, 1980). Pennycook and Samuels (1985) considered *B. dothidea sensu lato* to be a widespread species complex.

Botryosphaeria dothidea isolated from *Protea minor* has definite pseudoparaphyses in the pseudothecium. The conidium ontogeny is holoblastic, but also reveals a phialidic ontogeny that occurs simultaneously with the holoblastic ontogeny in the same conidioma (Cesare, 1982).

Ascostromata in stems are embedded in the cortex, becoming erumpent, sub-pulvinate, scattered, solitary, botryose, black, up to 4 mm wide, with individual ascogenous locules 170-250 µm diam., ostiolate and darker around the neck. Asci interspersed amongst filiform paraphyses, clavate, 100-110 x 16-20 µm, 8 spored, bitunicate. Ascospores irregularly biseriate, hyaline, unicellular, ovoid, 17-23 x 7-10 µm. Pycnidia on stems and leaves solitary or botryose, stromatic, globose, 150-250 µm diam., with papillate ostioles darker around the neck. Pycnidial wall composed of many cells, outer sclerotised cells and inner thin-walled cells, lining the entire cavity. Conidiogenous cells holoblastic, hyaline, arising from the inner lining of the pycnidial cavity. Macroconidia hyaline, fusoid, 17-25 x 5-7 µm. Microconidia (spermatia) hyaline, allantoid, 2-3 x 1 µm (Punithalingham & Holliday, 1973).

Symptoms - Cankers caused by *B. dothidea* are dark brown-black and sunken (Fig. 21) (Von Broembsen, 1986). Once the fungus invades the vascular tissue, the mycelium moves rapidly down the stem. Frequently, only one side of an affected branch shows the brown discoloration (Milholland, 1972).

The main characteristics of Botryosphaeria disease of silver tree (*Lcd. argenteum*) appear to be the killing of areas of bark, and a sudden wilt and death of branches above the site of infection. Sometimes the entire tree can die. Infection may take place through wounds, or the fungus may infect the young growing tips and cause a dieback of the shoot. After growing down the infected branch and reaching the trunk, it may cause a blighting of the rest of the branches above the point of infection. The growth of the fungus through tissue can be very rapid. A rate of 2 cm a day has been recorded. It is possible that infection takes place above soil level, and that the fungus grows simultaneously down into the roots and up through the stem (Olivier, 1951).

Benic (1986) isolated *Botryosphaeria* spp. from all sources of propagation material in the nursery bed and mistbed. Disease symptoms varied from seedling dieback or blight to stem cankers. Basal and tip dieback and leaf necrosis were the typical symptoms encountered in mistbeds.

Widespread and destructive necrotic lesions, associated with leaf spots caused by *C. senniana* on *P. magnifica*, are caused by secondary invasion of the Coleroa leaf spots by

Fusicoccum aesculi (Serfontein & Knox-Davies, 1990a). *Fusicoccum aesculi* assumably gains entry when the *C. senniana* pseudothecia burst through the cuticle, therefore necrosis is generally restricted to the side of the leaf with the pseudothecia. Sometimes necrosis extends through the leaf. The necrotic leaf spots are light brown with darker margins. Lesions can coalesce and extend beyond the area originally occupied by *C. senniana* (Serfontein & Knox-Davies, 1990a).

Host range and Geographic distribution (B. dothidea/B. ribis) **- South Africa: Cape province,** *Leucospermum cordifolium, Lsp. cordifolium* cv Flamespike, *Lsp. lineare x cordifolium* cv Succession, *Lcd. argenteum, Lcd. laureolum, Lcd. spissifolium, P. compacta, P. cynaroides, P. eximia, P. grandiceps, P. repens** (Oliver, 1951; Van Wyk, 1973a, b; Knox-Davies *et al.*, 1981; Benic & Knox-Davies, 1983b; Benic, 1986; Von Broembsen, 1986); **Gauteng, Brits,** *P. repens** (present study*).

Botryosphaeria Ces. & De Not. sp. (with *Sphaeropsis* anamorph) - **South Africa: Cape province,** *Protea longifolia, P. punctata* (Knox-Davies *et al.*, 1981).

Botryopshaeria sp. (without conidial stage, and differing from *Botryopshaeria dothidea* in ascospore size and shape): - **South Africa: Cape province,** *Leucospermum conocarpodendron, Lsp. cordifolium, P. cynaroides, P. neriifolia, P. punctata* (Knox-Davies *et al.*, 1981).

Dothiorella sp.: - **USA: Hawaii,** *Leucadendron laureolum, P. cynaroides* (Protea Disease Letter, 1991).

Botryosphaeria proteae and *Fusicoccum* anamorph - **Australia,** *Leucospermum, Leucadendron* and *Protea* spp. (Forsberg, 1993); **USA: California,** *Leucadendron discolor**, *Lcd. laureolum x salignum* cv Silvan Red*, *P. cynaroides**, *P. eximia**, *P. laurifolia**, *P. neriifolia**, *P. obtusifolia**, *P. scolymocephala** (present study*).

Host range **-** *Botryosphaeria ribis* is a cosmopolitan fungus with a wide distribution and is found in many of the temperate and subtropical countries (Punithalingam & Holliday, 1973). It has a wide host range, which includes at least 34 plant genera and 20 plant families (Smith, 1934).

The pathogen causes stem, trunk and branch canker on a wide range of woody plants such as *Grevillea* (Schieber & Zentmeyer, 1978), *Rhododendron* (Schreiber, 1964), *Macadamia* (Zentmeyer & Storey, 1965; Herbert & Grech, 1985), almonds (English *et al.*, 1975), apple trees (Latorre & Toledo, 1984), peach trees (Reilly & Okie, 1982) and *Eucalyptus* (Davison & Tay, 1983; Shearer, Tippett & Bartle, 1987). *Botryosphaeria ribis* has also been recorded as an

apparent saprophyte on the leaves of *Eucalyptus* in South Africa (Crous, Knox-Davies & Wingfield, 1989). The fungus, however, can cause a serious twig blight of Douglas-Fir and Arizona cypress (Luttrell, Davis & Murray, 1962; Shanin & Claflin, 1980). *Botryosphaeria dothidea* as a pathogen, is generally associated with branch and trunk cankers, but Hodges (1983) found that the fungus infects pine roots in Hawaii directly from the soil. *Botryosphaeria dothidea* was viewed as an opportunistic pathogen that infects trees only under favourable conditions. In contrast to these findings, Smith *et al.* (1996) showed that the fungus exists as a symptomless endophyte in healthy eucalypt trees.

Wound pathogen - The fungus is considered to be primarily a wound pathogen (Smith, 1934; Wiehe, 1952; Witcher & Clayton, 1963), especially of stressed plants (Crist & Schoeneweiss, 1975; Spiers, 1977; Schoeneweiss, 1981). The fungus penetrating through wounds, causes the perennial dieback and canker disease on *Rhododendron*. Wounds decrease in susceptibility with increasing age (Schreiber, 1964). The fungus has been reported to enter through natural growth cracks in almond (English *et al.*, 1975), stomata in blueberry (Milholland, 1972) and lenticels in peach (Weaver, 1974). However, Luttrell (1950), Milholland (1972) and Shanin and Claflin (1980) reported that *B. dothidea* can also invade unwounded elm, blueberry stems and Douglas-Fir, respectively. Milholland (1972) found that penetration of unwounded blueberry stems by *D. dothidea* occurs through open stomata. Natural infection of almond by *B. dothidea* appears to be through cortical growth cracks (English *et al.*, 1975). Brown and Hendrix (1981) reported that although wounded apple trees inoculated with *B. dothidea* showed disease symptoms more frequently than unwounded ones, wounding is not a prerequisite for infection.

Pathogen of Proteaceae – The fungus has been isolated form the leaves and stems of proteas (Protea Disease Letter, 1991) and occurs throughout the Cape province (Crous *et al.*, 1989). The stem cankers and dieback caused by the fungus, cause considerable losses in the production of cut-flower proteas in South Africa (Von Broembsen & Van der Merwe, 1990). *Botryosphaeria* causes canker and tip blight of proteas in Hawaii (Greenhalgh, 1981). *Botryosphaeria* spp. seem to be extremely successful opportunistic colonisers of protea shoots, flowers and seed, while environmental stress factors possibly increase the susceptibility of host tissue to canker and dieback (Knox-Davies *et al.*, 1986).

According to Knox-Davies *et al.* (1981), a variety of different *Botryosphaeria* spp. (some with *Dothiorella* anamorphs) have been isolated from shoot lesions, seed heads and seeds of

different proteas. Knox-Davies *et al.* (1981) examined 14 *Botryosphaeria* isolates, of which 10 were from twigs and leaves of indigenous Proteaceae. Three distinct groups of isolates were recognised on the basis of growth rate, optimum growth temperature, ascospore size and shape, and conidia. Only one group, with a *Dothiorella* anamorph, was typical of *B. dothidea*. It is not known whether any of these isolates were pathogenic to the hosts from which they were isolated, because no infection studies were done. Knox-Davies *et al.* (1981) suggested that the great variation in *Botryosphaeria* isolated from the SWCP points to the existence of a large gene pool of *Botryosphaeria* in this area.

Benic and Knox-Davies (1983b) frequently isolated *Botryosphaeria* from anthracnose lesions on *P. compacta* plants and suggested that it is a secondary invader of the canker and dieback disease of mature *P. compacta*, colonising tissue after infection by *Colletotrichum gloeosporioides*. Von Broembsen (1986) reported *B. dothidea* to be a wound pathogen of pincushions and an important secondary pathogen of the stem cankers caused by *Drechslera dematioidea*. Studies indicated that natural field inoculum levels were high. Orffer and Knox-Davies (1989) isolated a *Botryosphaeria* sp. together with a *Phomopsis* (Sacc.) Sacc. sp. from about a third of canker and dieback lesions on *P. repens* and *P. obtusifolia*.

Oliver (1951) inoculated *Lcd. argenteum* with *B. ribis* and concluded that *Botryosphaeria* is the primary cause of the silver tree disease. However, Oliver's (1951) work was inconclusive in that inoculations of fully-grown silver trees with *B. ribis* consistently failed to induce the disease. Van Wyk (1973a, b) inoculated *Lcd. argenteum* with *B. ribis* and *P. cinnamomi* and came to the conclusion that *B. ribis* causes branch dieback, whereas *P. cinnamomi* is the primary pathogen leading to tree death. The imperfect stage of *B. ribis*, corresponding with a *Dothiorella* sp., was isolated from lesions around cracks and from shoots showing dieback. It was also occasionally associated with *P. cinnamomi* in rotted crown tissues, especially in advanced stages of decay (Benic, 1986; Von Broembsen, 1986).

Aerially dispersed canker-causing fungi, including *Botryosphaeria* and *Diplodina* spp. have caused extensive damage to Proteaceae in the south-coastal areas of Western Australia. These fungi have a very broad host range. About 86% of the Proteaceae were damaged and often killed. The level of tolerance to the cankers varied between species. *Banksia coccinea* R. Br. and *B. baxteri* R. Br. are both highly susceptible to diseases caused by canker fungi. The canker fungi have the potential to cause serious damage in this region of Australia, and currently, the

only practical way of regenerating plant communities after infestation by canker, is fire (Lamont *et al.*, 1995).

The role of environmental stress - Although many isolates of *B. dothidea* are virulent pathogens on a wide range of host species (Smith, 1934), others cause cankers only on hosts that are weakened or stressed (Hutton, 1958; Toole, 1963; Schoeneweiss, 1965; Crist & Schoeneweiss, 1975; Neely, 1968). Stress can be an important factor affecting host susceptibility to infection by pathogens (Schoeneweiss, 1975; Wene, 1979). Organisms that attack plants predisposed by stress are often non-aggressive pathogens that enter plants through wounds or natural openings, but do not cause disease as long as host vigour is high. Many of these organisms are common saprophytes of dead and dying plant tissue, but have the ability to attack weakened or stressed plants (Wene, 1979; Schoeneweiss, 1981).

Certain plant diseases, particularly stem cankers, crown cankers and diebacks, are most prevalent on plants subjected to environmental stress (Schoeneweiss, 1981). *Botryosphaeria dothidea* may remain viable for many weeks in wounds on vigorous stems, without extensive colonisation, until plants are exposed to stress (Crist & Schoeneweiss, 1975). Fusicoccum canker of mountain ash trees did not develop as long as the inoculated trees were kept in a vigorous growing condition. When the trees were placed under stress, cankers developed rapidly (Schoeneweiss, 1965). This indicates a latent type of infection.

Drought and nutrition have been implicated as factors favouring *B. ribis* infection of *Macadamia* (Herbert & Grech, 1985) and pines (Ivory, 1967; Hodges, 1983). Wene (1979) found that colonisation of stems of woody plants are significant only in stems with plant water potentials lower than -12 bars. Following two dry years in South Africa, a severe branch dieback of macadamias was induced in all major production areas by *B. ribis*. It was indicated that water stress increased the susceptibility of these trees (Herbert & Grech, 1985). Drought and freezing appeared to be the most common stress factors predisposing woody plants to disease (Crist & Schoeneweiss, 1975).

Predisposition, caused by short-term droughts, is reversible, as cankers caused by non-aggressive pathogens often heal after irrigation is resumed. Prolonged drought over several consecutive years of below-normal precipitation, however, may result in irreversible predisposition (Crist & Schoeneweiss, 1975; Schoeneweiss, 1981; Hodges, 1983). Fungal colonisation is more drastic when plants are subjected to longer periods of water stress. Field-

grown plants are usually less subject to rapid depletion of soil moisture and may be below the threshold level of susceptibility for longer periods of time before signs of water stress appear. This may explain the sudden appearance of stem cankers on field-grown plants which have not displayed visible signs of water stress (Wene, 1979).

After an extensive drop in temperature during fall or winter, outbreaks of stem canker and dieback appear. The threshold temperatures required for predisposition are mostly between -20 and -30°C. The predisposing effects of freezing near the threshold are reversible, and stem cankers heal when growth resumes, but it may become irreversible when injury is severe (Schoeneweiss, 1981). The temperature required to predispose seedlings varies with the degree of cold hardiness the plants have acquired prior to exposure. The predisposing effect is not increased by the lengthening of the exposure period. The effect of freezing is a localised event. *Botryosphaeria dothidea* colonises only those portions of the plant exposed to freezing temperatures even though adjacent to unfrozen portions (Wene, 1979). Severely defoliated plants seem to be more susceptible to attack by canker and dieback pathogens (Wene, 1979; Schoeneweiss, 1981).

Bark and wood tissue near the cambium are extensively colonised in stems stressed by drought or defoliation. If the canker is expanding, the pathogen can often be isolated from bark and young wood beyond the canker margins, with much less colonisation in older wood. However, in stems stressed by freezing, the oldest wood tissue surrounding the pith is colonised, often for a considerable distance beyond the canker margins. Water stress usually predisposes the entire plant, including the root system, to attack by non-aggressive pathogens. Freezing stress, however, predisposes only that portion subjected to temperatures below the threshold temperature. Maintaining a high level of vigour and hardiness, is the most effective means of preventing disease predisposition by environmental stress (Schoeneweiss, 1981).

Control - All practices designed to maintain the tree in a vigorous condition must be implemented (Schoeneweiss, 1965). Overwatering of cuttings, soggy planting media, wounding and drought stress due to cuttings drying out, are all factors favouring infection (Benic, 1986). The fungus can spread from diseased to healthy plants by the use of contaminated pruning tools (Schreiber, 1964). Pruning should also be avoided during wet weather conditions (Schoeneweiss, 1965). Unnecessary wounding of plants should be avoided, and harvesting and pruning wounds

should be treated with a fungicidal spray or wound sealant (Von Broembsen & Van der Merwe, 1990).

The single, most effective control measure is pruning (Olivier, 1951; Von Broembsen & Van der Merwe, 1990). Sanitation practices reduce the levels of conidia and ascospores which can serve as a source of infection during the growing periods (Sutton, 1981). Dying trees should be cut down as soon as possible after the first signs of wilting appear, as pycnidia soon appear on the dead tissue. No stump should be left above ground level. The trunk should be removed and burned, as fruiting bodies will form and discharge their spores on any fragments of the dead tree left lying about. These measures will not necessary control the disease, but it will help to keep down the number of new infections (Olivier, 1951).

Dead wood left in or near orchards, is a major source of *Botryosphaeria* inoculum (Pusey, 1989). Natural infection under field conditions probably results from conidia that are produced in large numbers on dead stems (Witcher & Clayton, 1963). Benic and Knox-Davies (1983b) suggested that *Botryosphaeria* inoculum could be reduced by burning plantations as they become unproductive. Since *B. ribis* has such a wide host range, other infected nursery plants may serve as sources of inoculum for infection.

The effectiveness of protectant sprays in excluding the pathogen from healthy plants is limited by the long period of wound susceptibility (Schreiber, 1964). Schoeneweiss (1979) protected dogwood plants against stress predisposition to Botryosphaeria canker by soil injection with benomyl. Herbert and Grech (1985) found that the fungus is sensitive to benomyl. Pre-planting dipping of cuttings followed by regular sprays with fungicides such as difolitan, captan and benomyl can help to prevent cutting disease from developing in mistbeds (Benic, 1986). Dieback of proteas has been controlled to some extent by regular foliage applications of captafol, captab and mancozeb (Benic & Knox-Davies, 1983b). Since the fungus is also isolated from seeds, seed treatment appears to be advisable (Von Broembsen, 1986). It was also indicated that a spray programme of fungicides in combination with pesticides, gave better control than the fungicides alone. This supports the view that cankers are often associated with insect wounds (Von Broembsen & Van der Merwe, 1990).

Notes - Some authors (Punithalingam & Holliday, 1973; Davison & Tay, 1983; Webb, 1983) considered *B. ribis* to be distinct from *B. dothidea*, while others treated it as a synonym (Latorre & Toledo, 1984; Maas & Uecker, 1984). According to Von Arx and Müller (1954),

Botryosphaeria mali Putterill, *B. ribis* and *B. berengeriana* De Not. are synonyms of *B. dothidea*. Although Knox-Davies *et al.* (1981) considered several *Botryosphaeria* spp. to be present on Proteaceae, the synonymy proposed by Von Arx and Müller (1954) will be followed until more data becomes available.

Botryosphaeria dothidea grows under a wide range of temperatures (Brown & Hendrix, 1981). Optimum temperature for fungal growth *in vitro* is 28°C, but good growth is obtained at 36°C and slight growth at 38°C (Weaver, 1974). The minimum, optimum and maximum temperatures for growth and conidial production in culture of *B. dothidea* are 10, 28 and 32-35°C, respectively (Witcher & Clayton, 1963).

Botryosphaeria dothidea predominates in summer and fall (Pusey, 1989). Warm, wet weather favours the release and germination of conidia (Weaver, 1979; Pusey, 1989). White spore masses, containing conidia, exude from the pycnidia after rainy periods (Crist & Schoeneweiss, 1975). Optimum sporulation occurs at 25-30°C in 100% RH (Eid & Heuberger, 1959). Ascospores are also more abundant during the summer (Sutton, 1981) and spring (Pusey, 1989). Rainfall triggers the discharge of ascospores and conidia and is the most important mechanism for conidial dispersal. Conidia are primarily dispersed in rain splash. Ascospores are air- and water-borne, therefore conidia may be more important in intra-tree spread, while air-borne ascospores may be more important in inter-tree spread (Sutton, 1981; Pusey, 1989).

More inoculum is produced on newly infected trees than on trees that are diseased for several years. Because spore release and infection by *B. dothidea* may occur over a long period, a programme to control the disease with fungicides may last for several months of the year (Weaver, 1979). Studies performed by Von Broembsen and Van der Merwe (1990) on *P. grandiceps*, indicated that sporulation of *B. dothidea* occurs from spring to late summer following rain. Sporulation is negligible during the winter months despite the abundant rainfall. Average daily temperatures above 20°C and rainfall are the prominent factors affecting sporulation. Wounding is necessary for infection and infection is greatest at 25-30°C (Von Broembsen & Van der Merwe, 1990).

The mycelium of the fungus is found in the lumen of cells (largely intracellular) in both xylem and phloem, and are passed from cell to cell through pits. Some branching into adjacent parenchyma cells is evident (English *et al.*, 1975; Wene, 1979). The hyphae of water-stressed plants occur more often in the youngest xylem vessels; colonisation of seedlings subjected to

freezing stress, occur mainly in the older xylem. Once girdling cankers begin to form, hyphae grow throughout all woody tissue (cambium and phloem). The pathogen does not degrade the cell walls (Wene, 1979). The size of the bark cankers is not a good indication of internal colonisation. Viable mycelium of the pathogen is often present in xylem some distance beyond the bark canker margin (English *et al.*, 1975; Wene, 1979). Bark cankers are smaller than the internal colonisation (Wene, 1979). Milholland (1972) and Wene (1979) found that tyloses formed in the xylem vessels of infected stems, while protrusions were also observed in the xylem which lead to complete occlusion of the vessels.

Botryosphaeria cankers of almonds develop rapidly in spring, summer and early fall, but progress is halted in late fall or early winter. Field observations indicate that most cankers appear to be annual rather than perennial (English *et al.*, 1975), but some cankers on other hosts (Schreiber, 1964) seem to enlarge year after year. *Botryosphaeria ribis* is not normally a soil-inhabiting fungus, and is not capable of surviving in the soil for long periods of time (Olivier, 1951).

Phoma Sacc. spp.

Notes - In Hawaii, a disease that is of minor importance causes leaf spots, looking quite similar to *Botrytis*, and marginal leaf scorch. The leaf spot occurs when flower removal appears to allow entrance of a pathogen into the stem, moving down the stem, causing a canker and death of the leaves. A *Phoma* has been the major pathogen isolated from both types of diseased material. Whether this fungus is the cause of the disease, is uncertain (Rohrbach, 1983). Benic (1986) isolated *Phoma* spp. from seed of different species of Proteaceae, but not from damped-off seedlings.

Phoma sorghina (Sacc.) Boerema, Dorenb. & Kesteren, Persoonia 7: 134 (1973).

Taxonomy - The fungus produces abundant aerial mycelium on agar (Gorter, 1976). Pycnidia are scattered in spots, up to 140 µm broad (Sivanesan, 1984). Simple chlamydospores and dictyospores are formed in older cultures with cells that are typically wider than long. They are mainly intercalary (Gorter, 1976; Sivanesan, 1984). Chlamydospores are single-celled or tend to form strands, but formation is variable (Sutton, 1980). Conidiogenous cells are hyaline, short,

obpyriform, arising from the cells lining the inside of the pycnidium. Conidia are hyaline, non-septate, ellipsoid, usually biguttulate, 5.8 x 2-2.5 µm. Conidia may become 1-septate at germination (Sivanesan, 1984). Sutton (1980) measured conidia 4-5 x 2-2.5 µm, being ellipsoid and eguttulate.

Symptoms - The disease is characterised by a necrotic browning of the stems of young plants from soil level to about 30 cm high. The first symptoms are a few small, dark red-brown spots on the stem. These spots enlarge from pinhead size, coalesce and cover the entire stem. Continuous red-brown stem discoloration (bark rot) can reach a length of about 7 cm. Growth of the plants with dark brown girdled stems is severely restricted. With time, longitudinal and transverse cracks appear in the necrotic tissue to expose the wood. The cracks can also develop into cankers and the disease can spread to the axillary parts of the lowest leaves, especially on the underside. Spots on the leaves are seldom noticed because they remain small. The necrotic stem tissue detaches from the underlying wood and changes from brown to grey. No fructifications have been found in these lesions, but small structures, which look like fruiting bodies to the naked eye, are visible on the surface (Gorter, 1976).

Host range and Geographic distribution - **South Africa: Transvaal,** *Leucospermum cordifolium* (Gorter, 1976).

Notes - The pathogen causes brown stem or stem canker of *Lsp. cordifolium*. The disease was first noticed in 1972 in protea nurseries in the Transvaal (Gorter, 1976) and has not been recorded in the South-Western Cape or elsewhere (Knox-Davies *et al.*, 1986). Brown stem usually occurs in autumn. Cool, wet conditions apparently favour the disease (Gorter, 1976).

Phomopsis (Sacc.) Sacc. sp.

Taxonomy – Primary pycnidia are large, 408 x 287 µm, and flattened. Secondary pycnidia develop in the craters after disintegration of the primary pycnidia. The secondary pycnidia are smaller, 183 x 97 µm, more irregular in shape and frequently more rounded than the primary pycnidia. Pycnidia contain two types of conidia. The alpha conidia are hyaline, ellipsoidal, straight, both aseptate and septate, biguttulate, triguttulate or tetraguttulate, 16.5-25.0 x 2.9-5.1 µm. The beta conidia are hyaline, aseptate, filiform, curved or hamate, 17.7-33.8 x 1.5-2.9 µm.

Only one of the two spore types may be found in some cultures, whereas both may be found in others (Orffer & Knox-Davies, 1989).

Symptoms - The disease is characterised by dieback of young shoots, while diffuse and perennial cankers develop on the older twigs and branches. The cankers on the older parts are sunken and maroon-black, while the perennial cankers are sunken, cracked and corky. Cankers usually extend from wounds and old flower heads. A distinctly yellow to orange-red discoloration of the shoots and leaves develops above the cankers. Vascular tissues below and above the cankers also become discoloured. Pycnidia, and particularly the sunken areas left after disintegration of the pycnidia, are often seen on old lesions (Orffer & Knox-Davies, 1989).

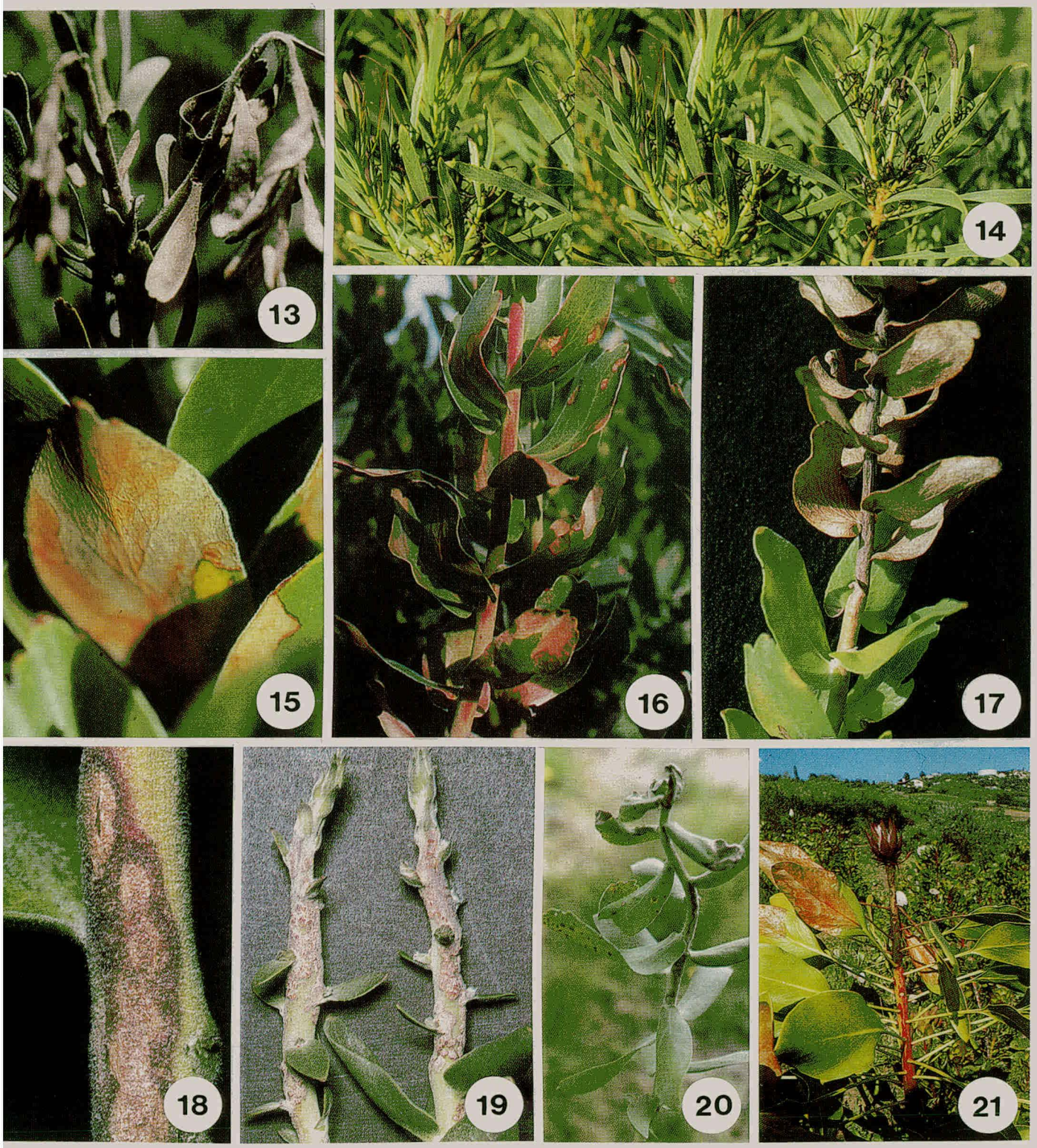
Host range and Geographic distribution - **Australia: Queensland,** *Leucadendron* cv Big Red, *P. cynaroides, P. neriifolia, P. repens, Protea* spp. (Greenhalgh, 1981; Forsberg, 1993); **South Africa: Cape province**, *Protea obtusifolia, P. repens* (Orffer & Knox-Davies, 1989).

Control - Sanitation pruning is the only means of control (Rohrbach, 1983).

Notes - The fungus causes a canker and dieback disease of *Protea* spp. in the Cape province of South Africa (Orffer & Knox-Davies, 1989). A *Phomopsis* sp. was recorded by Benic (1986) on dead *P. repens* cuttings from a mistbed, showing basal and tip dieback and necrosis of leaves, as well as on seeds of different species of Proteaceae. This suggests that the fungus may be seed-borne (Orffer & Knox-Davies, 1989). A Phomopsis shoot and stem canker of *Protea* spp. was also recorded in Queensland. The fungus enters through wounds and causes sunken lesions which result in death of branches and entire plants (Greenhalgh, 1981). Optimum growth of the fungus is at 26°C, with very slow growth below 15°C. This explains why symptoms develop more rapidly during spring than during winter (Orffer & Knox-Davies, 1989).

General control strategies

All unnecessary wounding and damaging of plants should be prevented. Other diseases and insects should therefore be controlled. Sanitation pruning should be implemented to remove diseased plant parts. Harvesting and pruning tools should be disinfested. Small wounds should be protected with a fungicidal spray, and the bigger pruning wounds should be protected with a wound sealant (Von Broembsen, 1989).



Figs 13-21. Stem and leaf symptoms of Proteaceae. **13.** Collapse of shoot tips infected by *Botrytis cinerea*. **14.** Tip dieback associated with *Colletotrichum gloeosporioides*. **15.** Characteristic leaf symptoms associated with *Drechslera dematioidea*. **16.** Red, superficial stem cankers caused by *D. dematioidea*. **17.** Extensive canker development following *D. dematioidea* infection. **18-20.** Stem and leaf symptoms caused by an *Elsinoë* sp. **21.** Botryosphaeria stem canker.

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2. FUNGI OCCURRING ON PROTEACEAE. I.

ABSTRACT

The present study has led to the description of several new fungi occurring on leaves of *Protea*, *Leucospermum*, *Telopea* and *Brabejum* collected from South Africa, Australia or New Zealand. *Cladophialophora proteae*, *Coniothyrium nitidae*, *Coniothyrium proteae*, *Coniothyrium leucospermi*, *Harknessia leucospermi*, and *Septoria protearum* spp. nov. are described from *Protea* and *Leucospermum* in South Africa, while *Phyllosticta owaniana* is redescribed from leaves of *Brabejum stellatifolium*. Furthermore, *Mycosphaerella telopeae* sp. nov. is described from leaves of *Telopea* collected in New Zealand, while *Phyllosticta telopeae*, which also occurs on this host, is described in culture from Australian material.

INTRODUCTION

The Proteaceae, which is the most unique family of the Cape Floral Kingdom, comprises approximately 8600 species, the majority of which are found in the southern hemisphere. About 360 proteaceous species occur in South Africa, of which 330 species in 14 genera are confined to the South-Western Cape province (Fynbos biome) (Rebelo, 1995).

Proteaceae, which are grown for cut-flowers in South Africa, form part of a large, economically viable, and expanding industry. Based on a survey conducted during the 1993/1994 season, approximately 2507 ha of veld are cultivated, while an additional 600626 ha are natural Fynbos vegetation. Export of fresh Fynbos from the Western and Eastern Cape for the 1993/1994 season amounted to nearly R9 million (Malan, 1995).

The proteaceous cut-flower industry has considerable potential, but diseases which blemish foliage and blooms are one of the problems which make this a high risk factor crop (Greenhalgh, 1981). Since South Africa is the centre of origin for the majority of proteaceous plants, pests and diseases that have evolved with these hosts have become a serious problem not only in South Africa, but to the industry internationally (Knox-Davies, 1981). Prior to 1970, the foliicolous fungi occurring on proteas in South Africa were poorly studied (Van Wyk, 1973). The description of *Cercostigmina protearum* (Cooke) U. Braun & Crous (=*Cercospora protearum* Cooke) by Cooke (1883), represents the first reference of a leaf pathogen occurring on the genera *Protea*, *Leucadendron* and *Leucospermum*. However, since the 1970's, numerous

diseases of the Proteaceae have been recorded and the causal fungi described (Van Wyk, 1973; Van Wyk *et al.*, 1975; Benic & Knox-Davies, 1983; Van Wyk *et al.*, 1985; Knox-Davies *et al.*, 1987; Orffer & Knox-Davies, 1989; Serfontein & Knox-Davies, 1990). Since the Proteaceae are amongst the most endangered species of the southern African flora, it is in the interest of the conservationist and the protea industry to record all new diseases and potentially important pathogens. In the present study, nine new fungi, most being associated with leaf spots, are described from leaves of Proteaceae collected in South Africa, New Zealand and Australia.

MATERIALS AND METHODS

Symptomatic leaves and leaf litter samples were incubated in Petri dish moist chambers at 25°C on the laboratory bench to induce sporulation. Single conidium colonies were established on 2 % malt extract agar (MEA; Oxoid), then transferred to divided plates containing fresh MEA in one half and carnation-leaf agar (CLA; Fisher *et al.*, 1982; Crous *et al.*, 1992) in the other half of the dish, and incubated at 25°C under continuous near-ultraviolet light. Linear growth of colonies growing on MEA at 25°C in the dark was measured after 1, 2 or 6 weeks, and colony colours were determined according to the charts of Rayner (1970). Leaf lesions with which species of *Mycosphaerella* were associated were excised and single ascospore cultures established on MEA using the technique described by Crous *et al.* (1991). For microscopic examination the fungi were mounted in lactophenol and measurements made at 1000x magnification. Averages were derived from at least 30 observations, and the ranges are given in parentheses. Reference cultures are maintained in the culture collection of the Department of Plant Pathology at the University of Stellenbosch (STE-U).

RESULTS AND DISCUSSION

Taxonomy

Cladophialophora proteae sp. nov. Figs 1, 13, 14.

Conidiogenae cellulae integratae, protuberationes breves truncatas formantes, 2-3 x 1.5-2 µm, mycelio concoloratae, subcylindracea. Conidia in catenis longis acropetalis (ad 20), simplicia vel ramosa, subcylindracea ad oblongo-doliiformia, (9-)13-17(-22) x 2.5-3(-4) µm *in vitro*, 0-1(-2)-

septata, pallide brunnea ad pallide olivacea, laevia, hilis subtruncatis ad truncatis, non crassis sed parum refractivis.

Sterile hyphae branched, septate, often forming strands, anastomosing, smooth to finely verruculose, frequently constricted at septa, olivaceous, 3-4 µm wide; hyphal cells in older cultures becoming swollen, up to 6 µm wide. Conidiophores reduced to conidiogenous cells. Conidiogenous cells integrated, forming short, truncate protuberances, 2-3 x 1.5-2 µm, concolorous with mycelium, subcylindrical. Conidia in long acropetal chains (up to 20), simple or branched, subcylindrical to oblong-doliiform, (9-)13-17(-22) x 2.5-3(-4) µm *in vitro*, 0-1(-2)-septate, light brown to pale olivaceous, smooth, hila subtruncate to truncate, not thickened, but somewhat refractive.

Cultural characteristics - Colonies erumpent, segmented, with smooth, sinuate, margins; fuscous black 7""k (surface and bottom); aerial mycelium absent. Colonies reaching 5 mm diam. on MEA after 6 weeks in the dark at 25°C.

Specimen examined - **South Africa: Western Cape province, Stellenbosch**, J.S. Marias Park, isolated as endophyte from leaves of *Protea cynaroides* (L.) L., L. Viljoen, Dec. 1996, PREM 55345 (holotype), cultures ex-type STE-U 1514-1516.

The genus *Cladophialophora* Borelli, which includes species associated with human disorders and others isolated as saprophytes or endophytes from plants, has teleomorphs placed in *Capronia* Sacc. in the Herpotrichiellaceae (Braun & Feiler, 1995; De Hoog *et al.*, 1995) and *Venturia* Sacc. in the Venturiaceae (Untereiner, 1997). Strains isolated as endophytes from leaves of *Protea cynaroides* fit this generic complex. It appears that *Cladophialophora* is heterogenous and it is possible that the saprophytic species will be placed in their own form genus, separate from the human pathogens. The present species is considered congeneric with *Cladophialophora* because of the flat, unthickened but somewhat refractive conidial scars, long conidial chains, and by being saprophytic. Morphologically *C. proteae* is most similar to *C. hachijoensis* (Matsush.) U. Braun & U. Feiler [conidia 1-3-septate, (4.5-)8-25(-35) x (1.5-)2-4(-5) µm)], but *C. proteae* has slightly smaller conidia. Braun and Feiler (1995) depicted a lot of variation between strains presently treated as *C. hachijoensis*, and it is possible that there are even more distinct species within this complex.

Coniothyrium nitidae sp. nov. Figs 3, 15.

Conidiomata pycnidialia, subepidermalia, globosa, discreta, brunnea, ad 200 µm diam., pariete in ex 3-4 stratis cellularum brunnearum texturae angularis constanti. Conidiogenae cellulae discretae, laeves hyalinae ad pallide olivaceae, doliiformes ad ampulliformes, 1-4 plo enteroblastice et percurrenter proliferantes, 5-8 x 5-10 µm. Conidia pallide ad medio brunnea, parietibus verruculosis, 0-1-septata ellipsoidea ad subcylindracea, apice obtuso, base obtusirotundata ad truncata, (6.5-)8-9(-11) x 3-4(-4.5) µm *in vivo*, (5.5-)6-8(-9) x 3-4 µm *in vitro*.

Leaf spots light brown, amphigenous, variable in shape and size, frequently associated with tip die-back or situated along leaf margins. Mycelium immersed, septate, medium brown, finely verruculose, 3-4 µm diam. *in vivo*, 2-5 µm *in vitro*, finely verruculose, light to medium brown, forming intercalary and terminal chains of globose chlamydospores. Conidiomata pycnidial, subepidermal, globose, separate, brown, up to 200 µm diam., wall consisting of 3-4 layers of brown cells of *textura angularis*. Conidiophores reduced to conidiogenous cells. Conidiogenous cells discrete, smooth, hyaline to pale olivaceous, doliiform to ampulliform, proliferating 1-4 times enteroblastically and percurrently, 5-8 x 5-10 µm. Conidia medium brown, thick-walled, verruculose, 0-1-septate, ellipsoidal to subcylindrical, apex obtuse, base bluntly rounded to truncate, (6.5-)8-9(-11) x 3-4(-4.5) µm *in vivo*, (5.5-)6-8(-9) x 3-4 µm *in vitro*.

Cultural characteristics - Colonies with irregular margins; grey olivaceous 21""b to cinnamon 13"b (bottom); aerial mycelium moderate, dirty pink to white. Colonies reaching 32 mm diam. on MEA after 2 weeks in the dark at 25°C.

Specimen examined - **South Africa: Western Cape province, Hermanus**, leaves of *Protea nitida* Mill., S. Denman, 29 Aug. 1996, PREM 55346 (holotype), cultures ex-type STE-U 1476-1478, 1531-1533.

Coniothyrium proteae sp. nov. Figs 2, 16.

Conidiomata pycnidialia, subepidermalia, globosa, discreta, brunnea, 60-120 µm in diam., pariete ex 2-3 stratis cellularum brunnearum texturae angularis constanti. Conidiogenae cellulae discretae, laeves hyalinae, doliiformes ad ampulliformes 1-2 plo enteroblastice et percurrenter proliferantes, 3-8 x 3-4 µm. Conidia pallide ad medio brunnea, parietibus tenuibus, laevia ad

subtiliter verruculosa, aseptata, ellipsoidea ad globosa, rare pyriformia, apice obtuso, base obtusirotundata ad truncata, (5-)5.5-7(-8) x 3.5-4 µm *in vivo*, (3-)3.5-4 x 2-2.5 µm *in vitro*.

Leaf spots light brown, amphigenous, variable in shape and size, frequently associated with tip die-back or situated along leaf margins. Mycelium immersed, septate, branched, pale to medium brown, smooth to finely verruculose, 3-4 µm diam. Conidiomata pycnidial, subepidermal, globose, separate, brown, 60-120 µm diam., wall consisting of 2-3 layers of brown cells of *textura angularis*. Conidiophores reduced to conidiogenous cells. Conidiogenous cells discrete, smooth, hyaline, doliiform to ampulliform, proliferating 1-2 times enteroblastically and percurrently, 3-8 x 3-4 µm. Conidia light to medium brown, thin-walled, smooth to finely verruculose, aseptate, ellipsoidal to globose, rarely pyriform, apex obtuse, base bluntly rounded to truncate, (5-)5.5-7(-8) x 3.5-4 µm *in vivo*, (3-)3.5-4 x 2-2.5 µm *in vitro*.

Cultural characteristics - Colonies with smooth, sinuate margins, olivaceous grey 23""i (bottom), smoke grey 21""f (surface); aerial mycelium sparse to moderate. Colonies reaching 4 mm diam. on MEA after 2 weeks in the dark at 25°C.

Specimens examined - **South Africa: Western Cape province, Hermanus**, leaves of *Protea nitida*, S. Denman, 29 Aug. 1996, PREM 55347 (holotype), cultures ex-type STE-U 1423-1425; **Stellenbosch**, leaves of *Protea mellifera*, L. Verwoerd, Apr. 1923, BPI 639096.

Coniothyrium leucospermi sp. nov. Figs 4, 17.

Conidiomata pycnidialia, subepidermalia, amphigena, discreta, globosa atrobrunnea ad 200 µm in diam., pariete ex 3-4 stratis cellularum brunnearum texturae angularis. Conidogenae cellulae discretae, laeves, pallide brunneae, doliiformes ad ampulliformes, 1-3 plo enteroblastice et percurrenter proliferantes, 9-11 x 5-7 µm. Conidia mediobrunnea, parietibus crassis, verruculosa, aseptata, ellipsoidea ad globosa, apice obtuso, base obtusirotundata ad truncata, 11-13 x 5-6 µm *in vivo*, (9-)10-13(-15) x 6-7 µm *in vitro*.

Leaf spots amphigenous, irregular, grey to light brown with a raised, dark brown border, frequently associated with tip blight or leaf margins. Conidiomata pycnidial, subepidermal, amphigenous, separate, globose, dark brown, up to 200 µm diam., wall consisting of 3-4 layers of brown cells of *textura angularis*. Conidiophores reduced to conidiogenous cells. Conidiogenous cells discrete, smooth, light brown, doliiform to ampulliform, proliferating 1-3 times

enteroblastically and percurrently, 9-11 x 5-7 µm. Conidia medium brown, thick-walled, verruculose, aseptate, ellipsoidal to globose, apex obtuse, base bluntly rounded to truncate, 11-13 x 5-6 µm *in vivo*, (9-)10-13(-15) x 6-7 µm *in vitro*.

Cultural characteristics - Colonies with smooth, regular margins, fucous black 7""k (surface), olivaceous black 27""m (bottom); aerial mycelium sparse. Colonies reaching 12-16 mm diam. on MEA after 2 weeks in the dark at 25°C.

Specimens examined - **South Africa: Western Cape province, Piketberg,** leaves of *Leucospermum conocarpodendron* (L.) H. Buek., S. Denman, 29 Aug. 1996, PREM 55348 (holotype), cultures ex-type STE-U 1426-1428; **Dominican Republic**, leaves of *Leucospermum* sp., L. Schroeder, 7 Jul. 1986, BPI 1107823.

Van Wyk (1973) listed several specimens from *Protea* and *Leucadendron* (PREM 44798, 44853, 44854) which he considered to represent a new species of *Coniothyrium*. An examination of these specimens found conidiomata to be present on PREM 44854 and 44798. Conidia were aseptate, finely verruculose, and resembled *C. protea* in shape, but were much larger [(7-)8-9(-10) x 4-5(-6) in PREM 44854; (5-)7-8(-9) x (2.5-)4-5(-6) in PREM 44798]. Although it appears that these collections represent yet another, distinct species, further collections and cultures are required to suitably characterise this pathogen.

As far as we could establish, only one other species of *Coniothyrium*, namely *C. proteae-abyssinicae* Bacc. has been described from these hosts. The latter species has conidia the differ in size (14.4 x 3.2 µm; Baccarini, 1917) to those of the species described in the present study. It is important to note, however, that major differences occurred in conidial shape and dimension in some species when cultured on agar, and these discrepancies will have to be carefully considered when comparing new species and isolates in the future.

Harknessia leucospermi sp. nov. Figs 5, 18.

Conidiomata discreta, immersa, globosa ad subglobosa, unilocularia, subepidermalia, ad 350 µm diam. Conidiogenae cellulae subcylindraceae ad lageniformes, hyalinae, laeves, 8-20 x 2.5-5 µm. Conidia holoblastica, late ventricosa, guttula media, granularia, laevia, irregulariter striata, apice obtusa ad obtusirotundato, base truncata (23-)25-28(-32) x (13-)15-17(-18) µm, appendicula basali hyalina non ramosa 4-8(-14) x 2-2.5(-3) µm.

Conidiomata separate, immersed, globose to subglobose, unilocular, subepidermal, up to 350 µm diam.; ostiole with light brown furfuraceous margin; basal and lateral walls 5-7 cells thick, composed of *textura angularis*, brown, becoming hyaline towards the interior. Conidiophores reduced to conidiogenous cells. Conidiogenous cells subcylindrical to lageniform, hyaline, smooth, 8-20 x 2.5-5 µm. Conidia holoblastic, broadly ventricose with a central guttule, granular, smooth, irregularly striate, apex obtuse to bluntly apiculate, base truncate (23-)25-28(-32) x (13-)15-17(-18) µm, with a hyaline, unbranched basal appendage 4-8(-14) x 2-2.5(-3) µm *in vivo*; conidia broadly ellipsoid, apiculate, (23-)25-27(-30) x (12-)13-15 µm, basal appendage 3-10 x 2-2.5 µm *in vitro*.

Cultural characteristics - Colonies with moderate, pale yellow aerial mycelium, luteus 21b (bottom); margins smooth to irregular. Colonies reaching 56 mm diam. on MEA after 1 week in the dark at 25°C.

Specimen examined - **South Africa: Western Cape province, Kirstenbosch**, leaf litter of a *Leucospermum* sp., P.W. Crous, 20 May 1996, PREM 55349 (holotype), cultures ex-type STE-U 1372-1374, IMI 375227, ATCC 201156, CBS 778.97.

Harknessia leucospermi is morphologically similar to *H. eucalypti* Cooke apud Cooke & Harkn., which has broadly ventricose, apiculate conidia (19-)20-25(-28) x (11-)13-15 µm with restricted striations and basal appendages (6-)8-13 x 2-4 µm, and *H. eucalyptorum* Crous *et al.*, which has broadly ventricose, astriate conidia with blunt apices, (16-)20-25(-29) x (9-)10-14(-16) µm *in vivo*, (14.5-)17-22(-24) x (10.5-)11-13(-14) µm *in vitro*, basal appendages 3-16 µm (Crous *et al.*, 1993). Sutton and Pascoe (1989) reported *H. eucalypti* occurring on *Banksia marginata* Cav. and *Lambertia formosa* Sm., both hosts in the Proteaceae. Conidia of *H. leucospermi* are larger than those of *H. eucalypti* and *H. eucalyptorum*. On leaf tissue the conidia resemble those of *H. eucalyptorum* in being more bluntly apiculate. In culture, however, conidia become more broadly ellipsoidal and sharply apiculate, distinct from conidia of *H. eucalyptorum* formed in culture (Crous *et al.*, 1993). Although Nag Raj (1993) illustrated conidia of *H. eucalypti* to be striate, striations were only observed in restricted areas on some conidia, and were not as prominant as in *H. leucospermi*. The latter feature was also found to be constant in culture for *H. leucospermi*.

Mycosphaerella telopeae sp. nov. Fig. 6.

Pseudothecia amphigena, sparse distributa, unica, nigra, erumpentia globosa ad 120 µm diam. Asci aparaphysati fasciculati bitunicati subsessiles obovoidei ad late ellipsoidei vel cylindracei, recti vel parum curvati, 8 sporis, 20-28 x 8-10 µm. Ascosporae multiseriatae imbricatae, hyalinae, guttulatae, parietibus tenuibus, rectae ad parum curvatae, fusoideo-ellipsoideae apicibus obtusis, latissimae in medio cellulae apicalis, mediano 1-septatae, magis prominanter ad basim contractae (9-)10-11(-12) x (2-)2.5(-3) µm.

Leaf spots circular, amphigenous, 1-4 mm diam., grey in centre, surrounded by a raised, dark brown border and a narrow chlorotic margin. Pseudothecia amphigenous, sparsely distributed, single, black, erumpent, globose, up to 120 µm diam.; apical papillate ostiole 5-10 µm in diam.; wall consisting of 3-4 layers of medium brown *textura angularis*. Asci aparaphysate, fasciculate, bitunicate, subsessile, obovoid to broadly ellipsoid or cylindrical, straight or slightly curved, 8-spored, 20-28 x 8-10 µm. Ascospores multiseriate, overlapping, hyaline, guttulate, thin-walled, straight to slightly curved, fusoid-ellipsoidal with obtuse ends, widest in the middle of the apical cell, medianly 1-septate, generally not constricted at septum, with some ascospores on the leaf surface appearing slightly constricted; ascospores tapering more prominantly towards the lower end (9-)10-11(-12) x (2-)2.5(-3) µm.

Specimens examined - **New Zealand**, leaf of *Telopea* sp., M. Abdelshife, 11 Sept. 1996, PREM 55350 (holotype); leaf of *Telopea* sp., M. Abdelshife, 5 Aug. 1996, BPI 806263; leaf of *Telopea* sp., M. Abdelshife, 6 Aug. 1996, BPI 806264.

Although no species of *Mycosphaerella* has been described from *Telopea* (Corlett, 1991, 1995), three species are presently known from leaves of *Protea* (Proteaceae), namely *M. proteae* (Syd.) Arx, *M. jonkershoekensis* Van Wyk *et al.* and *M. bellula* Crous & M.J. Wingf. (Figs 7-9) (Crous & Wingfield, 1993). Ascospores of *M. telopeae* are much smaller than those of the large-spored *M. proteae*, which measure 20-33 x 6-8 ($\bar{x}$ 1 = 26 x 7) µm. Morphologically ascospores of *M. telopeae* resemble those of *M. jonkershoekensis* which measure 11-23 x 4-6 ($\bar{x}$ 2 = 18 x 4.5) µm, but ascospores of the former differ in being much smaller and less prominently constricted at the septum. Ascospores of *M. telopea* are slightly larger than those of *M. bellula*, 7-11 x 2-3 ($\bar{x}$ 3 = 9 x 2.5) µm, and are not as prominantly constricted as in the latter species. The erumpent black

pseudothecia of *M. telopea* are also distinct from those of *M. jonkershoekensis* and *M. bellula*, which are subepidermal and generally not visible to the naked eye.

When Crous & Wingfield (1993) treated the species of *Mycosphaerella* occurring on *Protea*, little was known about their behaviour in culture. Subsequent to that study, fresh collections were obtained of all three of those species. As observed in the type collection of *M. jonkershoekensis* (PREM 44830), ascospores from fresh collections were frequently slightly olivaceous in their asci. When ascospores are shot out of the ascus and germinated on MEA (Crous *et al.*, 1991), ascospores become verruculose, brown, and constricted at the septum. Ascospores germinate initially with germ tubes growing parallel to the long axis of the spore (Fig. 8). After 48 h, however, ascospores usually have formed several germ tubes, and the germination is irregular. A peculiarity about *M. jonkershoekensis* is that ascospores germinate at 25°C, but die soon after germination if the plates are not incubated at 15°C for one to two weeks. After this initial phase the fungus will grow at most temperatures, and it is hypothesised that this low temperature requirement is a prerequisite for successful germination and infection of leaf tissue. The same phenomenon has also recently been reported for *M. juvenis* Crous & M.J. Wingf. on *Eucalyptus* (Crous & Wingfield, 1996).

Ascospores of *M. bellula* germinate with one to several germ tubes which grow irregular to the long axis of the spore. As with *M. jonkershoekensis*, spores darken and become verruculose at germination (Fig. 7). In the present study, *M. bellula* was also isolated from leaf lesions of *Leucospermum* spp. (STE-U 1321-1323), and it appears to be a very common species of *Mycosphaerella* on *Protea* spp., frequently also occurring in association with *Leptosphaeria protearum* Syd.

After several unsuccessful attempts, ascospores of *M. proteae* were finally induced to germinate in culture. Unlike *M. jonkershoekensis* and *M. proteae*, ascospores could never be induced to shoot out onto the agar surface, and the epidermis had to be cut open to expose the pseudothecia. This difference, as well as the distinct lesions and red-purple discoloration of the leaf tissue suggest that *M. proteae* is a fungus quite unrelated to the other species dealt with above. In culture germinating ascospores become constricted at their septum, brown in colour, and germinate with one germ tube generally parallel to the long axis of the spore (Fig. 9). Ascospores did not become as verruculose as those of *M. bellula* and *M. jonkershoekensis*. Colonies were extremely slow growing, and after about 6 months at 25°C on MEA had hardly

reached 5 mm in diam., suggesting that this fungus is more of an obligate pathogen than the other species of *Mycosphaerella* treated here.

Phyllosticta owaniana G. Winter, Hedwigia 24: 31 (1885) Fig. 10.

Leaf spots amphigenous, circular, 0.5-5 mm diam., light brown, becoming darker brown towards the raised, dark brown border; margins chlorotic when present. Conidiomata pycnidial, predominantly epiphyllous, clearly visible to the naked eye, scattered, immersed, becoming erumpent, globose to subglobose, up to 120 µm diam., unilocular, medium brown, ostiolate, becoming papillate; wall up to 15 µm thick, of *textura angularis*, with brown cells becoming lighter towards the interior. Conidiophores reduced to conidiogenous cells. Conidiogenous cells hyaline, smooth-walled, subcylindrical to doliiform, 5-8 x 3-6 µm. Conidia obovoid to ovoid *in vivo*, ovoid *in vitro*, apex rounded, base truncate to rounded, hyaline, guttulate, (10-)12-14(-15) x 7-8(-9) µm *in vivo* and *in vitro*, enclosed in a mucous sheath 0.5-3 µm thick, persistent on most conidia, bearing a single, unbranched, slightly tapering apical mucoid appendage 5-8(-14) x 1-1.5 µm.

Cultural characteristics - Colonies with irregular margins, devoid of aerial mycelium, black (surface), olivaceous black 27""m (bottom). Colonies reaching 9.5 mm diam. on MEA after 2 weeks in the dark at 25°C.

Specimen examined - **South Africa: Western Cape province, Jonkershoek**, leaf spots on *Brabejum stellatifolium* L., A. den Breeÿen, Mar. 1995, PREM 55351, cultures STE-U 1009-1010, IMI 375228, ATCC 201157, CBS 776.97.

Phyllosticta owaniana appears to be a well-established pathogen of *Brabejum stellatifolium* and is associated with prominent leaf spots on this plant throughout the Western Cape, where this host is planted as an ornamental. The present collection correlates well with the original description of Winter (1885), who cited conidia as being ovoid to subpyriform, 10-12 x 8 µm.

Phyllosticta telopeae H.Y. Yip, Mycol. Res. 93: 494 (1989) Figs 11, 19.

Leaf spots amphigenous, circular to somewhat irregular, often confined by leaf veins, 2-7 mm diam., grey-brown to grey olivaceous with a narrow, dark, slightly raised border on the adaxial surface. Conidiomata pycnidial, predominantly epiphyllous, clearly visible to the naked eye,

scattered, immersed, becoming erumpent, globose to subglobose, up to 150 µm diam., unilocular, medium brown, ostiolate, becoming papillate; wall up to 15 µm thick, of brown cells of *textura angularis*. Conidiophores reduced to conidiogenous cells. Macroconidiogenous cells hyaline, smooth-walled, subcylindrical to lageniform, 7-12 x 3-5 µm, often proliferating once enteroblastically and percurrently. Macroconidia ellipsoidal to obovoid with a rounded apex and a truncate base, rarely with a minute marginal frill, unicellular, hyaline, smooth-walled, guttulate, (12-)13-16(-18) x (7-)8-9 µm *in vitro*, 11.5-15.5 x 7-10 µm *in vivo*, enclosed in a thin mucoid sheath, 0.5-2 µm thick, bearing a single, unbranched, attenuated apical mucoid appendage 10-20 x 2-3 µm on MEA (up to 40 µm long when cultured on a medium consisting of 2% malt extract, 2% V8 juice and 4% agar), (6.5-)20-100 x 2-3 µm *in vivo*. Microconidiophores subcylindrical, hyaline, 0-2-septate, branched above, 15-20 x 2-3 µm. Microconidiogenous cells subcylindrical, ampulliform to lageniform with minute periclinal thickening, hyaline, smooth-walled, 5-12 x 2-2.5 µm. Microconidia bacillar with a rounded apex and swollen, truncate base, unicellular, hyaline, smooth-walled, (6-)8-10(-12) x 1.5 µm.

Cultural characteristics - Colonies with irregular margins, devoid of aerial mycelium, black (surface), olivaceous grey 25""m (bottom). Colonies reaching 35.2 mm diam. on MEA after 2 weeks in the dark at 25°C.

Specimens examined - **Australia**, leaves of *Telopea speciosissima* R. Br., coll. D. Koizumi, det. M. Palm, Oct. 1991, US1108807 (BPI), PREM 55352, cultures STE-U 1517, 1522, IMI 375229, ATCC 201158, CBS 777.97; leaves of *Telopea speciosissima* R. Br., coll. D. Koizumi & J. Van Dersal, det. M. Palm, Oct. 1997, US1108820 (BPI).

Phyllosticta telopeae is distinguished from *P. owaniana* by its larger conidia and much longer appendages. It was originally described by Yip (1989) from *T. speciosissima* leaves collected in Tasmania. Our material correlates well with the description given by Yip, with the only difference being the length of the mucoid appendages. The fact that appendages were observed to vary in length depending on the medium on which they were cultured, once again stresses the importance of standardising conditions and media when comparing species and descriptions of *Phyllosticta*. As far as we are aware, this is the first description of *P. telopeae* from culture, and the first record of its microconidial state.

Septoria protearum sp. nov. Figs 12, 20.

Conidiomata pycnidialia, globosa ad subglobosa, 65-200 µm diam. Conidiophorae hyalinae, laeves, subcylindraceae, nonramosae vel superne ramosae, 0-5-septatae, 8-30 x 1.5-3.5 µm. Conidiogenae cellulae terminales et laterales, hyalinae, subcylindraceae, non ramulosae, ad apices rotundatas planas contractae, 4-12 x 1.5-3 µm diam.; sympodialiter proliferantes. Conidia holoblastica, solitaria hyalina laevia guttulata, (0-)1-3(-4)-septata, subcylindracea ad anguste obclavata, apice subobtuso, base angusta, obconico-truncata ad truncata, recta ad curvata (6-)15-22(-30) x 1.5-2 µm *in vitro*.

Conidiomata pycnidial, associated with leaf spots, amphigenous, black on surface, subepidermal, becoming erumpent. Pycnidia globose to subglobose, 65-200 µm diam.; wall consisting of 3-4 layers of brown cells of *textura angularis*; ostioles slightly papillate, up to 60 µm wide. Conidiophores hyaline, smooth, subcylindrical, unbranched or branched above, 0-5-septate, 8-30 x 1.5-3.5 µm. Conidiogenous cells terminal and lateral, hyaline, subcylindrical, unbranched, tapering to rounded or flattened apices, 4-12 x 1.5-3 µm diam.; proliferating sympodially. Conidia holoblastic, solitary, hyaline, smooth, guttulate, (0-)1-3(-4)-septate, subcylindrical to narrowly obclavate, apex subobtuse, base narrow obconically truncate to truncate, straight to curved, (6-)15-22(-30) x 1.5-2 µm *in vitro*.

Cultural characteristics - Colonies with smooth margins, iron grey 23""'k (bottom), with moderate whitish aerial mycelium. Colonies reaching 10.3 mm diam. on MEA after 2 weeks in the dark at 25°C.

Specimen examined - **South Africa: Gauteng province, Pretoria**, leaves of *Protea cynaroides*, L. Viljoen, Sept. 1996, PREM 55353 (holotype), culture ex-type STE-U 1470, IMI 375230, ATCC 201159, CBS 778.97.

As far as we could establish, only one other species of *Septoria* has thus far been associated with leaf spots on, or described from leaves of *Protea*. *Septoria proteae* Ciccar. was described as having 1-3-septate conidia, 40-50 x 3-4 µm (Ciccarone, 1951), thus being much larger than those of *S. protearum*.

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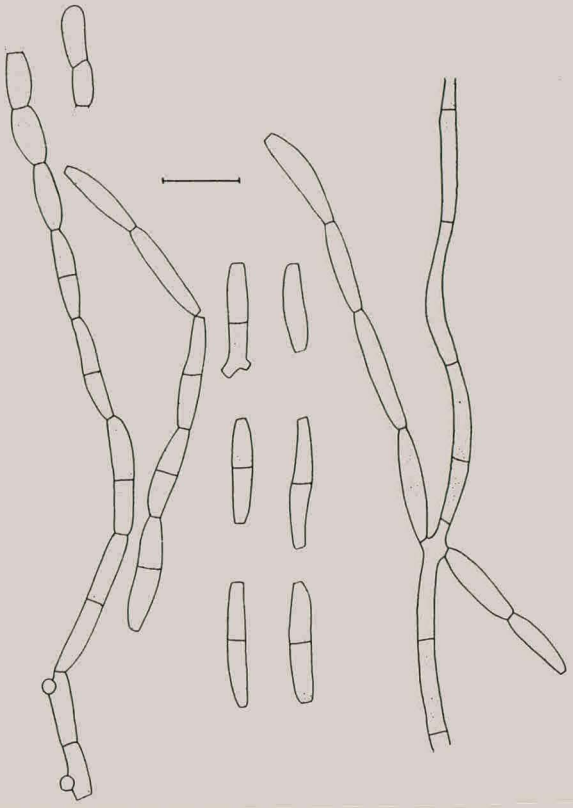
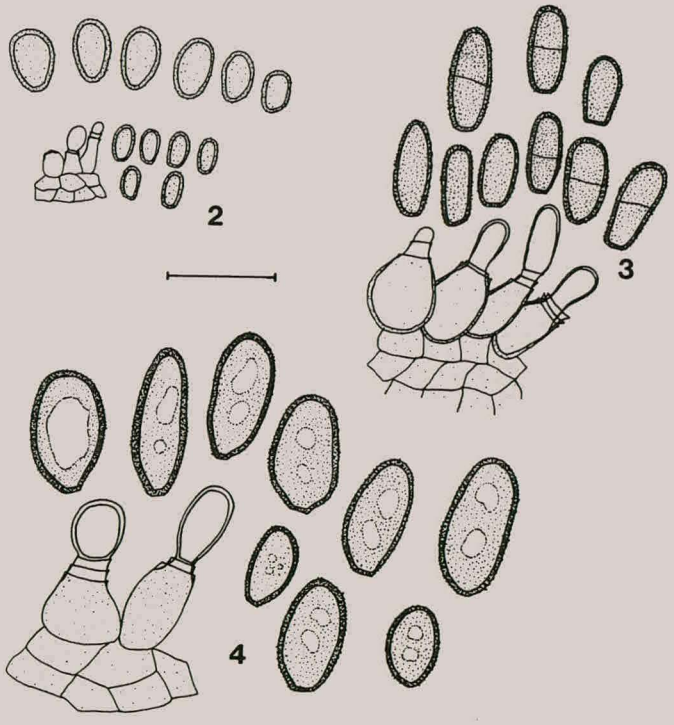


Fig. 1. *Cladophialophora proteae*. Chains of 0—1-septate conidia formed on malt extract agar (bar = 10 µm).



Figs 2-4. Conidia and conidiogenous cells of *Coniothyrium* spp. *in vivo*. **2.** *C. proteae*. **3.** *C. nitidae*. **4.** *C. leucospermi* (bar = 10 µm).

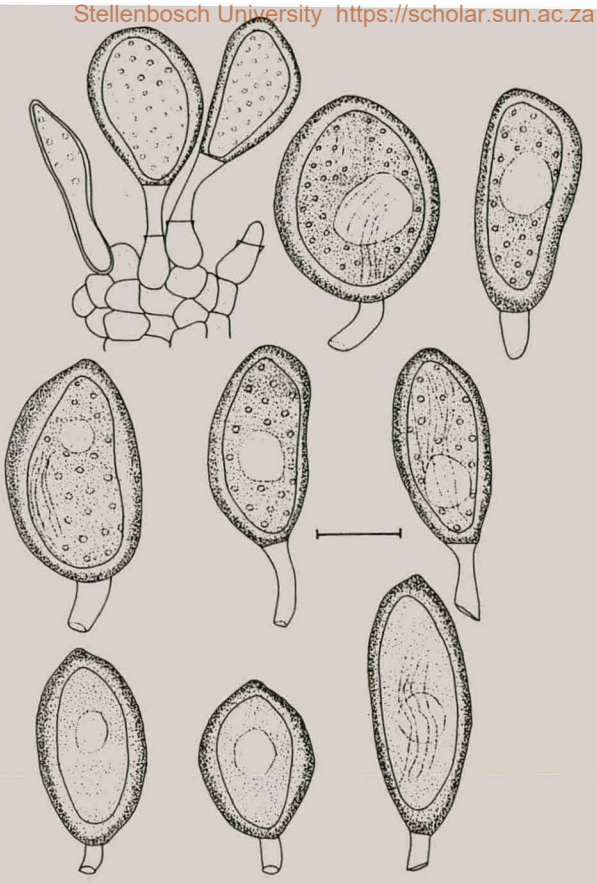


Fig. 5. *Harknessia leucospermi*. Conidia and conidiogenous cells *in vivo* (bar = 10 µm).

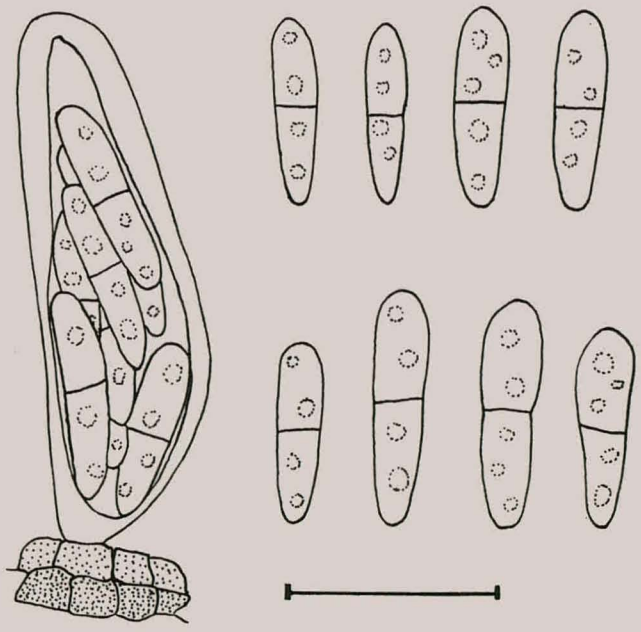
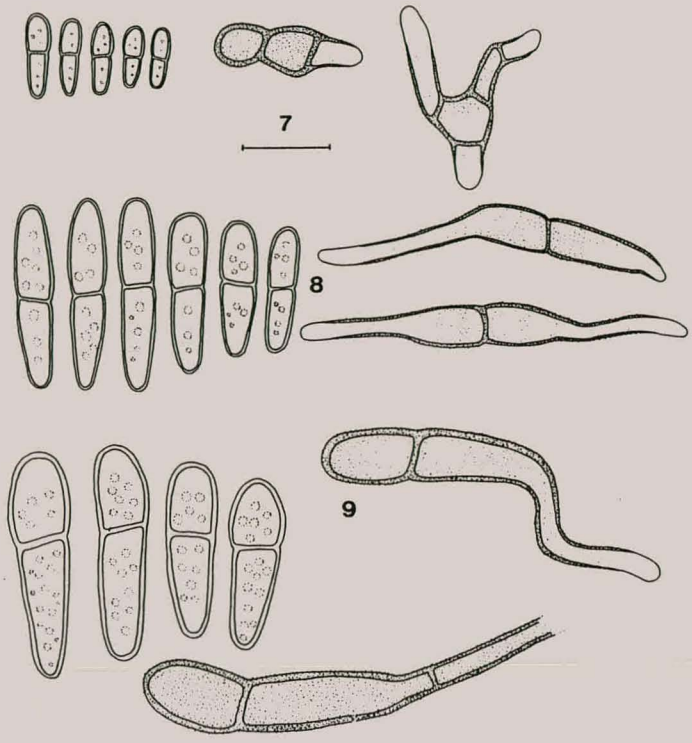


Fig. 6. *Mycosphaerella telopeae*. Ascospores and ascus in vivo (bar = 10 µm).



Figs 7-9. Ascospores, and germinating ascospores of *Mycosphaerella spp.* on malt extract agar after 24 h. **7.** *M. bellulae*. **8.** *M. jonkershoekensis*. **9.** *M. proteae* (bar = 10 µm).

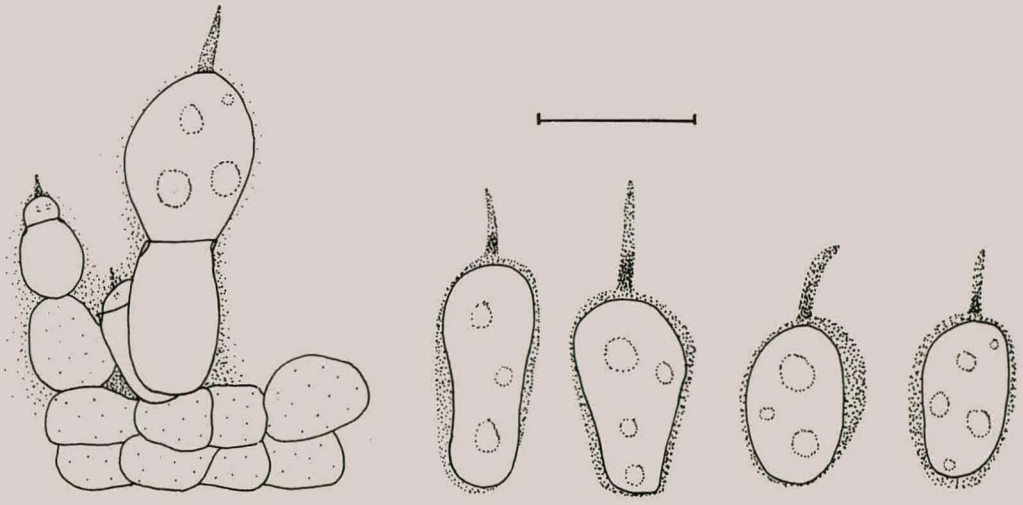


Fig. 10. *Phyllosticta owaniana* sporulating on malt extract agar (bar = 10 µm).

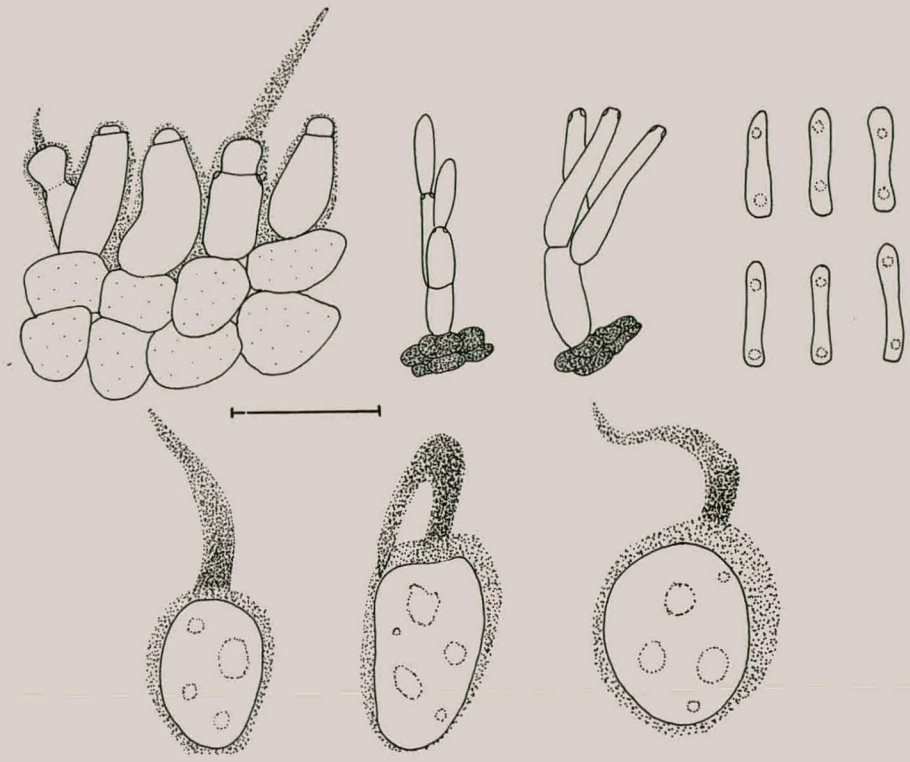


Fig. 11. *Phyllosticta telopeae* sporulating on malt extract agar (bar = 10 µm).

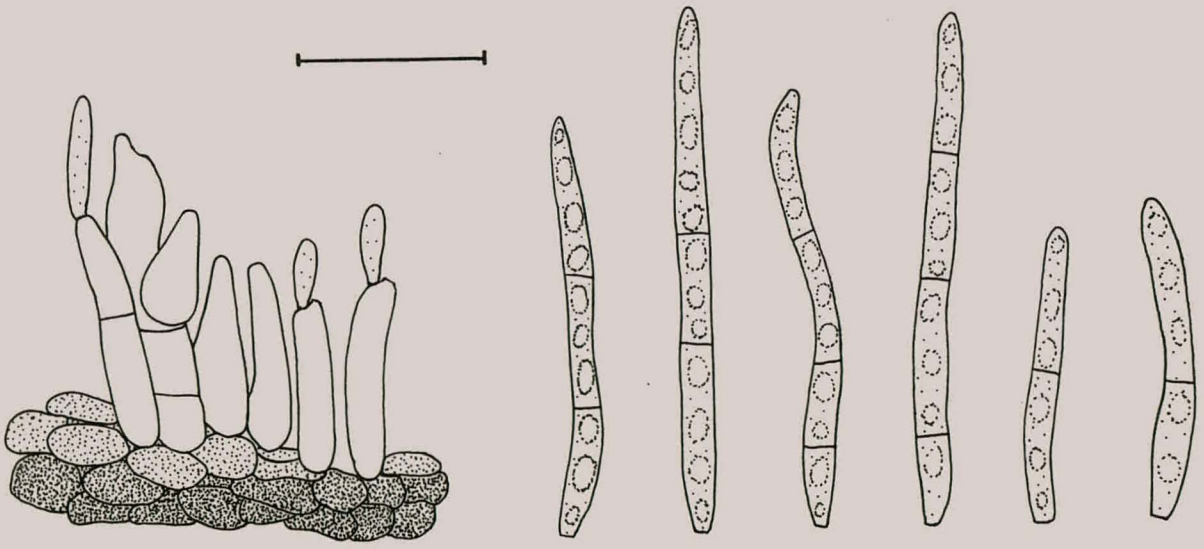
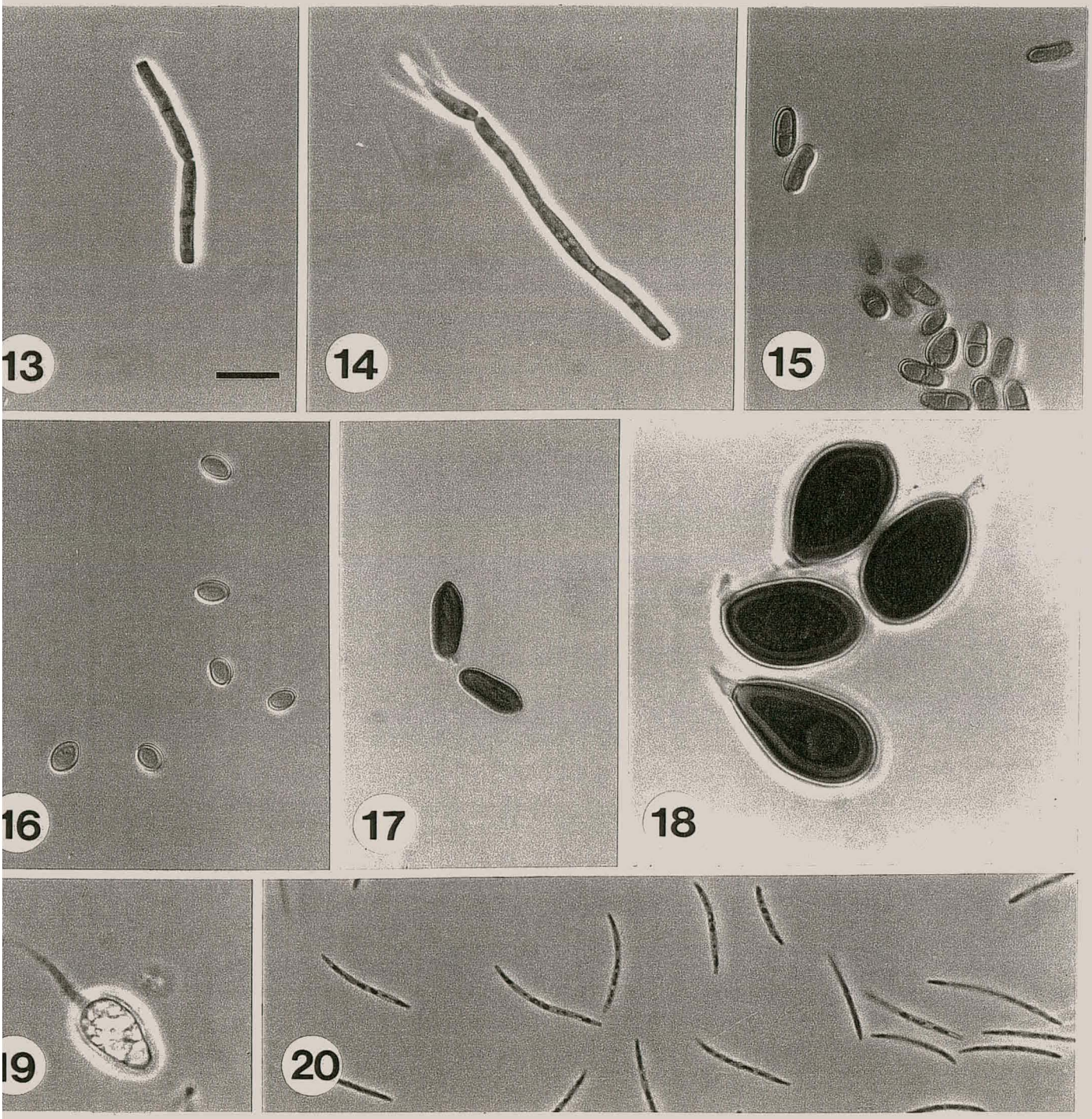


Fig. 12. *Septoria protearum*. Conidia and conidiogenous cells formed on carnation leaf agar (bar = 10 µm).



Figs 13-20. Conidia of microfungi occurring on Proteaceae. **13, 14.** Catenulate conidia of *Cladophialophora proteae*. **15.** *Coniothyrium nitidae*. **16.** *Coniothyrium proteae*. **17.** *Coniothyrium leucospermi*. **18.** *Harknessia leucospermi*. **19.** *Phyllosticta telopeae*. **20.** *Septoria protearum* (bar = 10 µm).

3. DIFFERENTIATION OF *ELSINOË* SPP. ASSOCIATED WITH SCAB DISEASE OF PROTEACEAE BASED ON MORPHOLOGY, SYMPTOMATOLOGY, RAPDS AND ITS SEQUENCE ALIGNMENT

ABSTRACT

Scab disease of Proteaceae, which was initially observed on *Leucospermum* in South Africa in 1981, has subsequently been reported on this host in Australia and Hawaii. The disease, commonly known as corky bark or scab, is associated with severe losses in commercial plantings of *Leucospermum* in South Africa, and has also been collected from species of *Leucadendron, Protea* and *Serruria* in South Africa, from *Banksia, Leucadendron, Mimetes, Protea* and *Serruria* in Australia, and from *Leucospermum* and *Protea* in California and Zimbabwe. The causal agent was determined to be a species of *Elsinoë*, which has not been formally described. The aim of the present study was to elucidate the taxonomy of the species of *Elsinoë* associated with scab disease of Proteaceae in these countries. General morphology, symptomatology and phylogenetic analysis based on random amplified polymorphic DNA (RAPD) profiles and DNA sequences of the 5.8S rDNA gene and its flanking ITS1 and ITS2 regions were used. Anamorph and teleomorph characteristics of isolates from *Leucospermum*, *Protea* and *Banksia* suggest that there are at least four distinct species involved. These findings are strongly supported by the phylogenetic trees inferred from DNA sequences and RAPD banding patterns. Furthermore, results obtained using DNA sequences suggest that the *Elsinoë* isolates from *Leucadendron, Leucospermum* and *Serruria* in South Africa and Australia, and the isolates from *Leucospermum* in California and Zimbabwe are representative of the same species.

INTRODUCTION

Elsinoë spp. are usually associated with leaf, stem and pod disease symptoms of various plants (Pan, 1994; Phillips, 1994; Gottwald, 1995). The disease, commonly referred to as scab, has been recorded worldwide on numerous crops important to the agricultural as well as forestry sectors (Farr *et al.*, 1989). In members of the Proteaceae Benth. & Hook. f. in South Africa, scab disease is common on *Leucospermum* R. Br. (*Lsp.*) and has also been recorded on *Leucadendron* R. Br. (*Lcd.*) (Benic & Knox-Davies, 1983; Von Broembsen, 1989).

Scab disease of Proteaceae was first observed in South Africa in 1981. The disease, locally known as corky bark or scab, is associated with severe losses to commercial pincushion plantings (*Leucospermum* spp.). The causal agent, which was identified as a species of *Elsinoë*, was never formally described (Benic & Knox-Davies, 1983). Similar disease symptoms have also been observed on *Leucospermum* spp. in Hawaii (Protea Disease Letter, 1991) and Australia (Ziehrl *et al.*, 1995). Similarities in disease symptoms and the appearance of the casual organism in South Africa and Australia have, however, led to speculation that the causal organism could possibly be the same species. In Australia, however, scab disease symptoms have also been observed on species of *Banksia* L. f., *Leucadendron*, *Mimetes* Salisb., *Protea* L. and *Serruria* Salisb. (Forsberg, 1993; Pascoe *et al.*, 1995). In the present study, scab infestations have been recorded on species of *Leucadendron*, *Leucospermum*, *Protea*, and *Serruria* in South Africa and Australia, on *Banksia* in Australia, and on species of *Leucospermum* and *Protea* in California and Zimbabwe.

Characterisation of *Elsinoë* spp. is difficult since their teleomorph states are rarely observed, and their *Sphaceloma* De Bary anamorphs are generally morphologically conserved. Molecular tools have become increasingly useful in confirming the interpretation of morphological variation. Random amplified polymorphic DNAs (RAPDs) with arbitrary primers (Welsh & McClelland, 1990; Williams *et al.*, 1990) have proven useful at taxonomic levels ranging from cultivars to species (Demeke & Adams, 1994). For example, RAPDs have been used to distinguish between species and pathotypes of *Elsinoë* isolates associated with scab disease of *Citrus* L. (Tan *et al.*, 1996) and *Phaseolus* (Tourn.) L. (Mchau *et al.*, 1998). The phylogenetic value of rDNA sequences has been discussed in previous reviews (Bruns *et al.*, 1991; Hibbett, 1992; Kohn, 1992; Kurtzman, 1992). Ribosomal DNA sequences, in particular the 5.8S rDNA and flanking internal transcribed spacer regions (ITS1 and ITS2) have also been used to study the phylogenetic relationships of a number of plant pathogens (Chen *et al.*, 1992; Lee & Taylor, 1992; Morales *et al.*, 1993; Zambino & Szabo, 1993; Morales *et al.*, 1995).

The aim of the present study, therefore, was to delineate species among the *Elsinoë* isolates from Proteaceae collected in Australia, California, South Africa and Zimbabwe, using general morphology, symptomatology and phylogenetic analysis based on RAPD profiles and ITS1, ITS2 and 5.8S rDNA gene sequences.

MATERIALS AND METHODS

Isolates and cultures - Single spore isolations were made from acervuli and ascomata that occurred on the stems and leaves of the various hosts. Conidia and ascospores were cultured on 2 % malt extract agar (MEA, Oxoid), and incubated at 25°C under near-ultraviolet light. Sub-cultures are maintained in the Culture Collection of the Department of Plant Pathology, University of Stellenbosch (STE-U). Accession numbers and other data pertaining to the various *Elsinoë* isolates examined are listed in Table 1. Outgroups chosen for molecular comparisons were an *Elsinoë* sp. from *Citrus* for the RAPDs, and *Amphisphaeria umbrina* (Fr.) De Not. for the sequencing reactions.

Morphological characterisation and cultural studies - Sporulation in culture was induced by using Whiteside's (1975) method, as isolates did not sporulate on MEA. Ten milliliters of the modified Fries's medium was poured into a 90 mm Petri dish. The liquid medium consisted of 5 g $(NH_4)_2C_4H_4O_6$, 1 g NH_4NO_3, 1 g K_2HPO_4, 0.5 g $MgSO_4 .7H_2O$, 0.1 g $CaCl_2$, 0.1 g NaCl, and 20 g sucrose in 1 liter of distilled water. Mycelial fragments were taken from colonies and distributed in Petri dishes containing Fries's medium. After 4 days at 20°C, small colonies (1 mm in diam.) developed on the bottom of the Petri dishes. Colonies were flushed with sterile distilled water, scraped from the dish with a scalpel and transferred to drops of distilled water on glass slides. Mounts from host tissue were prepared in lactophenol, and measurements made at 1000x magnification. Averages were derived from at least 30 observations, and the range given in parentheses. Cardinal temperature requirements for growth were determined on MEA after 1 mo in the dark at 5-35°C in 5° intervals, with three replicate plates per temperature. The experiment was repeated once. Colony colours were determined according to Rayner (1970).

DNA extraction - Total DNA of single conidial isolates was extracted from 1-mo-old colonies grown on potato dextrose agar (PDA, Biolab) at room temperature. Fungal colonies were scraped clean of agar, frozen in liquid nitrogen, and ground to a fine powder. DNA was subsequently extracted by the method of Dellaporta *et al.* (1983) and quantified by ethidium bromide fluorescence on an UV transilluminator with known quantities of lambda DNA (Sambrook *et al.*, 1989).

Polymerase chain reaction (PCR) amplification - PCR amplifications were carried out in 25 µl volumes in thin-walled Eppendorf tubes in an Idaho Technology Air Thermo Cycler, model 1605

(Idaho Technology, Idaho Falls, Idaho, USA). Reaction mixtures contained 1-5 ng template DNA, 100 µM each of dATP, dCTP, dGTP and dTTP, 2.5 µL 10x ammonium sulphate buffer (670 mM Tris-HCl, pH 8.8, 40 mM $MgCl_2$, 160 mM $(NH_4)_2SO_4$, 0.01% Tween 20), 0.2 µM oligonucleotide primers, and 0.5 units of Taq DNA polemerase (Boehringer, Mannheim Ltd., Mannheim, Germany). Mixtures were subjected to 45 cycles of denaturation at 94°C for 15 s, annealing at 37°C for 30 s, and elongation at 72°C for 35 s. The 45 cycles were followed by a final extension step of 4 min at 72°C. Eight 10-mer primers from Kit OPM (OPM-1, -2, -6, -7, -9, -14, -15 and -16) three primers from Kit OPE (OPE-7, -14 and -15) and one primer from Kit OPY (OPY-19) of Operon Technologies (Alameda, California, USA) were used. The primers and their sequences are listed in Table 2. The PCR amplification was repeated once for each primer. Products generated by PCR amplification were separated by electrophoresis according to size in 1.5% agarose gels, stained with ethidium bromide and visualised under an UV transilluminator (312 nm). All analyses included a negative control with all components except template DNA. The 5.8S ribosomal gene and the two flanking internal transcribed spacers (ITS1 and ITS2) were amplified with primers ITS1 (5'-dTCCGTAGGTGAACCTGCGG) and ITS4 (5'-dTCCTCCGCTTATTGATATGC) (White *et al.*, 1990). Thermal cycling conditions for amplification included an initial hold at 94°C for 2 min; followed by 30 cycles of 94°C for 15 s, 55°C for 30 s and 72°C for 35 s; and finally followed by a 4 min incubation at 72°C.

Sequencing reactions - PCR products of representative isolates from different proteaceous hosts were used for sequencing (Table 1). Prior to sequencing, the PCR products were purified from unincorporated nucleotides and primers using Wizard™ PCR Preps DNA Purification System (Promega Corporation, Madison, Wisconsin, USA). Both the forward and reverse strands of each fragment were sequenced to achieve greater confidence in the consistence of the sequence data, using an automated sequencer ABI Prism 377 DNA Sequencer (PE Biosystems, Inc., Foster City, CA. USA). The primers used for sequencing were the same as those used for PCR.

A Dye Terminator Cycle Sequencing Ready Reaction Kit containing AmpliTaq DNA Polymerase (Perkin-Elmer) was used for the sequencing reactions. The reactions were carried out with a concentration of 20 to 40 ng of DNA template and 3.2 pmol primer in a total volume of 10 µL. The cycle sequencing reaction was done by PCR under conditions of 96°C for 30 s, 50°C for 15 s, and 60°C for 4 min. This was repeated for 25 cycles. DNA was finally purified

using Centri-Sep Spin columns (Princeton Separations, Adelphia, New Jersey) and loaded onto the sequencing gel.

Statistical analysis of RAPD patterns - Both faint and intense bands shown to be reproducible in the two runs were scored. The presence or absence of bands was compared using a computer software program NTSYS-pc (Rohlf, 1990) to generate a data matrix (data not shown). This program analyses data by average linkage cluster analysis using shared fragments and simple matching similarity coefficients. The results obtained with all twelve primers were combined in a single analysis. Each band in each pattern was given a number to distinguish it from the other bands in all twelve patterns, and all the bands were included in the analysis. An Unweighted Pair Group Method with Arithmetic Mean (UPGMA) (Sneath & Sokal, 1973) analysis was performed on the matching coefficient, using the SAHN program of NTSYS. A dendrogram showing the relationship among the RAPD patterns was generated from these coefficients using the TREE program of NTSYS.

Phylogenetic analysis - The DNA sequences of the isolates in Table 1 obtained from this study (GenBank accession numbers: AF 097572-AF 097578; AF 131080-AF 131091) and the DNA sequence in the same region of *A. umbrina* obtained from GenBank (accession number: AF009805) as an outgroup, were edited with the Tex-Edit Plus program (Bender, 1995). All sequencing tracks were checked visually and minor manual modifications were introduced to improve alignment. Alignment of the sequences was conducted using the CLUSTAL W software program (Thompson *et al.*, 1994). A phylogenetic tree was constructed using PAUP 3.1.1 (Swofford, 1993). Unweighted parsimony analyses were performed on the DNA sequence data using the heuristic search option with 1,000 random addition sequences. Clade stability was assessed by 1,000 parsimony bootstrap replications. The best fit maximum likelihood method was also used to evaluate the topology of the tree using the program DNAML (Felsenstein, 1993) with 10 randomisations of sequence input order of the original data set and global rearrangement of the tree. Both the neighbour-joining and the best fit maximum likelihood methods produced identical topologies of the parsimony tree.

RESULTS

Phylogenetic analysis

RAPD-PCR - The twelve different random 10-mer primers that were tested all produced reproducible RAPD patterns and were used for a comparative analysis. The characteristic RAPD banding patterns generated by the twelve randomly selected primers are summarised in Table 2. With respect to the primers used, 11 to 32 DNA fragments, varying in intensity, were amplified. A total of 216 and 204 amplification and polymorphic products were respectively generated among the *Elsinoë* isolates. RAPD patterns obtained with primers OPM-9 and OPY-19 are shown in Fig. 1. The RAPD profiles broadly divided the isolates into 5 major groups. The isolates from *Leucospermum* and *Leucadendron* exhibited the same RAPD patterns, indicating that these isolates are very closely related. The isolates from *Protea, Banksia, Serruria* and *Citrus* represented four distinct groups. A dendrogram (Fig. 2), indicating the relationships among the *Elsinoë* isolates studied was constructed from a cluster analysis of the similarity matrix.

Sequencing - For each isolate 514-606 bp of PCR amplification product of the gene encoding 5.8S rDNA and ITS regions were sequenced. The sequence data were aligned for all isolates. The alignment of the sequences of the 5.8S ribosomal RNA gene and the flanking internal transcribed spacers (ITS1 and ITS2) had a total consensus length of 549 bp. Of the aligned sites, 215 (39%) showed homology, 54 (10%) were constant, 280 (51%) were variable and 78 (14%) were informative for use in parsimony. The ITS1 and ITS2 showed sequence variation.

The phylogenetic tree (Fig. 3) divided the isolates into five groups, group one representing an isolate from *Banksia*, group two isolates from *Leucadendron, Leucospermum* and *Serruria*, group three an isolate from South African *Protea*, group four isolates from Zimbabwean *Protea*, and group five an isolate from *Citrus*. These groups were generally consistent with the RAPD data, and correlated well with similarities in general morphology.

Description of species

Elsinoë banksiae sp. nov. Figs 4-8.

Anamorph - Sphaceloma sp.

Ascomata dispersa, separata, pulvinata, subcuticularia, ex textura angulari pseudoparenchymatica pallida vel media brunnea composita, ad 100 mm lata et alta. Asci diffusi irregulariter ubique in ascomate, ovoidei vel globosi, extremis rotundatis, crasso pariete, octospori, sessiles, hyalini, 20-30 x 20-27 mm. Ascosporae hyalinae, late ellipsoideae, extremis rotundatis vel obtusis, 2-3 septis transversalibus et 1-3 septis verticalibus et 0-2 septis obliquis praeditae; constrictae ad septum medianum, (15-)17-19(-20) x 6-7(-8) mm. Mycelium internum, compositum ex hyphis ramulosis, septatis, pallidis vel mediis brunneis, 3.5-4.5 mm diam. Conidiomata acervulata, foliicola et caulicola, subelevata, coalescentia in maturitate, composita e textura angulari media brunnea, usque ad 200 mm lata et 1 mm longa. Conidiophori subcylindrati, pallidi vel medii brunnei, verruculosi, 0-3 septa, 20-30 x 3-5 mm. Conidiogenae cellulae enteroblasticae, polyphialidicae, 1-2 locis integris praeditae, pallidae brunneae, verruculosae, doliiformes, 6-13 x 4-6 mm. Conidia hyalina, aseptata, ellipsoidea, obtuso apice, basi constricta ad locum subtruncatum, (3.5-)4-6(-7) x (1.5-)2-3(-3.5) mm.

Holotype – **Australia: New South Wales, Brisbane Waters National Park**, on leaves of *Banksia serrata* L. f., 23 Oct. 1986, B.C. Sutton, J. Walker & M. Priest, (VPRI 14815a).

Leaf spots circular to irregular, predominantly epiphyllous on leaves, extending partially through the lamina; initially small grey specks with a brown margin, surrounded by a chlorotic zone, 0.5-7 mm diam. Lesions also develop on stems and midribs of leaves, circular to irregular, medium brown, frequently with large black acervuli, appearing as longitudinal ruptures of the epidermis. *Ascomata* scattered, separate, pulvinate, subcuticular, composed of light to medium brown pseudoparenchymatic *textura angularis*, up to 100 µm wide and high. *Asci* distributed irregularly throughout the ascoma, ovoid to globose with rounded ends, thick-walled, 8-spored, sessile, hyaline, 20-30 x 20-27 µm. *Ascospores* hyaline, broadly ellipsoid with rounded to obtuse ends, with 2-3 transverse septa and 1-3 vertical septa and 0-2 oblique septa; constricted at median septum, (15-)17-19(-20) x 6-7(-8) µm. *Mycelium* internal, consisting of branched, septate,

medium to dark brown hyphae, 3.5-4.5 µm diam. *Conidiomata* acervular, foliicolous and caulicolous, raised, coalescing at maturity, composed of medium brown *textura angularis*, up to 200 µm wide and 1 mm long. *Conidiophores* subcylindrical, light to medium brown, verruculose, 0-3-septate, 20-30 x 3-5 µm. *Conidiogenous cells* enteroblastic, polyphialidic, with 1-2 integrated loci, light brown, verruculose, doliiform, 6-13 x 4-6 µm. *Conidia* hyaline, aseptate, ellipsoid, apex obtuse, constricting at base to a subtruncate locus, (3.5-)4-6(-7) x (1.5-)2-3(-3.5) µm *in vivo*. Could not be induced to sporulate *in vitro* on Fries's medium.

Cultures - On MEA colonies were irregular, erumpent, folded; margins sinuate, smooth, and aerial mycelium absent; surface blood red (71k), with a lighter red outer zone, surrounded by a diffuse red pigment. Colonies reached 10 mm in diam on MEA after 1 mo in the dark at 25°C.

Cardinal temperatures for growth - Min. 5°C, opt. 20-25°C, max. above 35°C.

Substrate - *Banksia* spp.

Distribution - Australia.

Additional specimens examined – **Australia: New South Wales, Durras**, on leaves of *Banksia serrata*, 23 May 1986, B. Fleming, (VPRI 13976a); **Adelaide S.A.**, on leaves of *Banksia* sp., 20 Oct. 1995, B. Hall, (VPRI 20732a); **Victoria, Longford**, on leaves and stems of *Banksia prionotes* Lindl., 5 Aug. 1996, D. Tricks & A. Ziehrl, (VPRI 21100a, VPRI 21100b).

Elsinoë leucospermi sp. nov. Figs 9-18.

Anamorph - *Sphaceloma* sp.

Ascomata dispersa, separata, pulvinata, subcuticularia, composita e textura angulari pseudoparenchymatica medio- vel atrobrunnea, ad 200 mm lata et 60 mm alta. Asci diffusi irregulariter ubique in ascomate, ovoidei vel globosi, crasso pariete, octospori, sessiles, hyalini, 16-28 x 13-18 mm. Ascosporae hyalinae, late ellipsoideae, extremis rotundatis vel obtusis, 1-4 septis transversalibus et 1-2 septis verticalibus praeditae, septo obliquo raro praesente; constrictae ad septum medianum, (10-)12-14(-19) x 4-5 mm. Mycelium internum, compositum ex hyphis ramulosis, septatis, hyalinis vel brunneis, 3.5-4.5 mm diam. Conidiomata acervulata, foliicola sed primum caulicola, subelevata, coalescentia in maturitate, composita ex textura angulari mediobrunnea, usque ad 200 mm lata et 1mm longa. Conidiophori subcylindrati, pallidi brunnei,

verruculosi, 0-2 septa, 20-30 x 3-6 mm. Conidiogenae cellulae enteroblasticae, polyphialidicae, 1-2 locis integris praeditae, pallidae brunneae, verruculosae, ampulliformes addoliiformes, 10-15 x 3-4 mm. Conidia hyalina, granulata, aseptata, ellipsoidea, obtuso apice, basi constricta ad locum subtruncatum, (2-)5-7(-8) x (1-)2.5-3 mm.

Holotype – **South Africa: Western Cape, Harold Porter Botanical Gardens, Betty's bay**, on stems and leaves of *Lsp. cordifolium* (Salisb. ex Knight) Fourc., 11 May 1996, L. Swart, PREM 54980 (holotype), ex-type (STE-U 1378-1380).

Leaf spots on *Leucospermum* and *Leucadendron* circular to irregular, amphigenous, 1-4 mm diam, extending through the lamina, but more prominent on the surface initially infected; translucent, becoming medium brown, slightly raised, rough and corky with age; border slightly raised, appearing whitish due to the ruptured epidermis; margin present, thin, red-purple. Lesions also develop on immature shoots, appearing as small, raised, yellow-brown to reddish spots, circular to irregular, 1-8 mm diam, enlarging and coalescing to form raised, irregular, medium brown lesions; borders raised, whitish to light brown due to the ruptured epidermis; red-brown margins frequently present; lesions become roughened and corky at maturity, and often have longitudinal cracks, the smaller of which frequently support acervuli of the asexual state. Severe infection can also lead to a twisting and distortion of the stem. Lesions on *Serruria* occurring on stems, consist of small specks to raised, circular spots, light brown, 1-3 mm diam, surrounded by a distinct, wide, red margin, up to 2 mm in diam. On leaves symptoms vary from small necrotic spots with red margins to a blighting of the whole lamina. *Ascomata* scattered, separate, pulvinate, subcuticular, composed of medium to dark brown pseudoparenchymatic *textura angularis*, up to 200 µm wide and 60 µm high. *Asci* are distributed irregularly throughout the ascoma, ovoid to subglobose, thick-walled, 8-spored, sessile, hyaline, 16-28 x 13-18 µm. *Ascospores* hyaline, broadly ellipsoid with rounded to obtuse ends, 1-4 transversely septate, and 1-2 vertical septa; oblique septa rare; constricted at median septum, (10-)12-14(-19) x 4-5 µm. *Mycelium* internal, consisting of branched, septate, hyaline to brown hyphae, 3.5-4.5 µm diam. *Conidiomata* acervular, foliicolous but primarily caulicolous, raised, coalescing at maturity, composed of medium brown *textura angularis*, up to 200 µm wide and 1 mm long. *Conidiophores* subcylindrical, light brown, verruculose, 0-2-septate, 20-30 x 3-6 µm. *Conidiogenous cells* enteroblastic, polyphialidic, with 1-2 integrated loci, light brown, verruculose, ampulliform to doliiform, 10-15 x 3-4 µm. *Conidia* hyaline, granular, aseptate,

ellipsoid, apex obtuse, constricting at base to a subtruncate locus, (2-)5-7(-8) x (1-)2.5-3 µm *in vivo*, 5-7 x 2-3 µm *in vitro* on Fries's medium.

Cultures - On MEA colonies were irregular, erumpent, folded; margins sinuate, smooth, and aerial mycelium absent; surface red (71i) to blood red (71k), sienna (13i) or coral (3'b). Colonies reached 7-12 mm in diam on MEA after 1 mo in the dark at 25°C.

Cardinal temperatures for growth - Min. above 5-10 °C, opt. 20-25° C, max. above 35 °C.

Substrate - *Leucadendron* spp., *Leucospermum* spp., *Serruria florida* (Thunb.) Salisb. ex Knight.

Distribution - Australia, South Africa, USA (California), Zimbabwe.

Additional specimens examined – **Australia: Victoria, Red Hill**, on stems and leaves of *Lcd. thymifolium* (Salisb. ex Knight) I. Williams, 8 Aug. 1994, I. Pascoe & A. Ziehrl, (VPRI 20330a); **Gembrook**, on stems and leaves of *Leucospermum* sp., 8 Mar. 1995, I. Pascoe & A. Ziehrl, (VPRI 20521a); on stems and leaves of *Leucadendron* sp., 8 Mar. 1995, I. Pascoe & A. Ziehrl, (VPRI 20522a); on stems and leaves of *Lsp. cordifolium*, 8 Aug. 1996, A. Ziehrl, (VPRI 21187a); on stems and leaves of *Leucadendron* sp., 8 Aug. 1996, A. Ziehrl, (VPRI 21189a); **Merricks North**, on stems and leaves of *Lcd. laureolum* (Lam.) Fourc., 9 Mar. 1995, I. Pascoe & A. Ziehrl, (VPRI 20515a); **Bunyip**, on stems and leaves of *Leucadendron* sp., 9 Mar. 1995, I. Pascoe & A. Ziehrl, (VPRI 20526a); **Victoria**, on stems and leaves of *Serruria florida* 12 May 1995, W. Tregea, (VPRI 20591a); on stems and leaves of *Serruria florida*, 8 Aug. 1996, D. Tricks & A. Zierhl, (VPRI 21188a); **South Africa: Eastern Cape, Humansdorp**, on stems and leaves of *Lcd. pubibracteolatum* I. Williams x *Lcd. chamelaea* (Lam.) I. Williams, 2 Sept. 1997, L. Swart, (STE-U 2033); on stems and leaves of *Lcd. linifolium* (Jacq.) R. Br., 2 Sept. 1997, L. Swart; **Western Cape, Kleinmond**, on stems and leaves of *Leucospermum* sp., 1983, L. Benic, (STE-U 1605-1606); on stems and leaves of *Lsp. cordifolium*, 8 May 1996, L. Swart, (STE-U 1610); **Stellenbosch**, on stems and leaves of *Lsp. cordifolium*, 6 Aug. 1996, L. Swart, (STE-U 1607-1608); **Constantia**, on stems and leaves of *Lsp. cordifolium*, 17 Sept 1996, L. Swart, (STE-U 1604); **McGregor**, on stems and leaves of *Serruria florida*, 8 Sept. 1998, L. Swart, (STE-U 2043-2044); **USA: California, San Diego**, on stems and leaves of *Lsp. cordifolium* cv Vlam, 9 Apr. 1998, L. Swart, (STE-U 2041); on stems and leaves of *Lsp. cordifolium*, 13 Apr. 1998, L. Swart, (STE-U 2042); **Zimbabwe, Rusape**, on stems and leaves of *Lsp. glabrum* E. Phillips x *Lsp. tottum* (L.) R. Br. cv Scarlet Ribbon, 6 Mar.

1998, L. Swart, (STE-U 2039); on stems and leaves of *Lsp. reflexum* H. Buek ex Meisn. var *luteum*, 6 Mar. 1998, L. Swart, (STE-U 2040).

Elsinoë proteae sp. nov. Figs 19-22.

Anamorph - Sphaceloma sp.

Ascomata dispersa, separata, pulvinata, subcuticularia, composita e textura angulari pseudoparenchymatica medio- vel atrobrunnea, ad 250 mm lata et 80 mm alta. Asci diffusi irregulariter ubique in ascomate, ovoidei vel globosi, crasso pariete, octospori, sessiles, hyalini, 24-45 x 19-24 mm. Ascosporae hyalinae ad olivaceae, late ellipsoideae, extremis rotundatis, 3-4 septis transversalibus et 1-3 septis verticalibus praeditae, septo obliquo interdum praesente; plerumque leviter constrictae ad septum medianum, (14-)16-17(-20) x (5-)6-7 mm. Sphacelomatis statu non observato in hospite, sed in agaro dicto "Fries" formato. Conidiophori reducti ad conidiogenas cellulas. Conidiogenae cellulae enteroblasticae, poliphialidicae, 1-3 locis integris, hyalinae, leves, subcylindratae ad doliiformes, 6-12 x 4-6 mm. Conidia hyalina, granulata, aseptata, ellipsoidea, obtuso apice, basi rotundata vel subtruncata, (5-)6-7(-8) x 2-3(-4) mm in vivo.

Holotype – **South Africa: Western Cape, Harold Porter Botanical Gardens, Betty's bay**, on leaves of *Protea cynaroides* (L.) L., 15 Feb. 1996, P.W. Crous, PREM 54979 (holotype), ex-type (STE-U 1348-1350).

Leaf spots occurring on leaves and petioles, separate, becoming confluent, circular, 2-12 mm diam, amphigenous, not extending through the leaf lamina, raised with a discrete border, white-grey, with black ascomata visible to the naked eye. *Ascomata* scattered, separate, pulvinate, subcuticular, composed of medium to dark brown pseudoparenchymatic *textura angularis*, up to 250 µm wide and 80 µm high. *Asci* distributed irregularly throughout the ascoma, ovoid to subglobose, thick-walled, 8-spored, sessile, hyaline, 28-45 x 19-24 µm. *Ascospores* hyaline to olivaceous, broadly ellipsoid with rounded ends, with 3-5-transverse septa, and 1-3 vertical septa; oblique septa sometimes present; mostly slightly constricted at the median septum, (14-)16-17(-20) x (5-)6-7 µm. *Sphaceloma* state not observed on host, but induced on Fries's medium. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* enteroblastic, polyphialidic, with 1-3 integrated loci, hyaline, smooth, subcylindrical to doliiform, 6-12 x 4-6 µm. *Conidia*

hyaline, granular, aseptate, ellipsoid, apex obtuse, base rounded to subtruncate, (5-)6-7(-8) x 2-3(-4) µm *in vivo*.

Cultures - On MEA colonies were irregular, erumpent, folded; margins sinuate, smooth, with a sparse, whitish aerial mycelium; surface rose to red (71b-71i); older colonies surrounded by a diffuse red pigment. Colonies reached 12 mm in diam on MEA after 1 mo in the dark at 20°C.

Cardinal temperatures for growth - Min. above 5°C, opt. 15-20°C, max. below 30°C.

Substrate - *P. cynaroides.*

Distribution - South Africa.

Sphaceloma protearum sp. nov. Figs 23, 24.

Mycelium internum, ex hyphis ramulosis compositum, septatis, hyalinis vel brunneis, 3-4 mm diam. Conidiomata acervulata in caulibus, sporodochia in foliis insita, composita e textura angulari mediobrunnea, ad 100 mm lata et 1 mm longa. Conidiophori subcylindracei ad doliiformes, pallidi vel mediobrunnei, verruculosi, 0-2 septati, 12-20 x 5-6 mm. Conidiogenae cellulae enteroblasticae, polyphialidicae, 1-2 locis integris praeditae, pallide brunneae, verruculosae, doliiformes, 5-12 x 4-6 mm. Conidia hyalina, granulata, aseptata, ellipsoidea, obtuso apice, basi constricta ad locum subtruncatum, (3.5-)5-6(-7) x (1.5-)2-2.5 mm.

Holotype – **Zimbabwe: Gomo Remiti Farm, Mutare**, on leaves and stems of *Protea eximia* x *susannae* cv Sylvia, 5 Mar. 1998, L. Swart, PREM 56301 (holotype), ex-type STE-U 2037.

Leaf spots circular, reddish, 5-15 mm diam, covered with erumpent conidiomata, appearing as reddish sporodochia on the necrotic tissue; commonly associated with Shepherd's crook symptoms on young shoots, leading to a blackening, withering and death of shoot tips. *Mycelium* internal, consisting of branched, septate, hyaline to brown hyphae, 3-4 µm diam. *Conidiomata* acervular on stems, or sporodochial on leaves, composed of medium brown *textura angularis*, up to 100 µm wide and 1 mm long. *Conidiophores* subcylindrical to doliiform, light to medium brown, verruculose, 0-2-septate, 12-20 x 5-6 µm. *Conidiogenous cells* enteroblastic, polyphialidic, with 1-2 integrated loci, light brown, verruculose, doliiform, 5-12 x 4-6 µm. *Conidia* hyaline, aseptate, ellipsoid, apex obtuse, constricting at base to a subtruncate locus, (3.5-)5-6(-7) x (1.5-)2-2.5 µm *in vivo*, not sporulating *in vitro* on Fries's medium.

Cultures - On MEA colonies were irregular, erumpent, folded; margins sinuate, smooth, aerial mycelium absent; surface blood red (71k-71m), with a slight diffuse red pigment becoming visible in the agar with age. Colonies reaching 11 mm in diam on MEA after 1 mo in the dark at 25°C.

Cardinal temperatures for growth - Min. above 5°C, opt. 20-25°C, max. below 30°C.

Hosts - *Protea* spp.

Distribution - Zimbabwe.

Additional specimens examined – **Zimbabwe: Mutare**, on stems and leaves of *Protea compacta* R. Br. *x Protea susannae* E. Phillips cv Pink Ice, 5 Mar. 1998, L. Swart, (STE-U 2035); on stems and leaves of *Protea eximia* (Salisb. ex Knight) Fourc. *x Protea susannae* cv Sylvia, 5 Mar. 1998, L. Swart, (STE-U 2037); on stems and leaves of *Protea magnifica* Link *x Protea susannae* cv Susara, 5 Mar. 1998, L. Swart, (STE-U 2038); **Harare**, on stems and leaves of *Protea eximia x Protea susannae* cv Sylvia, 6 Mar. 1998, L. Swart, (STE-U 2036); on stems and leaves of *Protea laurifolia* Thunb. cv Regal Pink, 6 Mar. 1998, L. Swart; on stems and leaves of *Protea neriifolia* R. Br. cvs Moonshine and Silvertips, 6 Mar. 1998, L. Swart; **Banket**, on stems and leaves of *Protea compacta x Protea susannae* cv Pink Ice, 9 Mar. 1998, L. Swart, PREM 56302, (STE-U 2034).

Undetermined specimens examined – **Australia: Victoria, Red Hill**, on leaves of *Protea* sp., 8 Aug. 1994, I. Pascoe & I. Porter, (VPRI 20320a, 20320b); on leaves of *Protea cynaroides*, 8 Aug. 1994, I. Pascoe, (VPRI 20321a); **Bunyip**, on leaves of *Protea compacta* 8 Mar. 1995, I. Pascoe & A. Ziehrl, (VPRI 20525a); on leaves of *Protea compacta*, 9 Mar. 1995, I. Pascoe & A. Ziehrl, (VPRI 20549a); **South Africa: Western Cape, Sir Lowry's Pass Villiage**, on stem of *Protea repens* (L.) L., 21 Feb. 1998, J.E. Taylor (deposited at PREM); **USA: California, San Diego**, on leaves and stem of *Protea mundii* Klotzsch, 8 Apr. 1998, L. Swart (deposited at PREM).

DISCUSSION

The present study provides the first evidence that several distinct *Elsinoë* spp. are associated with scab disease of Proteaceae. This is not totally unexpected, as the general symptoms of the disease on *Protea* and *Banksia* are totally different to those observed on *Leucospermum*, *Leucadendron* and *Serruria*. Moreover, the symptoms on *Protea* in South Africa differ

significantly from the symptoms on *Protea* in Zimbabwe. Material of an *Elsinoë* teleomorph was obtained on three host genera, namely *Leucospermum*, *Protea* and *Banksia*. The ascus and ascospore morphology of these collections suggests that they represent three distinct species. These findings are further corroborated by the distinct RAPD banding patterns generated by using twelve different primers and sequence data of the 5.8S rDNA and ITS regions. The analyses of the RAPD profiles and sequence variation of the 5.8S rDNA and ITS regions show that the isolates from *Leucospermum*, *Protea* and *Banksia* represent three distinct species. Furthermore, based on similar DNA sequences for the *Elsinoë* isolates from *Leucadendron*, *Leucospermum* and *Serruria* in South Africa and Australia, as well as the isolates from *Leucospermum* in California and Zimbabwe, it can be concluded that these isolates are representative of the same species. It further indicates that some *Elsinoë* species are non-host specific, occurring on more than one genus in the same family. The only exception is the Australian isolate from *Serruria*, which appears to be a separate species based on RAPD profiles, but clusters with isolates obtained from *Leucospermum*, *Leucadendron* and *Serruria* when ITS sequence data are compared. This is furthermore in agreement with its general morphology and cultural characteristics.

Contrary to earlier beliefs, the present study demonstrates that there are at least two respective *Elsinoë* spp. occurring on Proteaceae in South Africa. Scab, induced by *E. leucospermi*, is an important disease of *Leucospermum* spp., especially *Lsp. cordifolium* in South Africa. This disease has, however, been found to vary in severity on different cultivars and seedlings. Of the various *Leucospermum* cultivars, *Lsp. cordifolium* cv Vlam, Red Sunset and cv Gold Dust have been found to be extremely susceptible (Knox-Davies *et al.*, 1986). In knowing that it is the same pathogen occurring on *Leucospermum* and *Leucadendron* spp. in Australia, California, South Africa and Zimbabwe, possible communal programmes for the selection of resistant hybrids, and the implementation of different fungicide regimes for disease control could now be initiated in these countries.

The occurrence of an *Elsinoë* sp. on *Protea* cv Pink Ice in Australia was the first record of scab disease on this host (Ziehrl *et al.*, 1995). It has also been observed on *P. cynaroides*, *P. compacta* and on a *Protea* sp. in Australia, and on *P. mundii* in California, USA. The leaf symptoms on *P. cynaroides* from Australia correlate with those observed on this host in South Africa. Unfortunately, no cultures or teleomorph material from this host in Australia were available for comparison with the two species presently known from South Africa and

Zimbabwe, respectively. Similar symptoms to those on *P. cynaroides* were also observed on leaves of *P. repens* in South Africa, with ascospores (12-17 x 4.5-7 µm) resembling those of *E. proteae*. More isolates from *Protea* spp. in South Africa would also be required, however, to determine the variation present in this species.

The type material of *Elsinoë banksiae* was obtained from leaves of *Banksia serrata* (VPRI 13976). The culture used in the present study, however, was obtained from *Sphaceloma* acervuli occurring on *Banksia prionotes* (VPRI 21100b). Although there are some differences in the symptom expression between the two hosts, with stem lesions and acervuli being more prominant on *B. prionotes* than on *B. serrata*, the leaf spots appear similar in both collections. Nevertheless, it could be possible that more than one species of *Elsinoë* occurs on *Banksia*. Further collections and cultures would also be required to resolve this issue.

A comprehensive classification of *Elsinoë* species occurring on Proteaceae is attainable through a molecular phylogenetic approach. RAPD analyses and ITS sequence separate *Protea*, *Banksia* and *Leucospermum* isolates from each other and show a good correlation with general morphology. The value of these properties as taxonomic characters has thus been supported by the molecular data. As the *Elsinoë* state is seldom collected for these fungi, molecular studies are required to determine the genetic variation between isolates from various *Elsinoë* hosts in the family Proteaceae.

Although scab disease has also been reported from Proteaceae occurring in Hawaii (Protea Disease Letter, 1991), no cultures were available for inclusion in the present study. More detailed collections would therefore be required in the future from Australia, California, Hawaii, South Africa and Zimbabwe to suitably determine the molecular differences that exist between the isolates, as well as the host ranges and distribution of the various *Elsinoë* spp. associated with scab disease of Proteaceae in these countries.

Table 1. Accession numbers and collection data pertaining to the *Elsinoë* isolates used for molecular comparisons in the present study

Isolate	Collection	Host[c]	Geographic origin	Date isolated	Genbank
1[a,b]	VPRI 21100 STE-U 1508	*B. prionotes*	Australia	5 Aug. 1996	AF097572
2[a,b]	VPRI 21187 STE-U 1505	*Lsp. cordifolium*	Australia	8 Aug. 1996	AF097573
3[a,b]	VPRI 21188 STE-U 1511	*S. florida*	Australia	8 Aug. 1996	AF097574
4[a,b]	VPRI 21189 STE-U 1502	*Lcd.* sp	Australia	8 Aug. 1996	AF097575
5[a]	STE-U 1604	*Lsp. cordifolium*	South Africa	17 Sept. 1996	
6[a]	STE-U 1605	*Lsp.* sp.	South Africa	1983	
7[a]	STE-U 1606	*Lsp.* sp.	South Africa	1983	
8[a,b]	STE-U 1607	*Lsp. cordifolium*	South Africa	6 Aug. 1996	AF097576
9[a]	STE-U 1608	*Lsp. cordifolium*	South Africa	6 Aug. 1996	
10[a,b]	STE-U 1609	*Citrus* sp.	South Africa	1995	AF097577
11[a,b]	STE-U 1349	*P. cynaroides*	South Africa	15 Febr. 1996	AF097578
12[a]	STE-U 1610	*Lsp.* sp.	South Africa	8 May 1996	
13[b]	STE-U 2033	*Lcd. pubibracteolatum x chamelaea*	South Africa	2 Sept. 1997	AF131080
14[b]	STE-U 2034	*P. compacta x susannae* cv Pink Ice	Zimbabwe	9 Mar. 1998	AF131081
15[b]	STE-U 2035	cv Pink Ice	Zimbabwe	5 Mar. 1998	AF131082
16[b]	STE-U 2036	*P. eximia x susannae* cv Sylvia	Zimbabwe	6 Mar. 1998	AF131083
17[b]	STE-U 2037	cv Sylvia	Zimbabwe	5 Mar.1998	AF131084
18[b]	STE-U 2038	*P. magnifica x susannae* cv Susara	Zimbabwe	5 Mar. 1998	AF131085
19[b]	STE-U 2039	*Lsp. glabrum x tottum* cv Scarlet Ribbon	Zimbabwe	6 Mar. 1998	AF131086
20[b]	STE-U 2040	*Lsp. reflexum* var. *luteum*	Zimbabwe	6 Mar. 1998	AF131087
21[b]	STE-U 2041	*Lsp. cordifolium* cv Vlam	California	9 Apr. 1998	AF131088
22[b]	STE-U 2042	*Lsp. cordifolium*	California	13 Apr. 1998	AF131089
23[b]	STE-U 2043	*S. florida*	South Africa	8 Sept. 1998	AF131090
24[b]	STE-U 2044	*S. florida*	South Africa	8 Sept. 1998	AF131091

[a] Isolates used in RAPD analysis

[b] Isolates used for sequencing analysis

[c] *B. = Banksia; Lcd. = Leucadendron; Lsp.= Leucospermum; P. = Protea; S. = Serruria*

Table 2. RAPD products generated by 12 randomly selected primers used in this study

Primer	Nucleotide sequence (5' to 3")	Number of amplified products	Number of polymorphic products
OPM-1	GTTGGTGGCT	18	18
OPM-2	ACAACGCCTC	16	16
OPM-6	CTGGGCAACT	11	10
OPM-7	CCGTGACTCA	12	9
OPM-9	GTCTTGCGGA	19	19
OPM-14	AGGGTCGTTC	18	14
OPM-15	GACCTACCAC	15	15
OPM-16	GTAACCAGCC	13	13
OPE-7	AGATGCAGCC	28	28
OPE-14	TGCGGCTGAG	16	13
OPE-15	ACGCACAACC	18	17
OPY-19	TGAGGGTCCC	32	32

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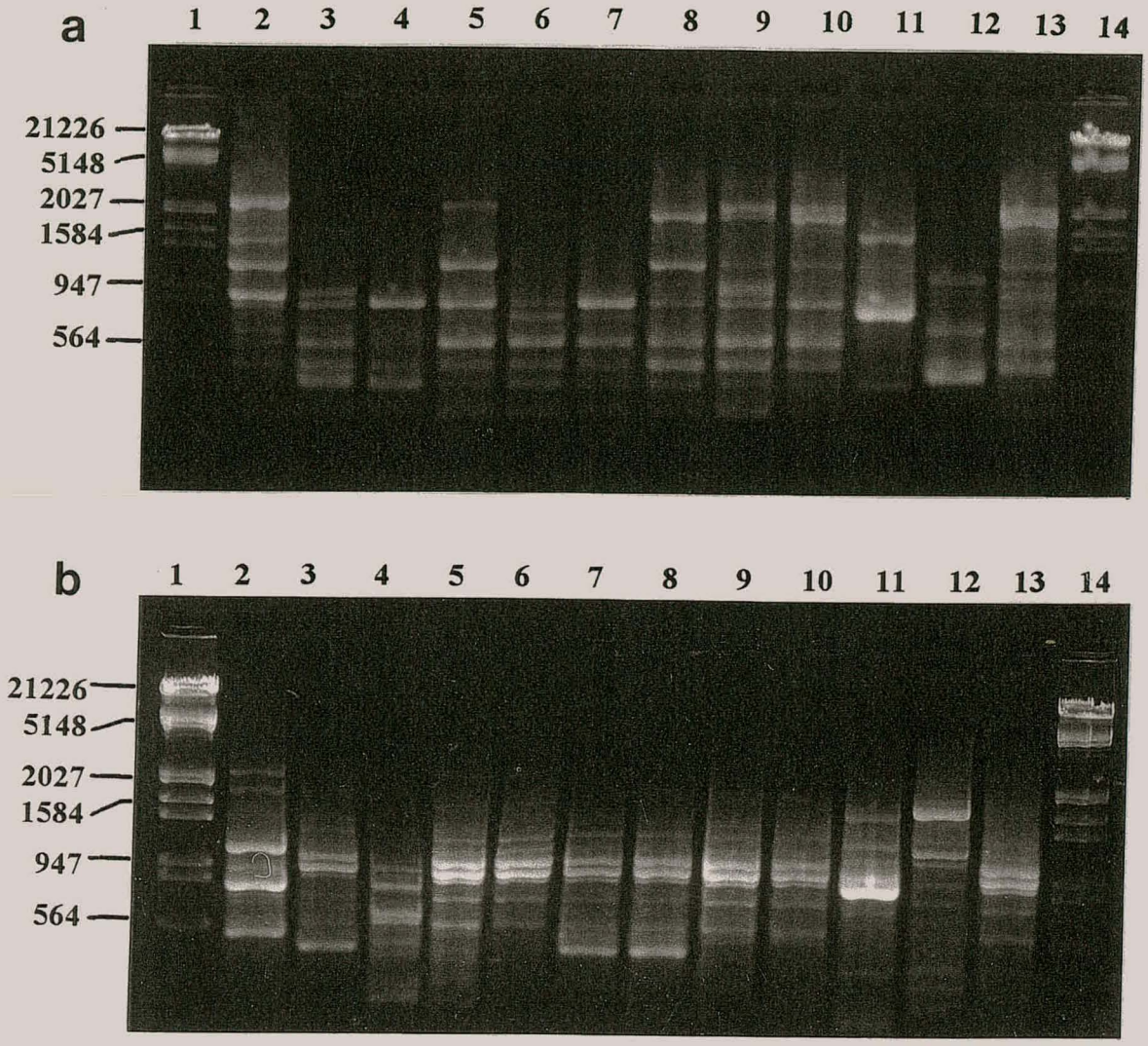


Fig. 1. RAPD banding patterns of 12 *Elsinoë* isolates generated using the primers a) OPM-9 and b) OPY-19. Lanes 1 and 14; lambda marker digested with *Eco*RI and *Hin*dIII. Lanes 2-12: 2, *E. banksiae* (STE-U 1508); 3, *E. leucospermi* from *Leucospermum* (STE-U 1505); 4, *E. leucospermi* from *Serruria* (STE-U 1511); 5, *E. leucospermi* from *Leucadendron* (STE-U 1502); 6-10, *E. leucospermi* from *Leucospermum* (STE-U 1604-1608); 11, *Elsinoë* sp. from *Citrus* (STE-U 1609); 12, *E. proteae* from *Protea* (STE-U 1349); 13, *E. leucospermi* from *Leucospermum* (STE-U 1610). The sizes of the fragments of the molecular markers are indicated in kb on the left.

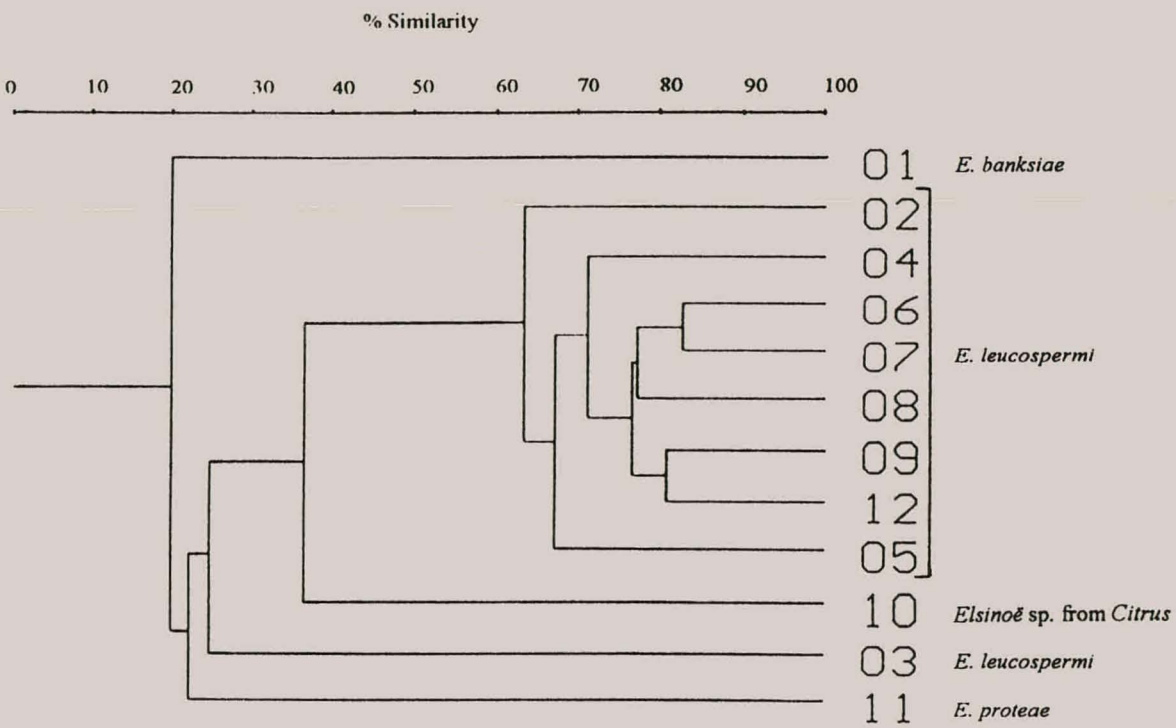


Fig. 2. UPGMA dendrogram of RAPD-PCR similarities between 12 *Elsinoë* isolates, using 12 different random primers. Isolates are numbered with accession numbers shown in Table 1.

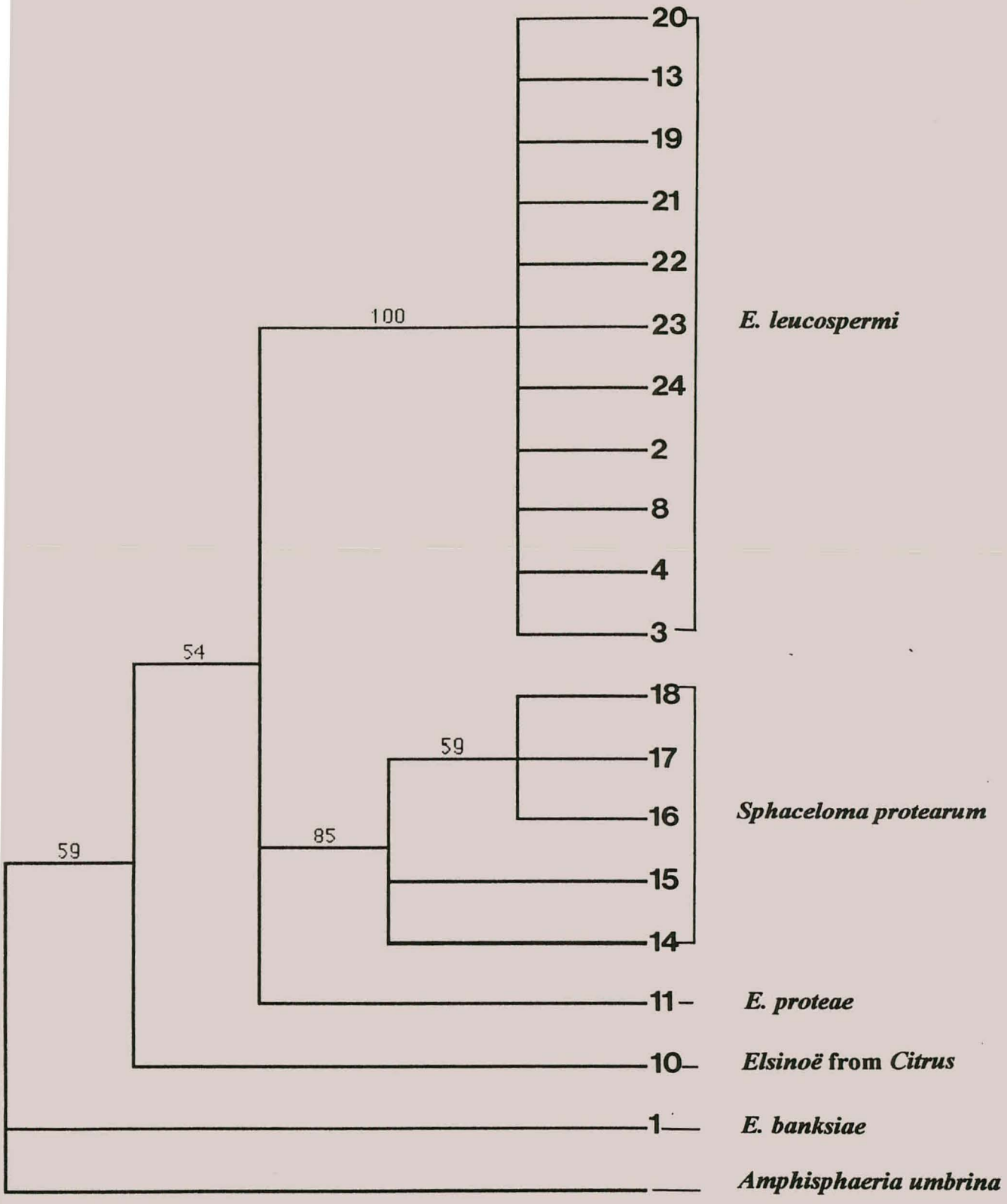
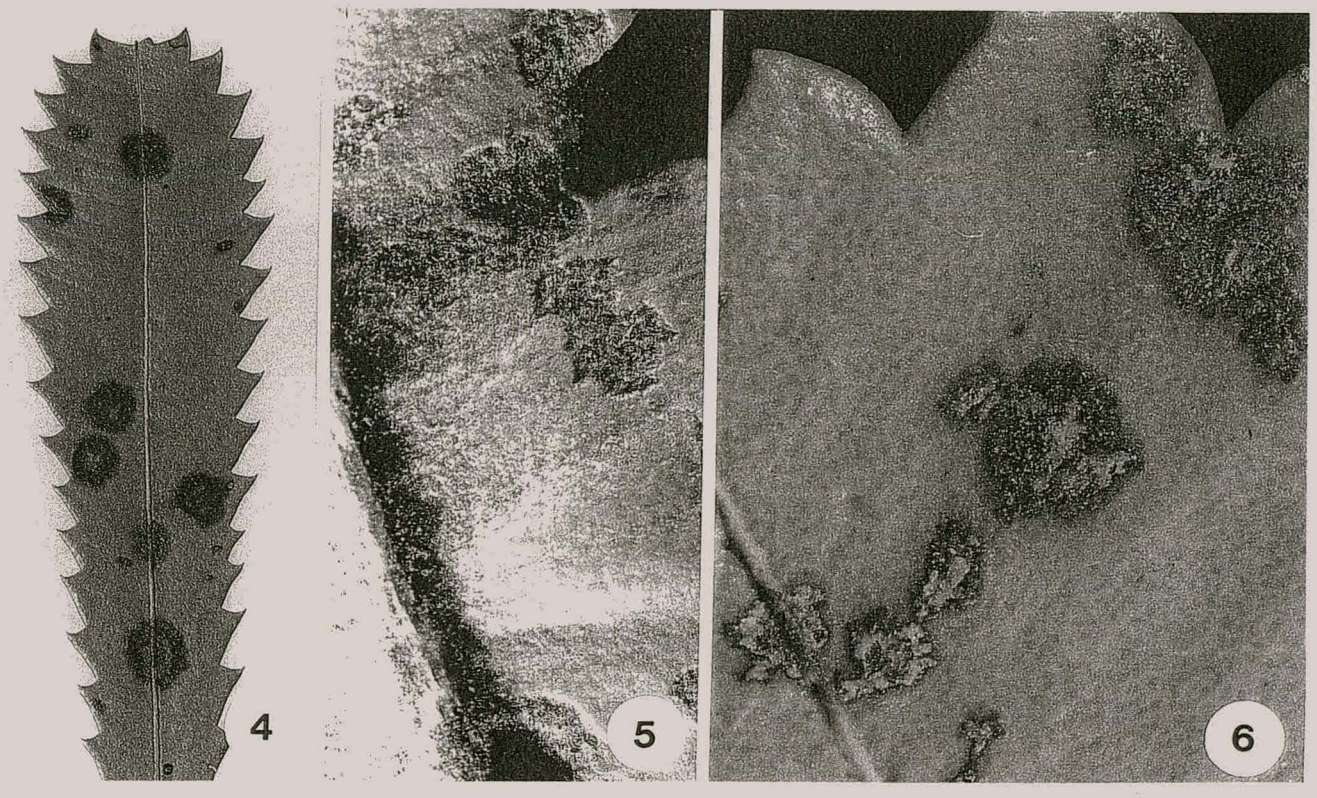
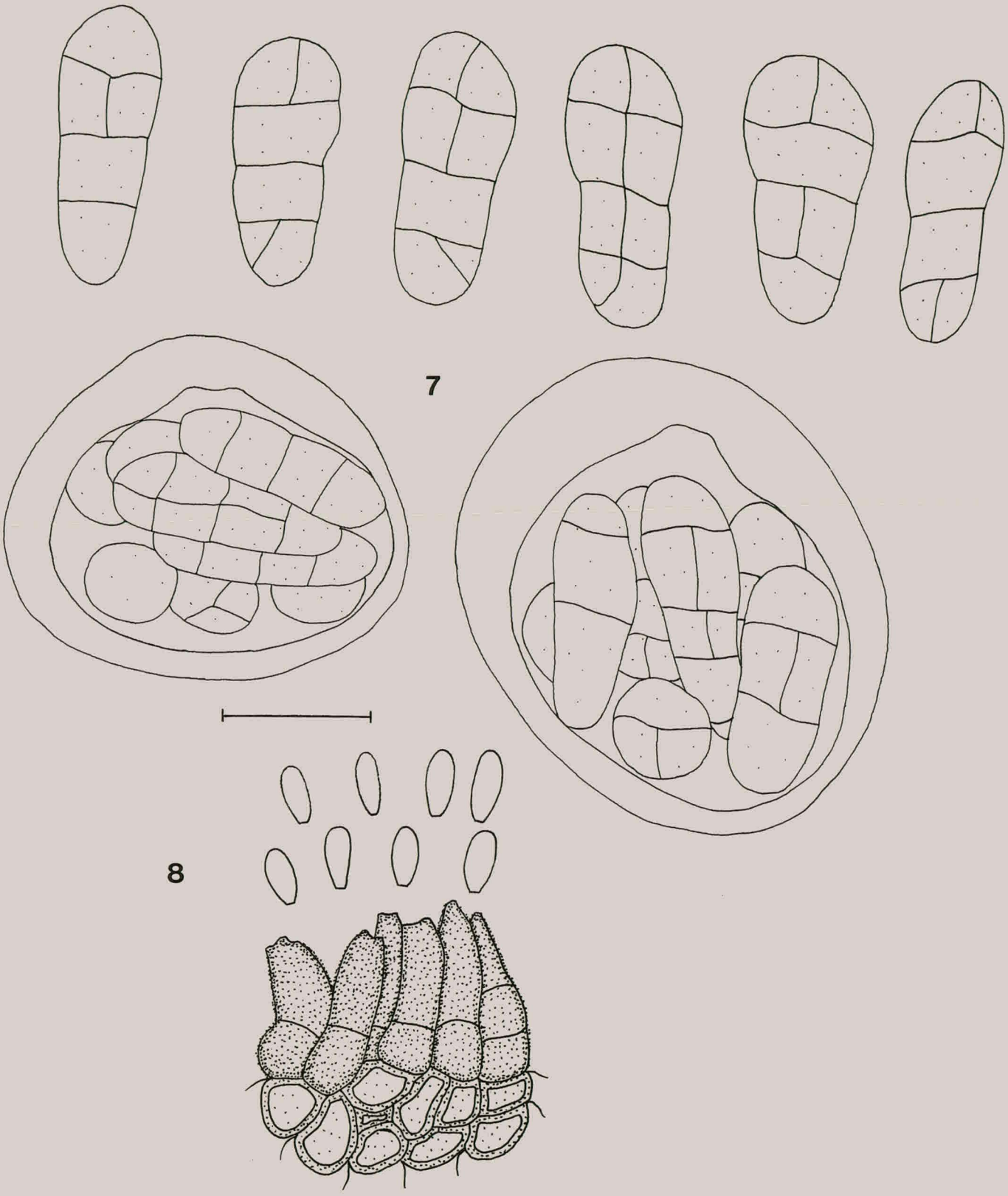


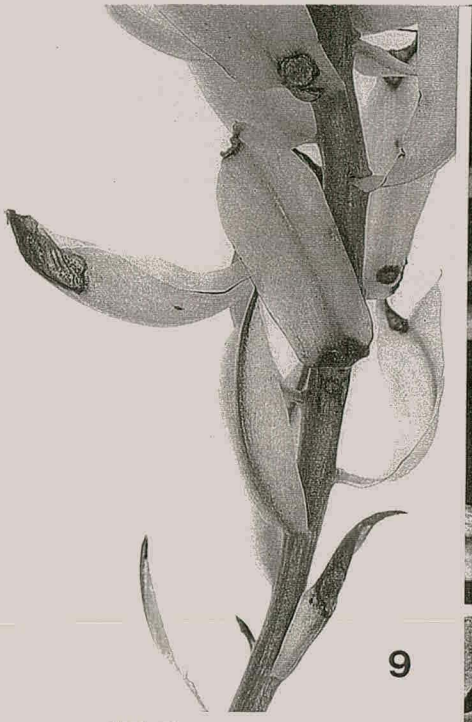
Fig. 3. Phylogenetic relationships of 19 *Elsinoë* isolates and *A. umbrina* as outgroup, based on rDNA ITS sequences. The tree illustrated was constructed using PAUP 3.1.1 (Swofford 1993). It is the consensus tree of 271 most parsimonious trees. The bootstrap value supports are shown on the branches.

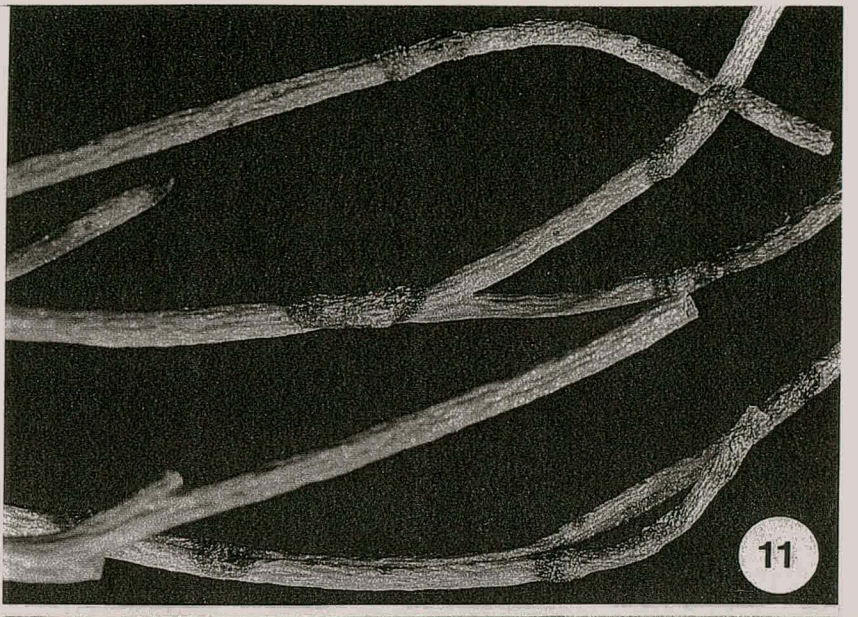


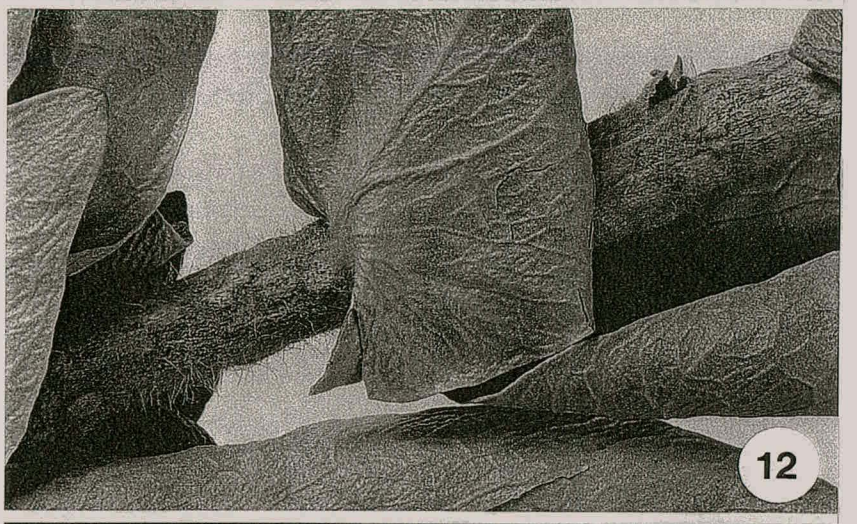
Figs 4-6. Foliar disease symptoms of *Elsinoë banksiae* on *Banksia* spp. **4.** *Banksia serrata*. **5.** *B. prionotes*. **6.** *B. serrata*.



Figs 7-8. *Elsinoë banksiae*. **7.** Asci and ascospores. **8.** Conidiophores and conidia of the *Sphaceloma* state (Bar = 10 µm).

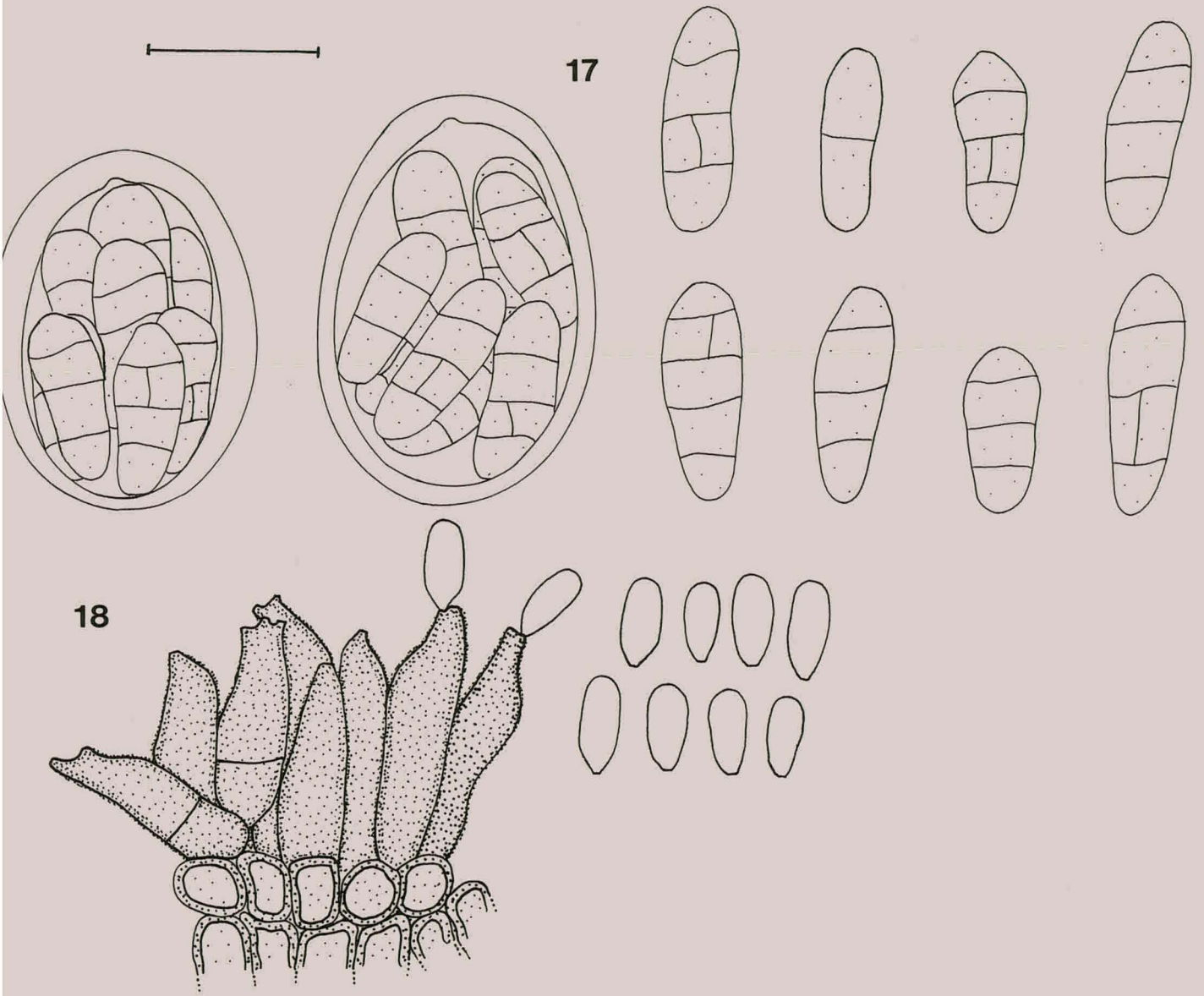




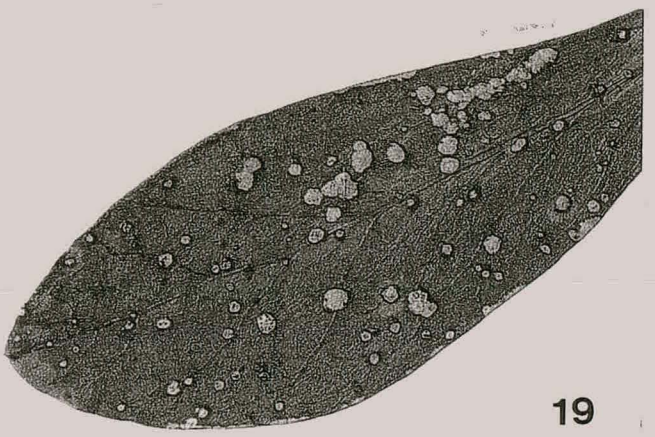


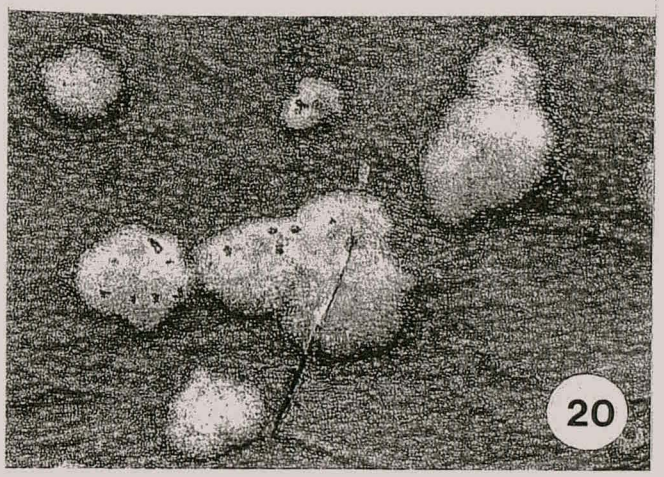
Figs 9-13. Foliar and stem disease symptoms of *Elsinoë leucospermi* on different hosts. **9-11.** *Serruria florida*. **12, 13.** *Leucospermum* hybrid.

Figs 14-16. Foliar and stem disease symptoms of *Elsinoë leucospermi* on *Leucadendron* spp. **14.** *Leucadendron* cv Silvan Red. **15.** *Lcd. thymifolium*. **16.** *Lcd. laureolum*.

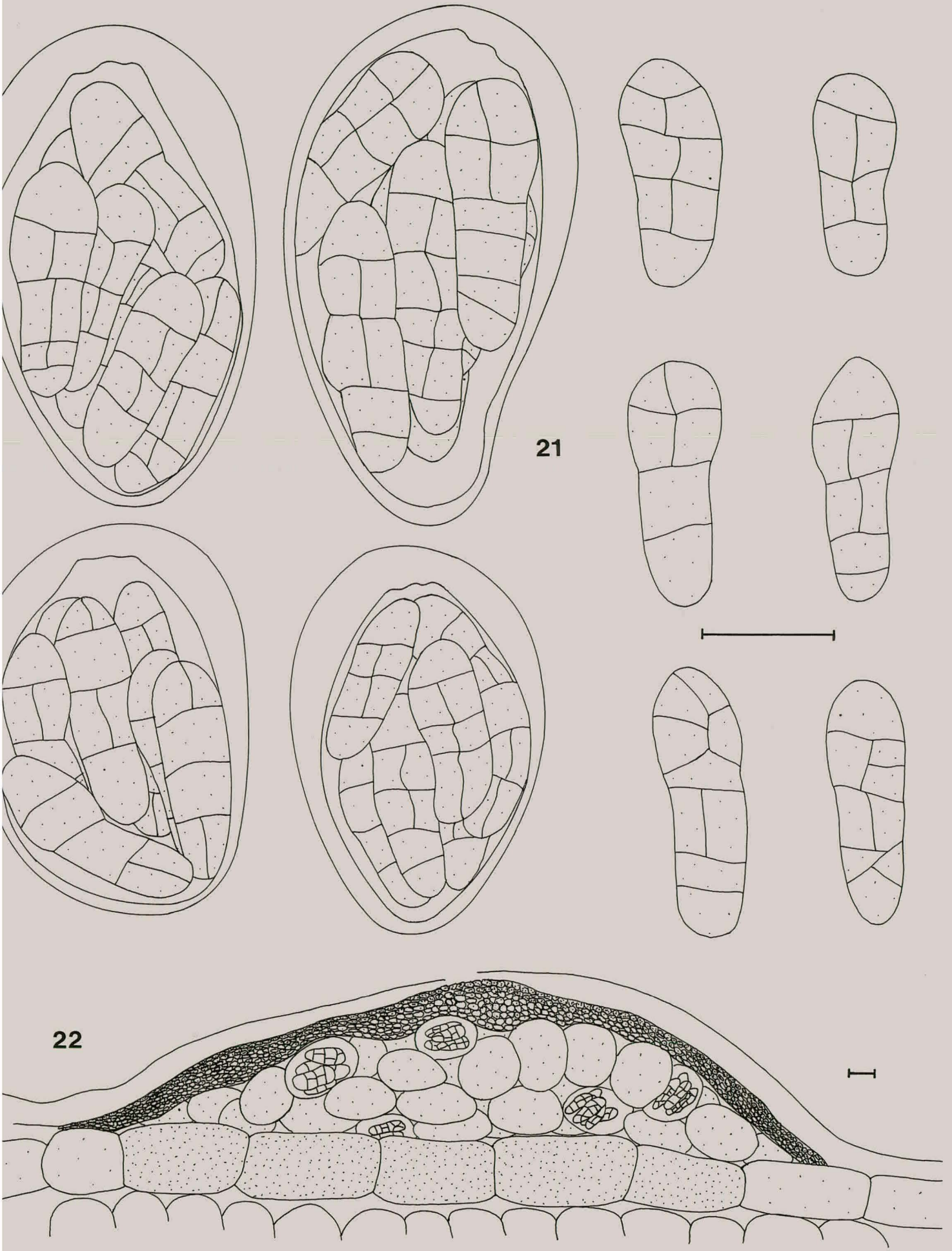


Figs 17, 18. *Elsinoë leucospermi*. **17.** Asci and ascospores. **18.** Conidiophores and conidia of the *Sphaceloma* state (Bar = 10 µm).





Figs 19, 20. Foliar disease symptoms of *Elsinoë proteae* on *Protea cynaroides*.



Figs 21, 22. *Elsinoë proteae*. **21.** Asci and ascospores. **22.** Vertical section through an ascoma on leaf tissue (Bars = 10 µm).

Figs 23, 24. Foliar and stem disease symptoms of *Sphaceloma protearum* on a *Protea* sp.

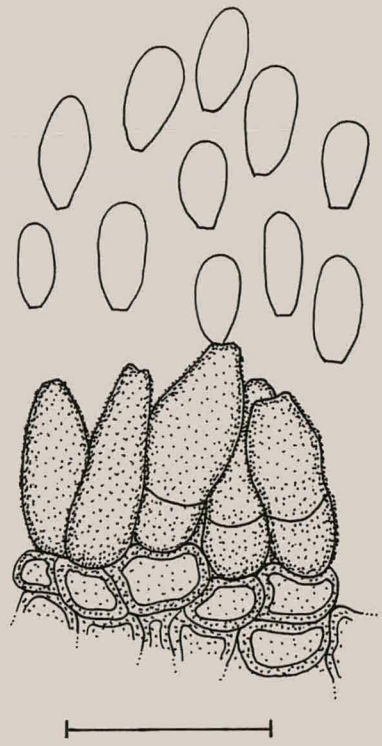


Fig. 25. Conidiophores and conidia of *Sphaceloma protearum* (Bar = 10 µm).

4. FUNGAL ENDOPHYTES OF PROTEACEAE, WITH PARTICULAR EMPHASIS ON *BOTRYOSPHAERIA PROTEAE* AND THE EFFECT OF WATER STRESS ON CANKER DEVELOPMENT

ABSTRACT

Fungal endophytes occurring in leaves and stems of a species of *Protea, Leucospermum* and *Leucadendron* were investigated in three locations in the Western Cape province of South Africa. The aim of this study was to determine if *Botryosphaeria proteae* was an endophyte of Proteaceae, and whether or not it was also an important canker pathogen. The pathogenicity of this fungus was evaluated on non-stressed plants, as well as on plants with a leaf water potential of –1.0 MPa (moderately stressed) and –2.0 MPa (severely stressed). Although *B. proteae* was routinely isolated as an endophyte, it was not regarded as a dominant taxon, and also did not occur in *Leucadendron*. Following inoculation trials with isolates obtained from *Protea* and *Leucospermum*, stem lesions were only induced in *Protea cynaroides* plants, and only on those severely stressed (-2.0 MPa). These results suggest that although *B. proteae* is an endophyte of Proteaceae, it is not an important stem canker pathogen. However, as *B. proteae* has primarily been collected from leaf spots in the past, further research is required to elucidate its role in this regard.

INTRODUCTION

The Proteaceae is one of the most prominent flowering plant families in the southern hemisphere with about 300 species confined to the South-Western Cape area alone (Rebelo, 1995). Proteas, which are considered as important niche products on the world floriculture market, are grown as cut-flower crops in Australia, California, Hawaii, Israel, New Zealand, South Africa (Forsberg, 1993) and Zimbabwe (Archer, 1998). The commercial trade in Fynbos in South Africa is composed of predominantly a fresh-cut-flower and also a dry-flower industry (Coetzee & Middelmann, 1997), of which about 70% of the fresh-cut-flowers and 80% of the dried flowers are exported (Wessels *et al.*, 1997).

Being indigenous to this region, members of the Proteaceae utilised in the cut-flower industry in South Africa are susceptible to many, apparently indigenous, diseases. Several species of *Botryosphaeria* Ces. & de Not. are recorded as being pathogenic to proteas (Van Wyk, 1973;

Knox-Davies *et al.*, 1981; Benic, 1986; Von Broembsen, 1986), of which *Botryosphaeria dothidea* (Moug.: Fr.) Ces. & De Not. is the most significant, and is also a well-known pathogen of numerous other hosts worldwide (Müller & Arx, 1962). Diseases caused by *B. dothidea* are devastating and difficult to control (Von Broembsen, 1986; Von Broembsen & Van der Merwe, 1990). The pathogen causes severe losses in species of *Protea, Leucospermum* and *Leucadendron* R. Br. (*Lcd.*) in South Africa, Western Australia, Hawaii and California (Greenhalgh, 1981; Lamont *et al.*, 1995). Another species of *Botryosphaeria*, *B. proteae* (Wakef.) Denman & Crous causes leaf spots and is associated with stem cankers of *Protea* and *Leucospermum* species (Denman *et al.*, 1999). Different *Botryosphaeria* spp. have also been isolated from shoot lesions, seed heads and seeds of different proteas in South Africa (Knox-Davies *et al.*, 1981).

Some plant diseases, particularly stem and crown cankers and die-back diseases are most severe on plants subjected to environmental stress (Schoeneweiss, 1981). Although species of *Botryosphaeria* are known to be virulent on a wide range of hosts, some cause cankers only on hosts that are weakened or stressed (Hutton, 1958; Toole, 1963; Schoeneweiss, 1965; Neely, 1968; Crist & Schoeneweiss, 1975). *B. dothidea* is normally viewed as an opportunistic pathogen that infects trees under conducive conditions (Crist & Schoeneweiss, 1975). However, Smith *et al.* (1996) showed that the fungus exists as an endophyte in healthy eucalypt trees. In previous surveys, *B. proteae* was found to be associated with stem canker and tip blight symptoms of Proteaceae, and in a few instances also isolated as an endophyte from apparently healthy tissue (S. Denman, pers. comm.). The aims of the present study were, therefore, to determine whether *B. proteae* is a common endophyte of Proteaceae, and if it could induce disease when re-inoculated into healthy tissue, and if so, what the effect of water stress on disease development would be.

MATERIALS AND METHODS

Endophytes

Collection - Plant material of *P. cynaroides*, pure and hybrid *Lsp. cordifolium* (Salisb. ex Knight) Fourc. and *Lcd. salignum* P. J. Bergius *x Lcd. laureolum* (Lam.) Fourc. cv Safari Sunset were collected in July 1997 from three localities in the Western Cape province. Samples were taken from five plants per genus at each locality, with two localities being situated near Stellenbosch (Elsenburg and Protea Heights, 15 km apart) and one in Hermanus (60 km distant). Specimens were transported

in paper bags in a cooler, stored overnight at 7 °C, and processed the following day.

Isolation - A stem piece (5 cm long) with two leaves from the previous growing season, was removed from each plant. Each leaf of *Lsp. cordifolium* and *Leucadendron* cv Safari Sunset was cut into 18 parts. Leaf laminas of *P. cynaroides* were cut into 33 parts, and their petioles cut transversely into five equal parts. Stem pieces of the three genera were cut transversely into five equal parts that were again split longitudinally. After dissection plant material was surface sterilised in 70% ethanol for 1 min, 3% NaOCl for 3 min, and finally in 70% ethanol for 30 s. Plant parts were plated on potato dextrose agar (PDA, Biolab, Randburg, South Africa), supplemented with cyclosporin (Sandimmun®, Sandoz, Randburg, Johannesburg, 50 $mgml^{-1}$) (5 mgl^{-1} PDA), to suppress the growth of fast growing isolates (Dreyfuss, 1987). Depending on the size of the leaf and stem pieces, four to seven pieces were plated on a 90 mm Petri dish. All dishes were incubated at room temperature. Petri dishes were checked every second day for signs of fungal development. Hyphal tip isolations were made from each colony and transferred to PDA slants.

Fungal colonies were transferred from PDA slants onto divided plates, containing PDA in one half of the dish and carnation leaf agar (CLA; Fisher *et al.*, 1982) in the other half. Plates were incubated at room temperature on the laboratory bench, and examined weekly for the presence of fungal structures to facilitate identification. Sterile isolates were checked every week for a period of up to three months. If they remained sterile after this time, they were assigned to "sterile morphotypes" depending on the characteristics of each culture.

Statistical analysis - A regression analysis was carried out to verify whether the raw colonisation rates and raw infection rates were correlated. In addition, confirmatory statistical analysis was carried out when appropriate on selected frequency data using Chi-square tests (P=0.05). These statistical analyses were carried out using Microsoft Excel Ver. 8.0. For the statistical evaluation, the infection frequency by a fungal species was defined as the total number of pieces of a given tissue colonised by a given taxon. Community ordination was performed on the complete matrix, containing all identified taxa, as well as on a reduced matrix of the raw data of the colonisation frequencies that contained only those taxa with a relative importance (dominance) index (Ludwig & Reynolds, 1988) of at least 5%. The resulting matrices were then analysed by simple correspondence analysis using the package SimCA 2.1 (Greenacre, 1990).

Inoculation experiments with *B. proteae*

Plant material - One hundred and ninety-two 5-month-old rooted plants of cultivars Red Rex (*P. cynaroides*), High Gold (*Lsp. patersonii* E. Phillips *x Lsp. cordifolium*) and Safari Sunset (*Lcd. salignum x Lcd. laureolum*) were supplied by the ARC Fynbos Nursery, Elsenburg, Stellenbosch, Western Cape province. The rooted plants were transplanted into 600 ml plastic pots containing growth medium (sand and composted bark mixture,1:1). A slow-release fertiliser, multi-coat (2500 gm^{-3}), dolomite (1000 gm^{-3}) and $FeSO_4$ (700 gm^{-3}) were added to the mixture before the plants were planted. The pots were elevated above the ground on iron grids in a glasshouse (23-28°C, daily temperatures) and watered every second day to field capacity. The plants were carefully maintained and were disease-free when the inoculation commenced. The day before inoculation, the diameter of each stem was measured 6 cm above soil level.

Stress regimes - The day after the plants were potted, all of the plants were watered to field capacity. Thereafter, one third of the plants of each of the three cultivars was watered every second day with 70 ml water to field capacity (non-stressed), water was withheld from another third of the plants until the leaf water potential reached -1.0 MPa (moderately stressed), and the last third of the plants were watered only when the leaf water potential fell below -2.0 MPa (severely stressed). Plants were given 70 ml water to alleviate stress. The leaf water potentials of these plants were determined with a pressure bomb as described by Scholander *et al.* (1965). For each watering regime, readings were taken twice a week from non-inoculated plants of all three cultivars. These plants were placed randomly among the inoculated plants. The use of the pressure bomb required destructive sampling of leaves. To avoid any effect of repeated leaf removal, a new plant was used for every reading. The watering-drying regime was continued over a period of 12 weeks until the conclusion of the trial.

Inoculation - Inoculation of plants with *B. proteae* was performed 10 weeks after the watering regimes had been implemented. Four stem treatments were respectively applied: non-wounded control, wounded control, and wounding followed by inoculation with isolates obtained as endophytes of leaves from *Leucospermum* and *Protea*. Inocula of *B. proteae* were prepared by culturing the two single conidium isolates in Petri dishes on PDA. A total of 54 plants for all the watering regime/cultivar combinations were respectively inoculated with the *Protea* isolate, the *Leucospermum* isolate, and a non-colonised PDA plug. A further 10 plants of each of the three cultivars were used as non-wounded controls. All inoculated plants were wounded by making a 3

mm deep incision into the stem, 6 cm above soil level. A mycelium plug (5 mm x 5 mm), taken from the margins of an actively growing colony was put mycelial side against the stem into the wound. Parafilm (American National Can™, Neenah, WI, USA) was used to seal the wounds and removed one week after inoculation.

Experimental design - The experimental design was a randomised block design with 27 treatment combinations. The treatment design was a 3 x 3 x 3 factorial with genera (*Leucadendron, Leucospermum, Protea*), watering regimes (severely stressed, moderately stressed, non-stressed) and stem treatments (wounded control, wounding and inoculation with *Leucospermum* isolate, wounding and inoculation with *Protea* isolate) as factors. Non-wounded control plants were places randomly between treated plants. The inoculation studies were conducted twice (14 May and 17 August 1998) using a new batch of the same cultivars for the second study.

Evaluation - Symptom development was recorded two weeks after inoculation. Observations included the presence of cankers as well as the colour of the wounds. The surface of the stem was removed and the total length of the either the wound or the associated stem discoloration was measured, which ever was the longest. The stem diameter of each stem was measured and the percentage of the stem girdled was calculated.

Isolations were made from half of the lesions to verify the presence of the pathogen. Stem pieces were surface sterilised as described previously, and isolations made from the lesion margins. Isolations were also made from the wounded controls. Tissue segments (1 mm^2) were plated on Petri dishes containing PDA and cyclosporin. Plates were incubated for three weeks and checked for the presence of *B. proteae*.

RESULTS

Endophytes

Identification - A total of 2700 samples from *Leucospermum, Leucadendron* and *Protea* from three sites were processed and 1279 isolates were recovered. Of the 1279 isolates recovered, 25 genera consisting of 31 species were recorded. This total includes fertile, and therefore identifiable cultures, which represented 954 (74.6%) of the total isolates recovered. It does not include sterile mycelia of which there were 325 (25.4%) isolates, consisting of eight recognisable

morphospecies. The total number of isolates from the Hermanus samples was at least twice (678) that recovered from the Elsenburg samples (265) and the Protea Height samples (336) (Table 1). Seventeen species were recorded from Elsenburg, and 20 and 26 species respectively from Protea Heights and Hermanus.

Fig. 1 shows that infection rates and colonisation rates were highly correlated and the dependence of both variables was linear, therefore the raw infection data were used for further analyses. Of the 39 different taxa isolated in this study, including sterile mycelia, 14 were present at RI values of more than 5%. The results of the correspondence analysis carried out on this reduced matrix showed that some samples were characterised by rather homogeneous fungal assemblages (Fig. 2). The percentage of inertia explained by the first four factors was approximately 75%, which indicates a rather good fit of the model to the data. Thus, the clustering of samples of *Lcd*/stem/Elsenburg, *Protea*/stem/Elsenburg, *Protea*/stem/Protea Heights and *Lsp*/stem/Elsenburg is caused by *Epicoccum purpurascens*, while samples of *Protea*/petiole/Protea Heights and *Protea*/leaf/Elsenburg cluster due to a *Phoma* and *Alternaria* sp. *Lcd*/leaf/Protea Heights is characterised by *Sporormiella isomera* and *Stemphylium vesicarium*, while *Lsp*/leaf/Protea Heights and *Lcd*/leaf/Hermanus are characterised by Sterile sp. 1. *Lsp*/leaf/Hermanus is associated mainly with *Fusarium oxysporum*, Sterile sp. 4, and *Coniothyrium* sp. 1. *Botryosphaeria proteae* is the main coloniser of *Protea*/leaf/Protea Heights and can be found, however, also in the samples that lie close to *Protea*/leaf/Protea Heights in the lower left quadrant.

The distribution of the fungal taxa is shown in Fig. 3. A Chi-square analysis of the distribution reveals that the three hosts are colonised by different fungal assemblages ($P<0.005$). These results have, however, to be examined with great caution as only one sampling time was considered and only three sites were included in the sampling.

The *Botryosphaeria* isolates isolated were identified as *B. proteae* according to Denman et al. (1999). *Botryosphaeria proteae* (BP) was not one of the most relevant endophytes (Figs 3, 4), being the 7th most frequent endophyte of *Protea* and only the 11th most frequent in *Leucospermum*. It was not present in *Leucadendron*. It was also predominantly isolated from the leaves of *Leucospermum* and *Protea* collected at Protea Heights and Hermanus, and from the petioles of *Protea* collected at Protea Heights.

Inoculation experiments with *B. proteae*

Symptoms and pathogenicity - Ten days after inoculation, cankers were observed on inoculated, severely stressed (-2.0 MPa) *Protea cynaroides* plants. No symptoms were observed on inoculated *Leucospermum* and *Leucadendron* plants with either of the isolates. Cankers continued to expand until evaluation 14 days after inoculation, when the largest canker lesion covered the total length of the cutting. Dark brown, sunken lesions developed on the stems, extending up- and downwards from the point of inoculation (Fig. 5). The lesions eventually girdled the stem and some of the petioles and leaves turned dark brown. Some of the plants were killed by girdling cankers. The plants of *P. cynaroides* developed canker symptoms with the *Botryosphaeria* isolates from *Protea* as well as those from *Leucospermum*. Of the severely stressed plants inoculated with *B. proteae* isolates from *Protea* and *Leucospermum*, a respective average of 75% and 50%, for the two trials, developed cambial lesions. The mean respective lesion length for the two trials with the isolates from *Protea* and *Leucospermum* was 62.8 mm and 33.8 mm and the mean percentage stem girdling was 15 and 30%. Wound-inoculated, and non-wounded, water-stressed plants displayed no cambial discoloration or girdling.

Stress regimes - Canker symptoms (cambial lesions and stem girdling) only developed when plants were severely stressed (-2.0 MPa) (Figs 6, 7). Leaves of *P. cynaroides* plants started to shrivel and drop at water potentials more negative than -2.0 MPa. No cankers were observed at levels above -2.0 MPa.

Re-isolation - The watering regime did not influence the re-isolation of *B. protea* from the point of inoculation. *Botryosphaeria proteae* was re-isolated from the margins of lesions of all three watering regimes, except for the controls.

DISCUSSION

Although not one of the most prominent endophytes in the studied plant material, *Botryosphaeria proteae* does occur as an endophyte in leaves and petioles of some members of the Proteaceae. Although there were some differences in the colonisation of samples at the three different sites, it was apparent that endophytes are relatively common in the Proteaceae. The reason for the low colonisation of plant material at the Elsenburg location is unknown. The high level of colonisation of endophytes at the Hermanus location could possibly be ascribed to the high

moisture level of this site near the coast. Moisture levels in the form of rain, dew and fog affect endophyte assemblages by favouring high infection rates (Carroll & Carroll, 1978; Carroll, 1995). Furthermore, the fungicide spraying practices at the Hermanus site differ from those of the other two sites. The low frequency at which plants are sprayed at the Hermanus site could also have contributed to the higher endophyte colonisation.

The endophytes showed a remarkable degree of similarity when compared over the three locations, possibly because all three locations were in a continuous distribution within the native habitat of the proteas in the South-Western Cape. The majority of dominant species e.g *Alternaria* sp., *Cladosporium cladosporioides*, *Epicoccum purpurascens*, *Gliocladium roseum*, *Gliocladium* sp. 2 and sterile sp. 1, occurred in all three locations, but variations lay with the composition of the less abundant and rare species (Fig. 3). Some evidence of specificity was indicated from the ordination of the data by correspondence analysis (Fig. 2). *Ascochyta* spp., *B. proteae*, *Fusarium oxysporum* and *Sporormiella isomera* only occurred in Protea Heights and Hermanus. *Coniothyrium* sp. and *Phomopsis* spp. did not occur in Protea Heights, while sterile sp. 5 and the *Pleospora* sp. only occurred in Hermanus. *B. proteae* showed a degree of host specificity only being recorded from *Protea* and *Leucospermum*. *Fusarium oxysporum* was mainly associated with leaves of *Leucospermum* in Hermanus. *Stemphylium vesicarium* and *Sporormiella isomera* only occurred in leaves and petioles, and *Coniothyrium* sp. 2, *Pestalotiopsis* sp. and *Pleospora* sp. only occurred in leaves. *Chaetomium* sp. was only associated with stems. As the sampling was carried out only once, however, it cannot be fully excluded that these results may also be random occurrence of the fungi or at least that some geographic factors may be involved.

Of the species encountered, several have been reported before as plant pathogens of ornamental plants. These include *B. proteae* (Van Wyk, 1973; Van Wyk *et al.*, 1975; Denman *et al.*, 1999), *Cladosporium cladosporioides* (Arx, 1987), *Colletotrichum gloeosporioides* (Benic & Knox-Davies, 1983; Arx, 1987) and *Fusarium oxysporum* (Arx, 1987; Swart *et al.*, 1999).

The results of the inoculation studies indicate that host water stress of young, rooted plants of *Protea cynaroides* enhances disease development and colonisation by endophytic isolates of *Botryosphaeria proteae*. *Protea* plants only reached susceptibility when water potential dropped below -2.0 Mpa. This water stress level is, however, not typically observed in the field (Van Zyl *et al.*, 1999). These results are consistent with the general conclusion that

environmental factors, especially drought and freezing stress, strongly influence both incidence and severity of woody plant diseases (Crist & Schoeneweiss, 1975). Threshold levels of water stress must be exceeded before woody plants are predisposed to colonisation by many non-aggressive pathogens (Crist & Schoeneweiss, 1975; Schoeneweiss, 1978; Schoeneweiss, 1981). In most cases, water stress leads to increased severity of canker development (Schoeneweiss, 1975; Pusey, 1989).

Cankers caused by *Septoria musiva* Peck. on poplar trees were significantly larger when water potential fell below -1.0 MPa than those on non-stressed trees (Maxwell *et al.*, 1997). However, in the case of cankers caused by *Diplodia pinea* (Desm.) Kickx, Petrak & Sydow f. sp. *cupressi* on cypress, trees had to be stressed below -4.5 to -5.5MPa for increased severity (Madar *et al.*, 1989). Schoeneweiss (1975) reported that European white birch infected by *B. dothidea,* was predisposed by -1.2 MPa of water potential stress. However, there are some exceptions to this generalisation. Drought stressed (-2.5 MPa) *Eucalyptus* inoculated with *Cryphonectria cubensis* (Bruner) Hodges developed smaller cambial lesions than non-stressed plants (-1.0 Mpa) (Swart & Conradie, 1992).

Compared to *Leucadendron* and *Leucospermum*, young *Protea* plants tend to be more susceptible to water stress (Van Zyl *et al.*, 1999). Significant differences in water requirements have been observed even between species in the same genus. *Protea cynaroides* has a water requirement twice that of *P. eximia* (Salisb. ex Knight) Fourc. (M. Montarone, INRA-URIH, Route des Colles, Sophia-Antipolis Biot France 06410, pers. comm.). The interaction between genera and water stress levels, indicates that *Leucadendron* and *Leucospermum* may be less susceptible to canker development by virtue of their ability to withstand drought. The water potentials for *Leucadendron* and *Leucospermum* were possibly not low enough (more negative than -2.0 MPa), to stimulate disease development during this experiment. Only when the plants are severely stressed to levels that do not normally occur in nature, the possibility exists that canker symptoms may develop.

In the past, pycnidia of *B. proteae* were observed on symptomatic stems of *Lsp. lineare* R. Br. *x Lsp. cordifolium* cv Succession, *P. cynaroides, P. grandiceps* Tratt., *P. repens* (L.) L. and *P. magnifica* Link. However, in some cases, the canker infection was secondary, resulting from colonised insect wounds. Since *B. proteae* is known to be a leaf pathogen (Van Wyk, 1973; Van Wyk *et al.*, 1975; Denman *et al.*, 1999), it is possible that stem cankers associated with *B.*

proteae are secondary. The absence of canker symptoms on *Leucospermum* and *Leucadendron*, and the fact that only unnaturally low water stress levels could incite canker development in *P. cynaroides*, indicates that *B. proteae* cannot be considered as a primary stem canker pathogen.

Since severe water stress conditions can predispose plants to attack by non-aggressive pathogens, all practices designed never to subject plants to severe water stress levels must be employed (Schoeneweiss, 1975, 1981). Results obtained under such rigidly controlled conditions as in the glasshouse tests, are, however, not consistent with that found in field conditions (Schoeneweiss, 1975). Stem cankers caused by *B. proteae* are therefore no threat for protea cultivation under field conditions. On the other hand, leaf spots caused by *B. proteae* appear to be of economic significance (Denman *et al.*, 1999). The effect of water stress on the development of leaf spots caused by *B. proteae*, therefore, needs to be further investigated.

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Table 1. Number of isolates recovered from *Protea*, *Leucospermum* and *Leucadendron* at three different locations in the Western Cape province

Locality	**Elsenburg**				**Protea Heights**				**Hermanus**			
Genus	**Protea**	**Lsp**[a]	**Lcd**[b]	**Total**	**Protea**	**Lsp**	**Lcd**	**Total**	**Protea**	**Lsp**	**Lcd**	**Total**
No of samples	230	230	440	900	230	230	440	900	230	230	400	900
No of isolates	158	83	24	265	162	103	162	427	322	180	176	678

[a] *Lsp = Leucospermum*

[b] *Lcd = Leucadendron*

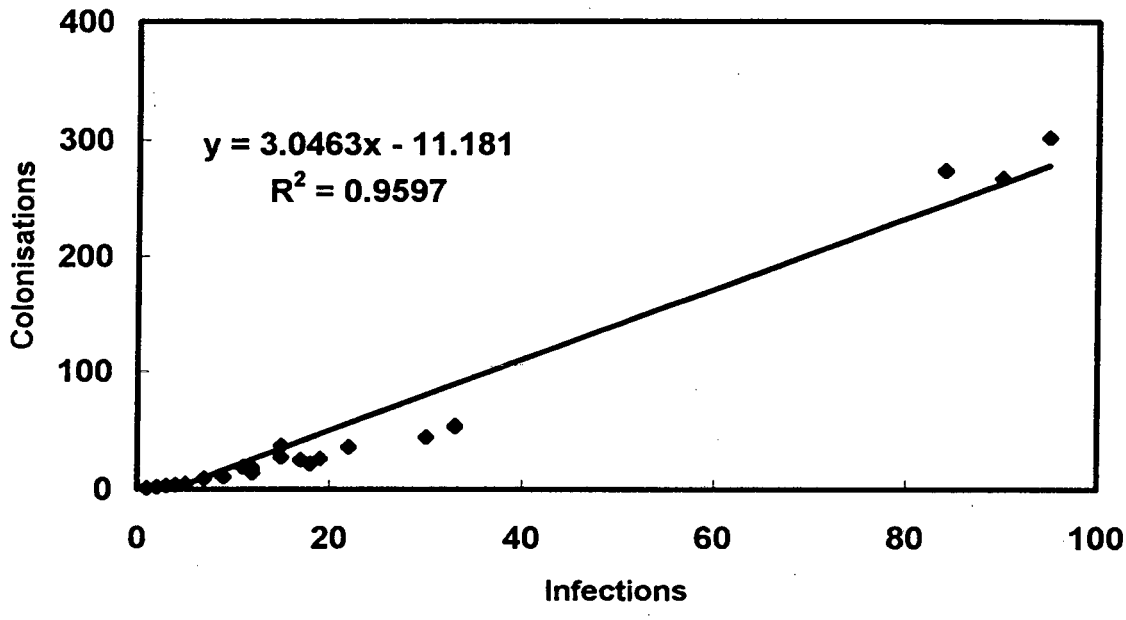


Fig. 1. Regression analysis of raw colonisation and infection rates of fungal endophytes isolated from Proteaceae.

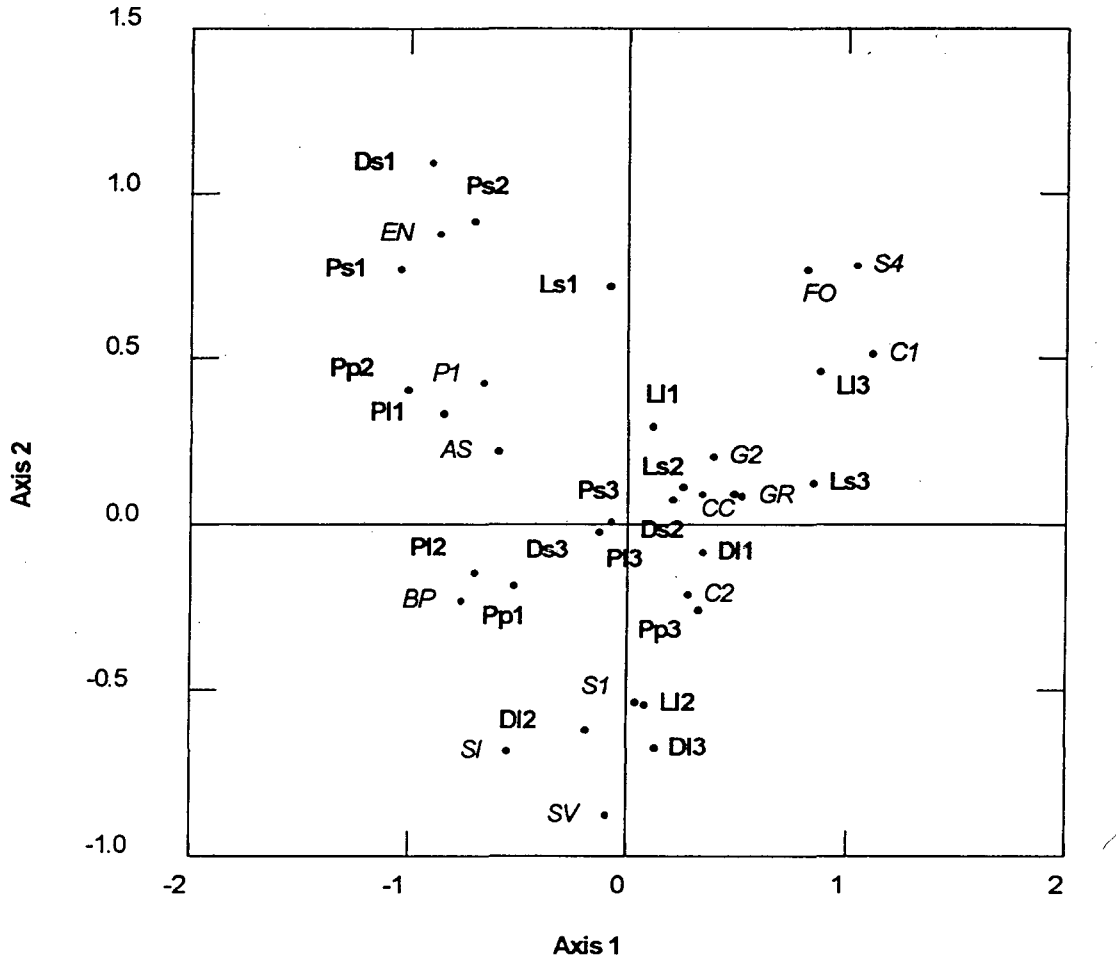


Fig. 2 Correspondence analysis of colonisation frequencies of taxa with a relative importance index of at least 5%.

Codes used in the analysis:

P = *Protea*; L = *Leucospermum;* D = *Leucadendron*; l = Leaves; p = Petiole; s = Stem; 1 = Elsenburg; 2 = Protea Heights; 3 = Hermanus; AS = *Alternaria* Nees sp.; BP = *Botryosphaeria proteae*; CC = *Cladosporium cladosporioides* (Fresen.) de Vries; C1 = *Coniothyrium* Corda sp. 1; C2 = *Coniothyrium* sp. 2; EN = *Epicoccum purpurascens* Ehrenb. ex Schltdl.; FO = *Fusarium oxysporum* Schltdl.: Fr.; GR = *Gliocladium roseum* Bainier; G2 = *Gliocladium* Corda sp. 2; P1 = *Phoma* Sacc. sp. 1; SI = *Sporormiella isomera* S.I. Ahmed & Cain; SV = *Stemphylium vesicarium* (Wallr.) Simmons; S1 = Sterile sp.1; S4 = Sterile sp. 4.

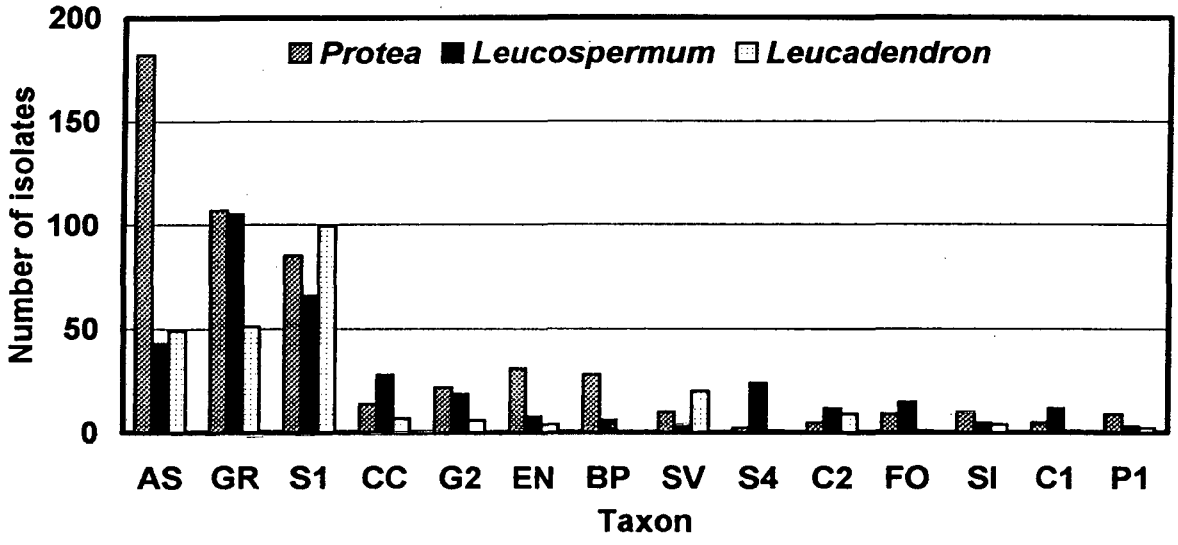


Fig. 3. Frequency of the most important fungal endophytes in *Protea*, *Leucospermum* and *Leucadendron*.

Codes used in the analysis:

AS = *Alternaria* sp.; BP = *Botryosphaeria proteae*; CC = *Cladosporium cladosporioides*; C1 = *Coniothyrium* sp. 1; C2 = *Coniothyrium* sp. 2; EN = *Epicoccum purpurascens*; FO = *Fusarium oxysporum*; GR = *Gliocladium roseum*; G2 = *Gliocladium* sp. 2; P1 = *Phoma* sp. 1; SI = *Sporormiella isomera*; SV = *Stemphylium vesicarium*; S1 = Sterile sp.1; S4 = Sterile sp. 4.

Taxa less than 5% of relative importance:

Protea: *Ascochyta* Lib. sp. 1.; *Ascochyta* sp. 2; *Bipolaris australiensis* (M. B. Ellis) Tsuda & Ueyama; *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc.; *Pestalotiopsis* Steyaert sp.; *Phomopsis* (Sacc.) Bubák sp. 1; *Phomopsis* sp. 2; *Pleospora* Rabenh. ex Ces. & De Not. sp.; *Sordaria* Ces. & De Not. sp.; Sterile sp. 2; Sterile sp. 3; Sterile sp. 5; Sterile sp. 6; Sterile sp. 7; *Thielavia hyrcaniae* Nicot ex Nicot & Longis; unidentified hyphomycete; *Xylaria* Hill ex Schrank sp.

Leucospermum: *Ascochyta* sp. 2; *Chaetomium* Kunze sp.; *Gilmaniella subornata* Morinaga; *Lophotrichus* R. K. Benj. sp.; *Pestalotiopsis* sp; *Phomopsis* sp. 1; *Pleospora* sp.; *Sordaria* sp.; Sterile sp. 2; Sterile sp. 7; unidentified hyphomycete; *Xylaria* sp.

Leucadendron: *Arthrinium* Kunze sp.; *Ascochyta* sp. 1; *Ascochyta* sp. 3; *Chaetomium* sp.; *Penicillium* Link sp. 1; *Periconia* Tode sp.; *Phomopsis* sp. 2; Sterile sp. 2; Sterile sp. 5; Sterile sp. 7; Sterile sp. 8; *Thielavia hyrcaniae*; unidentified hyphomycete.

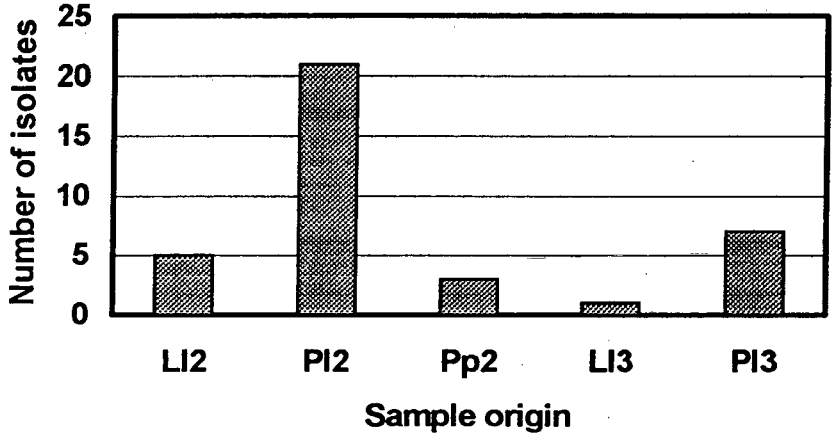
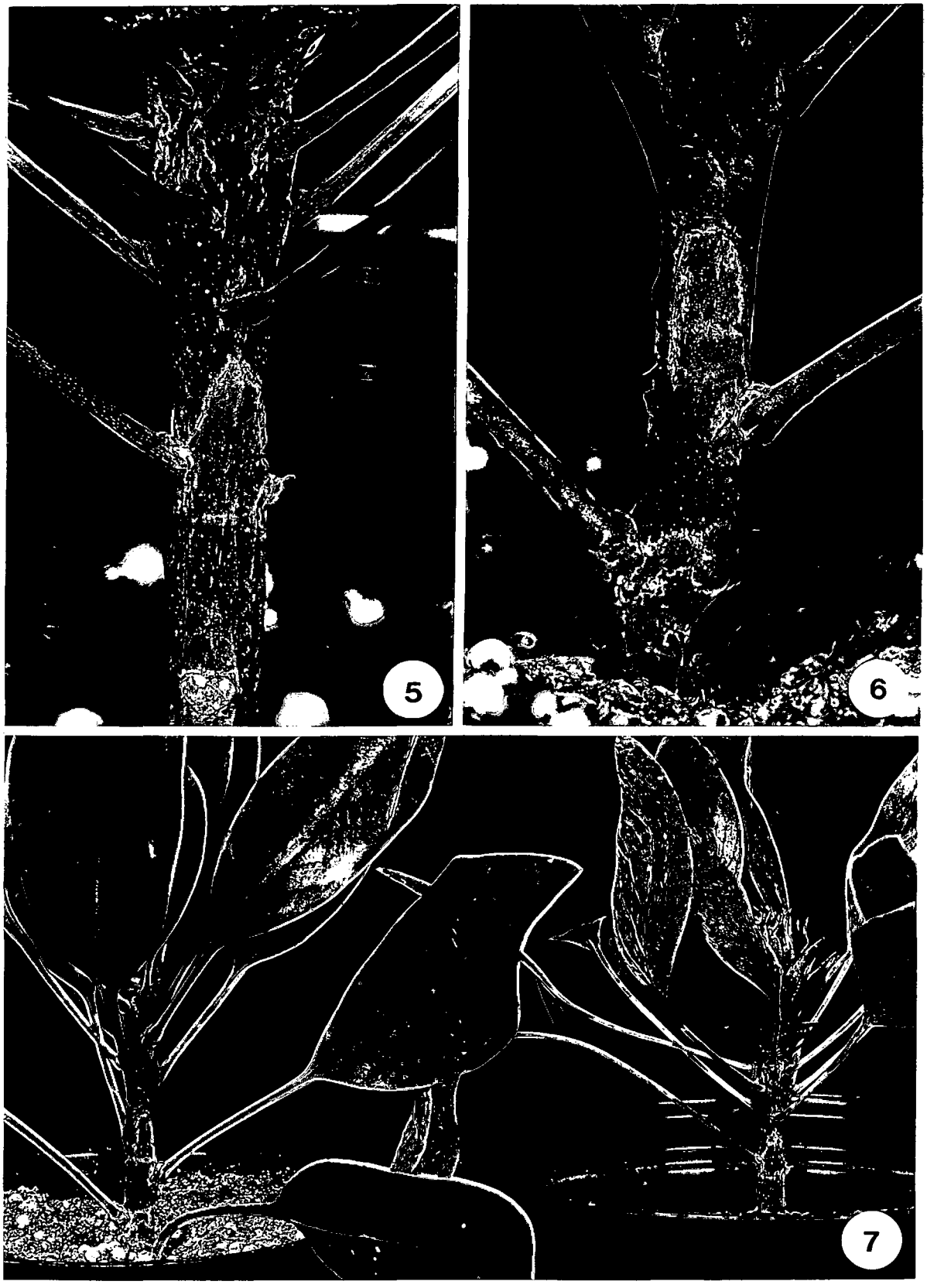


Fig. 4. The distribution of *Botryosphaeria proteae* in the different plant parts and sites.
P = *Protea*; L = *Leucospermum*; l = leaves; p = petiole; 2 = Protea Heights; 3 = Hermanus.



Figs 5-7. *Protea cynaroides* plants inoculated with *Botryosphaeria proteae*. **5.** Dark brown, sunken lesion associated with water stress. **6.** Apparently healthy, non-stressed, inoculated plant. **7.** Non-stressed *P. cynaroides* (left) vs severely stressed plant (right).

5. FUSARIUM WILT: A NEW DISEASE OF CULTIVATED *PROTEA* IN SOUTHERN AFRICA

ABSTRACT

A newly recorded disease of cultivated *Protea*, Fusarium wilt, is described and shown to be caused by *Fusarium oxysporum*. The disease occurs on mature (2-year-old) *P. aristata x repens* cv Venus, *P. compacta x susannae* cv Pink Ice, *P. cynaroides*, *P. eximia x susannae* cv Cardinal, *P. eximia x susannae* cv Sylvia, *P. magnifica x susannae* cv Susara and *P. repens* cv Sneyd plants in the summer rainfall areas of the North-Western province of South Africa and in Zimbabwe. Disease symptoms first become visible as necrotic leaves on infected plants. Subsequently, a dark lesion develops from the roots along the stem, usually visible only on one side of the stem. Occasionally the lesion develops in the upper part of the stem. The vascular tissue is discoloured leading to branch die-back and plant death. *F. oxysporum* was readily isolated from the roots, crown and vascular tissues of infected plants. Glasshouse trials were conducted to prove Koch's postulates on six *Protea* cultivars. Forty-five rooted plants of each of the six cultivars were inoculated with isolates of *F. oxysporum* derived from the same cultivar. Control plants were inoculated with potato-dextrose broth only. Disease symptoms similar to those observed in the field developed 6 weeks after inoculation on all of the cultivars. The fungus was re-isolated from the roots, crown and vascular tissues of inoculated plants. Fifty-one of the 54 *F. oxysporum* isolates tested induced disease. This is the first record of Fusarium wilt on *Protea* plants.

INTRODUCTION

Cultivation of Proteaceae for export as cut-flowers is a rapidly expanding industry in South Africa, Australia, California, Hawaii, Israel, New Zealand (Forsberg, 1993) and Zimbabwe (Archer, 1998). In South Africa, Proteaceae are sold as fresh-cut-flowers and dried flowers, and about 70% of the fresh-cut-flowers and 80% of the dried flowers are exported (Wessels *et al.*, 1997). *Protea* L. plants are propagated mostly as rooted and unrooted cuttings, but seedlings and seed are also used (Wessels *et al.*, 1997).

During December 1997, a disease of two-year-old *P. aristata* E. Phillips *x repens* (L.) L. cv Venus, *P. compacta* R. Br. *x susannae* E. Phillips cv Pink Ice, *P. eximia* (Salisb. ex Knight) Fourc. *x susannae* cv Cardinal, *P. eximia x susannae* cv Sylvia, *P. magnifica* Link *x susannae* cv

Susara and *P. repens* was observed on a *Protea* farm in the North-Western province of South Africa with an estimated disease incidence of 15%. Subsequent to the first observations in the North-Western province, the disease was also observed on *Protea* cultivars Pink Ice and Sylvia and also on *P. cynaroides* (L.) L. on protea farms in Zimbabwe. The disease was characterised by the blackening of the leaves on some of the stems of plants, and the development of a necrotic lesion, extending from the roots up the stem. The disease caused die-off of stems, leading to plant death. The stems of infected plants were discoloured (Fig. 1).

A *Fusarium* sp. was consistently isolated from all symptomatic *Protea* plants. The aim of the present study, therefore, was to establish whether the *Fusarium* species isolated was the pathogen causing the wilt disease of proteas in southern Africa.

MATERIALS AND METHODS

Isolation and identification - Diseased material was collected from 2-year-old *Protea* cultivars Cardinal, Pink Ice, Sneyd, Susara, Sylvia and Venus in the North-Western province, and from cultivars Pink Ice, Sylvia and *P. cynaroides* in Zimbabwe. Isolations were made from the roots, crown and vascular tissue of three plants per cultivar. Diseased material was surface disinfested in 70% ethanol for 30 s, 3% NaOCl for 1 min, and again in 70% ethanol for 15 s. Three pieces each of the root, crown and vascular tissue were plated on potato dextrose agar (PDA; Biolab Diagnostics, Midrand, South Africa) for each plant, and incubated at room temperature (approximately 23°C) under cool white and near-ultraviolet lights with a photoperiod of 12 h.

All fungal colonies developing from plated tissue were transferred to divided Petri dishes containing PDA in one half of the dish and carnation leaf agar (CLA; Fisher *et al.*, 1982) in the other half. The plates were then incubated in a growth room under near-ultraviolet and cool white light with a 12 h photoperiod to induce sporulation. Single conidial cultures were made from conidia formed on the CLA, and these were incubated as described above for 3 weeks. Colonies obtained from single conidial isolates were lyophilised. Isolates were identified according to Nelson *et al.* (1983).

Pathogenicity tests - Pathogenicity tests were conducted on 5-month-old rooted plants of the cultivars Cardinal, Pink Ice, Sneyd, Susara, Sylvia and Venus. The rooted plants were

transplanted into 600 mL plastic pots containing sterilised growing medium [Hygro-mix, Hygrotech, Pty (Ltd), South Africa]. Plants were inoculated 7 d after transplanting.

Inoculum and inoculation method - Nine single conidial isolates (three isolates each from root, crown and stem tissues) were used to inoculate 45 rooted plants of each cultivar (five replicates per isolate). The isolates were obtained from the same cultivars as those inoculated. Fifty-four isolates were used in total (nine isolates per cultivar, for six cultivars), and in total 270 plants were inoculated. Mycelial plugs (3 mm x 3 mm) taken from 7-day-old colonies growing on PDA were transferred to 250 ml flasks containing 100 ml of Difco potato-dextrose broth (10 g/l). Flasks were placed on a rotary shaking incubator, operating at 96 rpm at 23°C. After 5 days, the contents of each of the flasks were filtered separately through layers of sterile cheesecloth. The filtrate (mostly microconidial) was diluted with sterile distilled water to obtain inocula at a concentration of (1-10) x 10^6 microconidia per ml. A haemocytometer was used to quantify inoculum. Fifteen milliliters of spore suspension was pipetted around the base of each plant. Controls were inoculated with potato-dextrose broth only. The experiment was laid out in a glasshouse as a completely randomised design, with night and day temperatures of 16 and 28 °C, respectively.

Eight weeks after inoculation, disease assessments were made. Leaf necrosis was rated on a scale of 0 – 3, where 0 = healthy; 1 = less than 50% necrotic; 2 = more than 50% necrotic, and 3 = dead. The plants were removed from the pots and the soil carefully washed from the roots. Each plant was split open longitudinally and vascular discoloration was recorded. The pathogen was re-isolated from surface disinfected root and crown tissues. Pieces of the vascular tissue were excised at 2 cm intervals along the entire length of the plant, both from the sides of the stem i.e. the side of the stem with the necrotic cortical lesion and the side of the stem without an externally visible lesion. Tissue pieces were plated on PDA and incubated at room temperature as described previously. Colonies that developed from excised tissue pieces were identified.

RESULTS

Isolation and identification - The fungus isolated was identified as *Fusarium oxysporum* Schlecht. emend. Snyd. & Hans. on the basis of its morphological characteristics (Nelson *et al.*, 1983). Production of characteristic 3-septate macroconidia with a foot-shaped basal cell and an

attenuated apical cell, and single-celled microconidia borne on short monophialides were produced abundantly on CLA. Thick-walled chlamydospores, single or paired, were also observed. Colony colour ranged from peach, white-pink to purple on PDA (Fig. 2). Bluish sclerotia were formed at the base of PDA slants and orange sporodochia were abundant on the surface of cultures. *F. oxysporum* was consistently isolated from the roots, crown and vascular tissues of each cultivar collected in the field.

Pathogenicity - All cultivars inoculated with *F. oxysporum* developed severe disease symptoms similar to those observed in the field. Symptoms developed 6 weeks after inoculation. Disease symptoms were initially observed as a blackening of the leaves (Fig. 3). Irregular, black patches developed on the leaves, which finally coalesced and caused the leaves to die off. Necrotic leaves remained attached to the stem long after the plant had died. Soon after the first signs of leaf necrosis, a black lesion, visible on one half of the stem, started to develop from the soil level, extending upwards (Fig. 3). The discoloured area on the surface of the stem correlated with vascular discoloration inside the stem (Fig. 4). The lesion extended from the bark, through the phloem, to the xylem tissue. Sometimes the lesion developed in the upper parts of the stem only. Eventually the entire plant turned black and died. The leaves did not become chlorotic and flaccid, but became stiff and dry after necrosis. *Fusarium oxysporum* was re-isolated from the vascular tissue adjacent to the necrotic cortical lesion, above the necrotic cortical lesion, and from opposite and beneath the lesion.

Isolations were also made from inoculated plants with no external disease symptoms. The crown and vascular tissue of the majority of these plants were discoloured and *F. oxysporum* was re-isolated from the roots, crown and vascular tissues of these plants. Control plants showed no disease symptoms and remained healthy (Fig. 5).

Protea cv Pink Ice showed the lowest percentage of leaf necrosis, lesion development, vascular discoloration, and mortality of all the inoculated cuttings (Table 1). The highest mortality rate was found in cv Venus (Table 1). Almost all the Sylvia plants developed lesions (93.3%) and there was a high percentage vascular discoloration in both cvs Sylvia and Venus, which were the most severely affected (Table 1). Moderate levels of symptoms were recorded for cvs Cardinal, Sneyd and Susara (Table 1).

DISCUSSION

Symptoms similar to those observed in the field were observed on inoculated plants in the glasshouse, and the inoculated pathogen was re-isolated, confirming Koch's postulates. Although there are reports of *Fusarium* species cause damping-off (Greenhalgh, 1981; Benic, 1986) and blight of propagation material of the Proteaceae (Benic, 1986), this is the first record of *Fusarium oxysporum* causing wilt of commercially cultivated *Protea* cultivars in South Africa and Zimbabwe.

Fusarium oxysporum is one of the most important wilt pathogens and is also a common soil-borne fungus that can be saprophytic or parasitic under a wide range of environmental conditions (Booth, 1971). The most prominent feature of wilt pathogens is their colonisation of vascular elements, mostly the xylem, of their host (MacHardy & Beckman, 1981). Although many records of Fusarium wilt caused by various formae speciales of *Fusarium oxysporum* on woody hosts are found in the literature, including Mexican lime (Timmer, 1982), grapevine (Andrade *et al.*, 1993), guavas (Dwivedi, 1996), Hibiscus (Gangopadhyay & Kapoor, 1977) and coffee (Cardoso, 1986; Negrón & Acosta, 1989), few of these records include descriptions of symptoms, making comparison of the symptoms found on *Protea* difficult. In woody angiosperms, the primary symptoms include progressive foliar chlorosis, frequently accompanied by transient wilting, followed by necrosis and defoliation (Green, 1981). Vascular discoloration is common to most, if not all, vascular wilt diseases of woody perennials caused by fungi (Green, 1981).

The wilt disease on *Protea* is aggressive, causing leaf necrosis, the development of a black stem lesion, and vascular discoloration in the corresponding area. The symptoms on the stem of *Protea* plants closely resemble those of wilt caused by *F. oxysporum* f. sp. *chrysanthemi* Litt., Armst. & Armst. on *Chrysanthemum morifolium* (Ramat.) Hemsl., where black stem necrosis may develop, and sometimes occurs as a streak up one side of the stem (Engelhard & Woltz, 1971). On *Albizia julibrissin* Durazz. (mimosa tree), brown discoloured streaks are observed on symptomatic branches and when cut in cross section, a ring or partial ring of discoloured sapwood can be observed just under the bark in the outer wood of wilted branches (Tattar, 1978). In the case of the *Protea*, the necrotic area extends from the bark, through the phloem, into the xylem tissue. On *Eucalyptus* L' Hér., brown or black coloured streaks, possibly

representing discoloration of the vascular tissues, are clearly visible under the bark on the basal portion of infected plants (Arya & Jain, 1962).

The discoloured vascular tissue of inoculated *Protea* plants and the reisolation of the pathogen from these tissues, indicate that the pathogen invades the vascular system. Since vascular discoloration is the most typical symptom of a wilt disease, it seems appropriate to also refer to the disease of *Protea* as a wilt. No records of similar symptoms have been recorded on these affected cultivars in the South-Western Cape *Protea* growing region. Evidence thus points to this disease being limited to the summer rainfall area, where infection probably occurs during the warmer summer months. The fact that certain cultivars developed more severe symptoms than others could be a reflection of the aggressiveness of the isolates, rather than differences in susceptibility between cultivars. The woody nature of the plants could be the reason that no typical wilt symptoms (drooping leaves of young shoots) were observed on *Protea*.

Most of the Fusarium wilt diseases are caused by formae speciales of *Fusarium oxysporum* (Pennypacker, 1981). However, in the literature root rot of pine seedlings is reported to be caused by *F. oxysporum* f. sp. *pini* (Farquhar & Peterson, 1991) and root rot of coffee caused by *F. oxysporum* f. sp. *coffeae* (Dhaliwal *et al.*, 1963; Anon., 1985), thus some formae speciales appear to cause root rot rather than wilt. Historically, strains of *F. oxysporum* have been divided into formae speciales on the basis of virulence on a particular host or group of hosts (Armstrong & Armstrong, 1981). Further subdivisions of formae speciales into races often are made based on virulence on differential host cultivars carrying specific single dominant resistance genes (Bournival & Vallejos, 1991). More recently, strains of *F. oxysporum* of various formae speciales have been grouped on the basis of vegetative compatibility (Puhalla, 1985). The latter approach characterises subspecific groups on the genetics of the fungus rather than on the host-pathogen interaction (Correll, 1991). Further research is therefore needed to determine the pathogenicity of the isolates obtained from *Protea* to other genera and species of the Proteaceae, such as *Leucadendron* R. Br., *Leucospermum* R. Br. and *Serruria* Salisb. and to determine whether this pathogen of *Protea* should be described as a new forma specialis of *F. oxysporum*.

Fusarium wilt disease of *Protea* has significant economical implications for crop production. Plant and branch death results, but there is also a reduction in the numbers of flowers produced by infected plants. The disease may also spread if infected mother plants or cuttings

are used. It is therefore important that the highest level of hygiene is maintained in nurseries and that no cuttings are made from diseased material.

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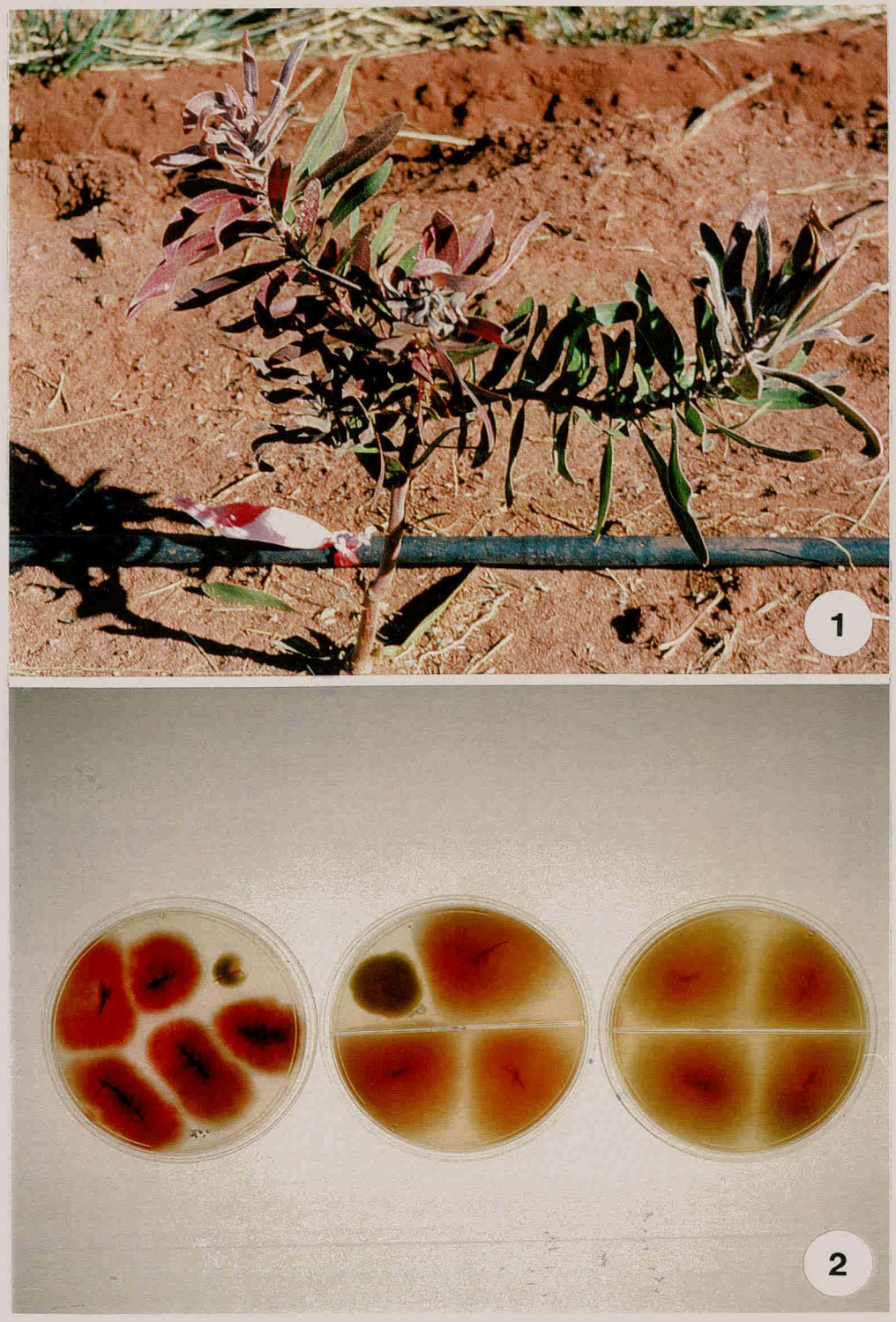
Table 1. Fusarium wilt disease ratings of six *Protea* cultivars

Cultivar	Leaf necrosis (%)[a]				Lesion (%)[b]	Vascular discolouration (%)[c]
	0	1	2	3		
Cardinal	40.0	8.9	31.1	20.0	42.2	60.0
Pink Ice	77.8	0.0	15.6	6.6	20.0	22.2
Sneyd	33.3	2.3	33.3	31.1	57.8	66.7
Susara	35.6	11.1	40.0	13.3	57.8	64.4
Sylvia	6.6	15.6	37.8	40.0	93.3	95.6
Venus	22.2	6.7	20.0	51.1	80.0	80.0

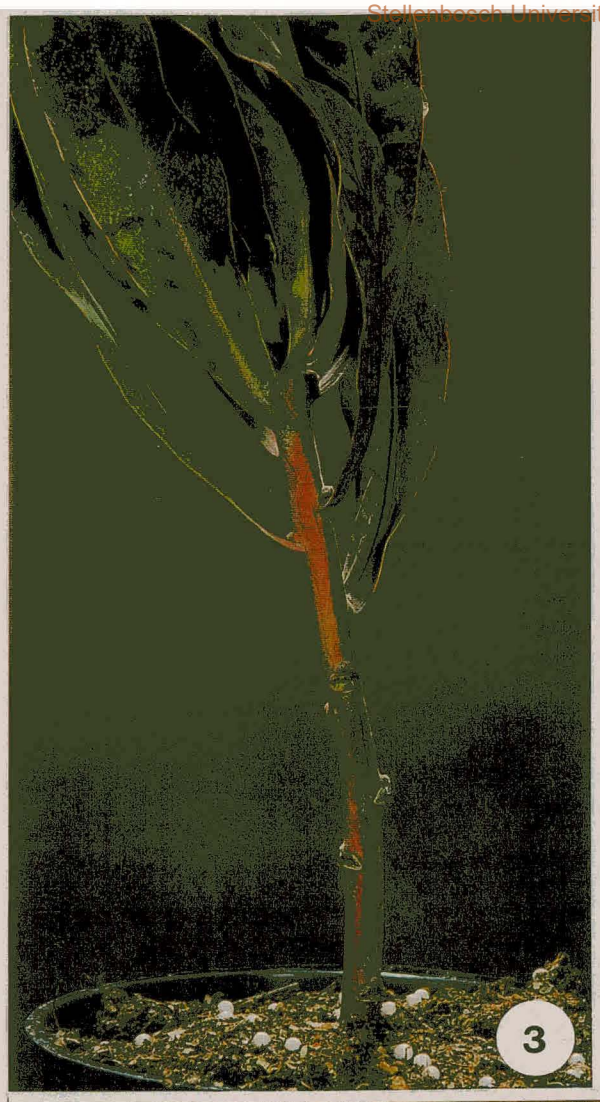
[a]Percentage of inoculated plants rated according to the state of leaf necrosis: 0 = healthy; 1 = <50% necrotic; 2 = >50% necrotic; 3 = 100% necrotic.

[b]Percentage of inoculated plants that developed a necrotic lesion on the stem.

[c]Percentage of inoculated plants that showed vascular discolouration.



Figs 1, 2. Symptom and cultural characteristics associated with *Fusarium oxysporum*. **1.** A plant infected with *F. oxysporum* in the North-Western province of South Africa. **2.** Isolations of *F. oxysporum* made on PDA from infected plant tissue.



Figs 3-5. Disease symptoms associated with Fusarium wilt. **3.** A diseased *Protea* cv Sneyd plant showing stem and leaf necrosis. **4.** Vascular discoloration. **5** *Protea* plant inoculated with *F. oxysporum* (left) vs control (right).

6. PESTALOTIOPSIS LEAF SPOT DISEASE OF PROTEACEAE IN ZIMBABWE

ABSTRACT

A species of *Pestalotiopsis* Steyaert was consistently isolated from necrotic leaf spots on *Leucospermum* R. Br. and *Protea* L. species in Zimbabwe. Inoculation studies were conducted to prove pathogenicity, and it was confirmed that the *Pestalotiopsis* sp. was the causal agent of the disease. A description of this fungus is given, and it is compared to other *Pestalotiopsis* spp. isolated from Proteaceae.

INTRODUCTION

The Proteaceae is well represented in southern Africa and is one of the most prevalent plant families in the southern hemisphere. The most prominent genera, *Protea* L., *Leucospermum* R. Br. and *Leucadendron* R. Br. have been extensively utilised in the cut-flower industries of Australia, Israel, New Zealand, southern Europe, South Africa, U.S.A. (California and Hawaii) and Zimbabwe (Regional Reports, 1998). In Zimbabwe, the genus *Leucospermum* comprises 35% of the total area commercially cultivated with these three genera of Proteaceae (Archer, 1998).

During March 1998 large, irregular, light brown leaf spots were noticed for the first time on plantings of *Leucospermum* spp. in Zimbabwe. The lesions were prominent on *Leucospermum cuneiforme* (Burm. f.) Rourke cv Sunbird, but other species such as *Leucospermum vestitum* (Lam.) Rourke also showed similar symptoms. Lesions were characterised by irregular necrotic leaf spots, in which black fruiting structures were prominent and arranged in concentric zones. On some of the leaves, the lesions enlarged and the entire leaf died off. The fungus was identified as a species of *Pestalotiopsis* Steyaert, and was subsequently recorded on leaves of *Protea* sp. in South Africa. Although *Pestalotiopsis* spp. are generally accepted as common secondary invaders of diseased or dead plant tissue, some have been shown to be primary pathogens (Arx, 1987). The aims of the present study were to test the pathogenicity of the fungus, identify the causal agent of the disease, and compare it with other *Pestalotiopsis* spp. known from Proteaceae.

MATERIALS AND METHODS

Isolation - Single spore isolations were made from sporulating conidiomata on the leaf surface. Cultures were grown on divided Petri dishes containing 2% potato dextrose agar (PDA; Biolab. Midrand, South Africa) in one half of the dish, and carnation leaf agar (CLA; Fisher *et al.*, 1982) in the other.

Identification - Mounts of the fungus, prepared from the host tissue and from colonies sporulating on the CLA, were made in water, and all measurements and photographs were made in this medium under oil immersion with a Zeiss Axioscope MC80 light microscope. Illustrations were made from mounts preserved in lactophenol. Minimum and maximum dimensions are given in parentheses, and the 95% confidence intervals determined from at least 30 observations. Cardinal temperature requirements for growth were determined on malt extract agar (MEA; Biolab) after one week in the dark at 5-35 °C in 5 °C intervals with three replicates per plate for each temperature. *Pestalotiopsis* spp. that have previously been recorded on protea hosts were compared with the species described in the present paper. These include *P. montellicoides* Mordue (IMI 155522, IMI 155539) and a *Pestalotiopsis* sp. (Samuels *et al.*, 1987).

Inoculation – Five cuttings each of *Leucospermum cuneiforme* and *L. glabrum* E. Phillips x *tottum* (L.) R. Br. cv Scarlet Ribbon were taken from the current season's growth and placed in small glass bottles filled with water. Each stem had 10 leaves on it. A spore suspension of the fungus (1×10^6 spores/ml) was made from sporulating cultures, and a drop applied to the upper surface of each leaf. Cuttings were covered with plastic bags for 48 hours to ensure a high humidity, and maintained in a humid growth room. Controls were treated in a similar fashion, but inoculated with water only. Results were assessed daily for two months.

RESULTS

Symptom development - Pin-head size spots developed 15 days after inoculation on the upper surfaces of the leaves. After 27 days the spots enlarged to form reddish lesions (Fig. 1). The reddish lesions coalesced to eventually cover the entire leaf surface, and after 34 days the leaves turned necrotic and died off. Fifty-five days after inoculation, the fungus began to form conidiomata in the necrotic lesions (Fig. 2).

Taxonomy

***Pestalotiopsis* sp.** Figs 3, 4.

Leaf spots irregular, necrotic, associated with leaf margins or causing tip dieback; slightly sunken, pale brown with reddish brown margins that are mostly raised and distinct, rarely diffuse, 2-35 mm (Figure 3a). *Conidiomata* amphigenous, pycnidioid to acervular, immersed, becoming erumpent, unilocular, dark brown to black, dehiscence by irregular splits in the apical wall and overlying host tissue, scattered, (100-)195-240(-400) µm (Figure 3b); pyriform or conical in section, base applanate, intraepidermal in origin (125-)138-165(-180) µm wide, and (125-)138-165(-180) µm high (Figure 3c). *Peridium* comprising two strata of *textura angularis*, an outer stratum of pale brown, thick-walled cells becoming hyaline inwardly, apical and lateral walls composed of slightly compressed, thinner-walled cells; thicker basal wall (13-)17-21(-23) µm, thinner apical wall (7-)11-17(-19) µm (Figure 3d). *Conidiophores* peripheral, reduced to conidiogenous cells, invested in mucus. *Conidiogenous cells* discrete, ampulliform, hyaline, smooth, (4-)5.5-6.5(-8) x (2-)4-5(-6) µm; conidiogenesis initially holoblastic, with up to two enteroblastic, percurrent proliferations to produce additional conidia at slightly higher levels (Figure 3e-i, Figure 4b). *Conidia* ellipsoidal to obovoid, euseptate, 4-septate, the second and third septa often darkened and indistinct, cells unequal, without constrictions at the septa, versicoloured, bearing appendages; basal cell obconic with a truncate base, bearing minute marginal frills, hyaline below, thin-walled, (3.5-)5-6(-7.5) x 4-4.5(-5) µm; second cell subcylindrical, pale brown, verruculose, third and fourth cells doliiform to subcylindrical, dark red-brown, verruculose, combined dimensions of median cells (14-)16-17(-18) x (6.5-)8-9(-10) µm [length of second cell from base (4-)5-5.5(-6) µm; central cell (4-)5-6(-7) µm; fourth cell (4-)5.5-6(-7) µm] apical cell subconical, hyaline, collapsed at maturity, thin-walled, smooth, (3-)3.5-4.5(-6) x (3-)3.5-4(-5) µm; appendages tubular, branched or not, straight to flexuous; 2-4 appendages arising apically, tip rounded, (15-)26-32(-43) µm long; basal appendage occasionally absent, filiform, flexuous, slender, centric, (2-)4.5-6(-9) µm (Figure 4a).

Morphological characteristics in vitro - *Conidia* ellipsoidal to obovoid, 4-euseptate, the second and third septa often darkened and indistinct, cells unequal, without constrictions at septa, versicoloured, bearing appendages; basal cell obconic with a truncate base, bearing minute marginal frills, hyaline below, thin-walled, (4-)5-6(-8) x 4-4.5(-5) µm; second cell subcylindrical, pale brown, faintly verruculose, third and fourth cells doliiform to subcylindrical, medium brown,

verruculose, combined lengths of median cells (14.5-)16-17(-19) x (6-)7-7.5(-8) µm [length of second cell from base (5-)5.5-6(-7) µm; central cell, (4-)5-5.5(-7) µm; fourth cell, (4.5-)5-5.5(-6) µm] apical cell subconical, hyaline, collapsed at maturity, thin-walled, smooth, (3.5-)4-4.5(-5) x 3.5-4(-5) µm; appendages tubular, branched, straight to flexuous; 2-4 appendages arising apically, tip rounded but often absent due to frequent breakage of appendage, (10-)15-17(-22) µm long; basal appendage occasionally absent, filiform, flexuous, slender, centric, (2-)3-3.5(-5) µm.

Cultural characteristics in vitro - Colonies circular with undulate margins; mycelium of medium density, woolly, with white aerial mycelium; white in reverse. Colonies fast growing, reaching 69 mm in 7 days on MEA at 25°C (min ≥ 10°C; opt 25°C; max ≤ 30°C). Fertile after 12 days, with conidiomata developing over the entire surface of the colony and producing black, wet spore masses.

Material examined – **South Africa: Stellenbosch**, Protea Heights Farm, on a living leaf of *Protea* sp., J. Taylor, 6 Mar. 1998, JT158, PREM 56187, culture STE-U 1749; **Zimbabwe: Harare**, Aveley Farm, on living leaves of *Leucospermum cuneiforme* cv Sunbird, L. Swart, 6 Mar. 1998, JT212, PREM 56186, culture STE-U 1765; *ibid,* **Banket**, Mariondale Farm, 9 Mar. 1998, JT213, PREM 56188, culture STE-U 1777; *ibid,* **Juliasdale**, Zorora Farm, *Leucospermum vestitum*, 5 Mar. 1998, JT203, PREM 56189, culture STE-U 1783; **Karoi**, Glenellen Farm, on living leaves of *Protea eximia* (Salisb. ex Knight) Fourc., L. Swart, 10 Mar. 1998, JT211, PREM 56190, culture STE-U 1779.

Host range - *Leucospermum cuneiforme* cv Sunbird, *Leucospermum glabrum x tottum* cv Scarlet Ribbon, *Leucospermum vestitum, Protea eximia, Protea* sp.

Known distribution - South Africa, Zimbabwe.

DISCUSSION

The genus *Pestalotiopsis* Steyaert represents anamorphs of *Pestalosphaeria* M.E. Barr in the Amphisphaeriaceae (Nag Raj, 1993; Hawksworth *et al.*, 1995). It is a widespread genus and consists of at least 50 species, many of which are plant pathogens (Hawksworth *et al.*, 1995). There has been much confusion over the taxonomy *Pestalotiopsis,* especially with regard to its separation from *Pestalotia* De Not. (Sutton, 1969, 1980; Nag Raj, 1985, 1993). The concept

generally agreed upon was proposed by Steyaert (1949) who accepted a single species, *Pestalotia pezizoides* De Not., and reassigned many species previously placed in *Pestalotia* to other genera. However, as illustrated by Nag Raj (1993), there are many species which remain in *Pestalotia* that should be transferred to *Pestalotiopsis* or other allied genera.

The main difference between *Pestalotiopsis* and *Pestalotia* is in the nature of their conidial septa, with the former genus possessing eusepta and the latter, distosepta (Sutton, 1969, 1980; Nag Raj, 1985, 1993). Other differences have been noted such as the structure of the conidiomata (Guba, 1961; Sutton, 1969), but are not considered to be of primary importance in distinguishing these two genera. The number of septa has also been regarded as a defining feature in the taxonomy of these genera (Guba, 1961; Sutton, 1969). In the present study, the generic concept proposed by Nag Raj (1993) was followed, whereby species with three- or four-septate conidia are accepted in *Pestalotiopsis*.

Two species of *Pestalotiopsis* have previously been described from Proteaceae. *Pestalotiopsis montellicoides* Mordue was isolated from *Protea cynaroides* (L.) L. leaves from South Africa (Mordue, 1986), and a *Pestalotiopsis* sp., the anamorph of *Pestalosphaeria leucospermi* Samuels, E. Müll. & Petrini, was described from living leaves of a *Leucospermum* sp. in New Zealand (Samuels *et al.*, 1987). *P. montellicoides,* when compared to the collection in this study, differed mainly in the larger dimensions of its conidia (e.g. three median cells, 26-35 x 7.5-10.6 µm). The *Pestalotiopsis* sp. from *Leucospermum,* however, has conidia of similar dimensions to the *Pestalotiopsis* sp. in this study, but possesses concolourous median cells, and more cylindrical conidiogenous cells (11-18 x 2-2.5 µm). In culture, it becomes greenish-yellow (also noted for some isolates in this study e.g. STE-U 1783), and produces conidiomata in distinct concentric rings all over the surface of the colony. The combination of these features indicates that the collections in the present study represent a distinct species.

When compared to the *Pestalotiopsis* spp. in the key provided by Nag Raj (1993), this collection does not correspond to any previously described species, although it is most similar in dimensions and morphology to *P. macrospora* (Ces.) Steyaert. However, as mentioned previously, *Pestalotiopsis* remains in disarray, and there are a large number of additional species, listed by Nag Raj (1993), that need to be re-examined before new taxa can be described in this genus. Therefore, a species name will not be given to this collection, but the material will be deposited in PREM and cultures maintained at STE-U for future reference.

Samuels *et al.* (1987) noted that the conidia produced in culture differ somewhat to those from the host material. It was observed here that, in culture, the conidia of this species possessed medium brown rather than dark red-brown third and fourth cells, and the second cell appeared less verruculose than those of the conidia on the host material. In addition, the apical appendages of conidia in culture tended to be shorter, but this was often due to the appendages breaking.

During studies of fungal pathogens of Proteaceae in South Africa, a collection of *Pestalotiopsis* was made from a necrotic leaf spot on a living leaf after incubation of the leaf material. The morphology, dimensions and culture characteristics were identical to those of the collections from Zimbabwe, and this collection is thus considered conspecific. There have been no reports of extensive disease outbreaks on plantings in South Africa, and this is possibly due to climatic differences.

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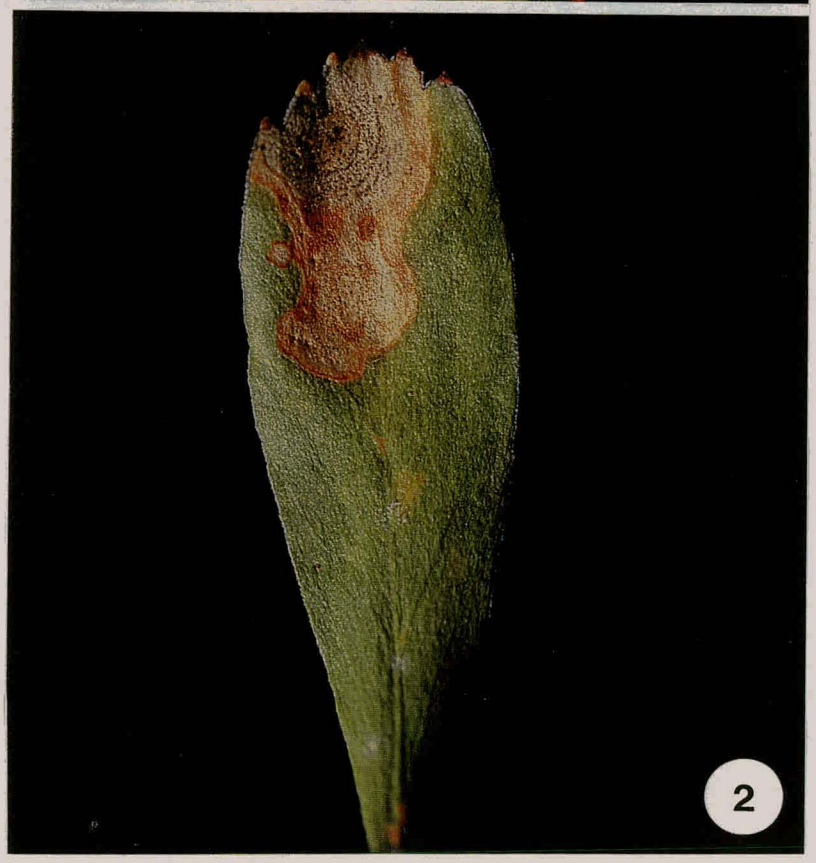

Figs 1, 2. Leaf spot symptoms associated with *Pestalotiopsis* infections. **1.** Inoculated leaves showing reddish lesions. **2.** Older, necrotic lesion with erumpent conidiomata.

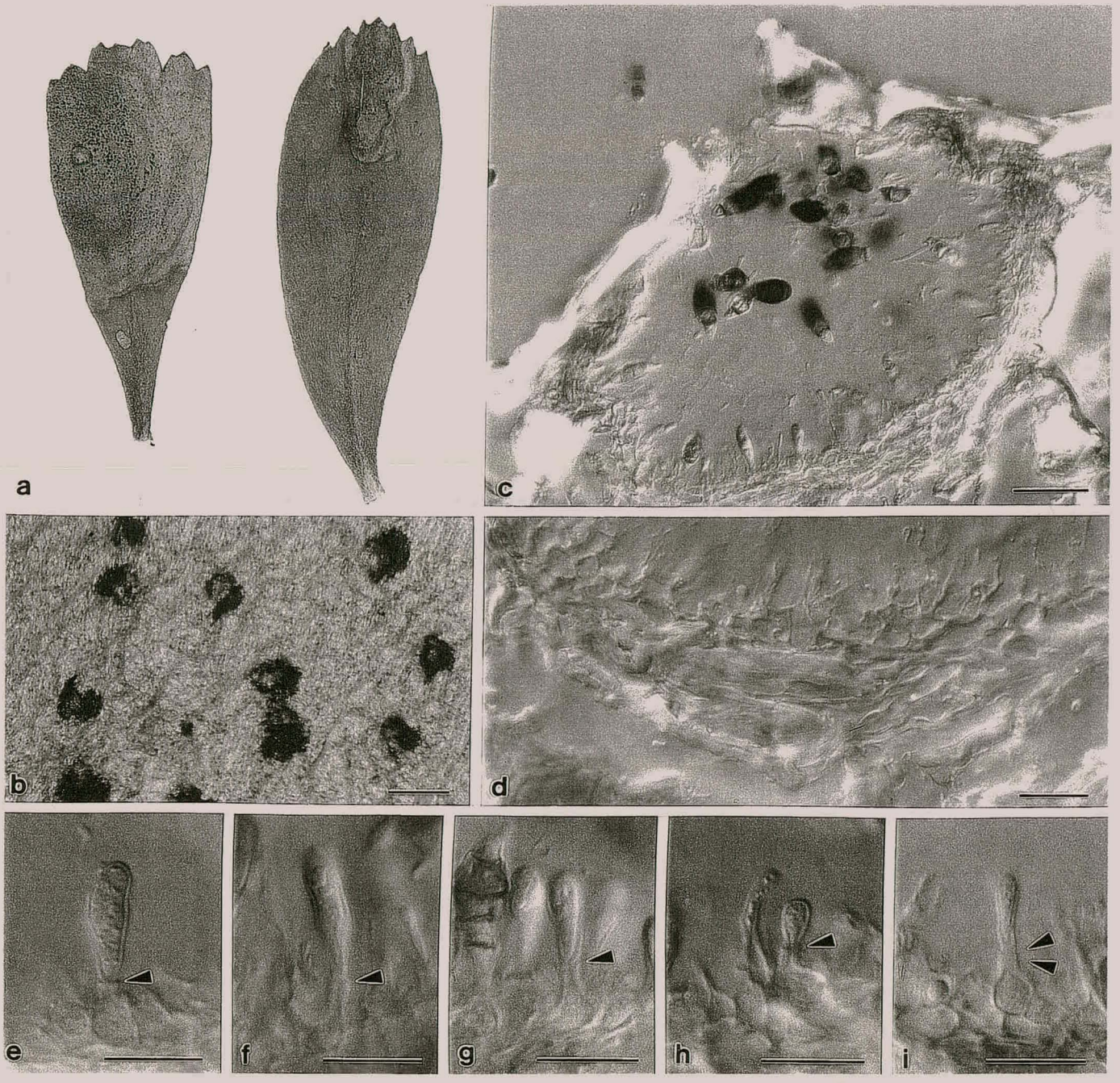

Fig. 3. *Pestalotiopsis* sp. (PREM 56186). **a.** Lesions on living leaves of *Leucospermum cuneiforme*. **b.** Erumpent conidiomata on leaf surface (Scale bar = 200µm). **c.** Transverse section of conidiomata (Scale bar = 20µm). **d.** Peridium (Scale bar = 10µm). **e-i.** Conidiogenous cells showing one or two enteroblastic, percurrent proliferations (arrowed) (Bar = 10µm).

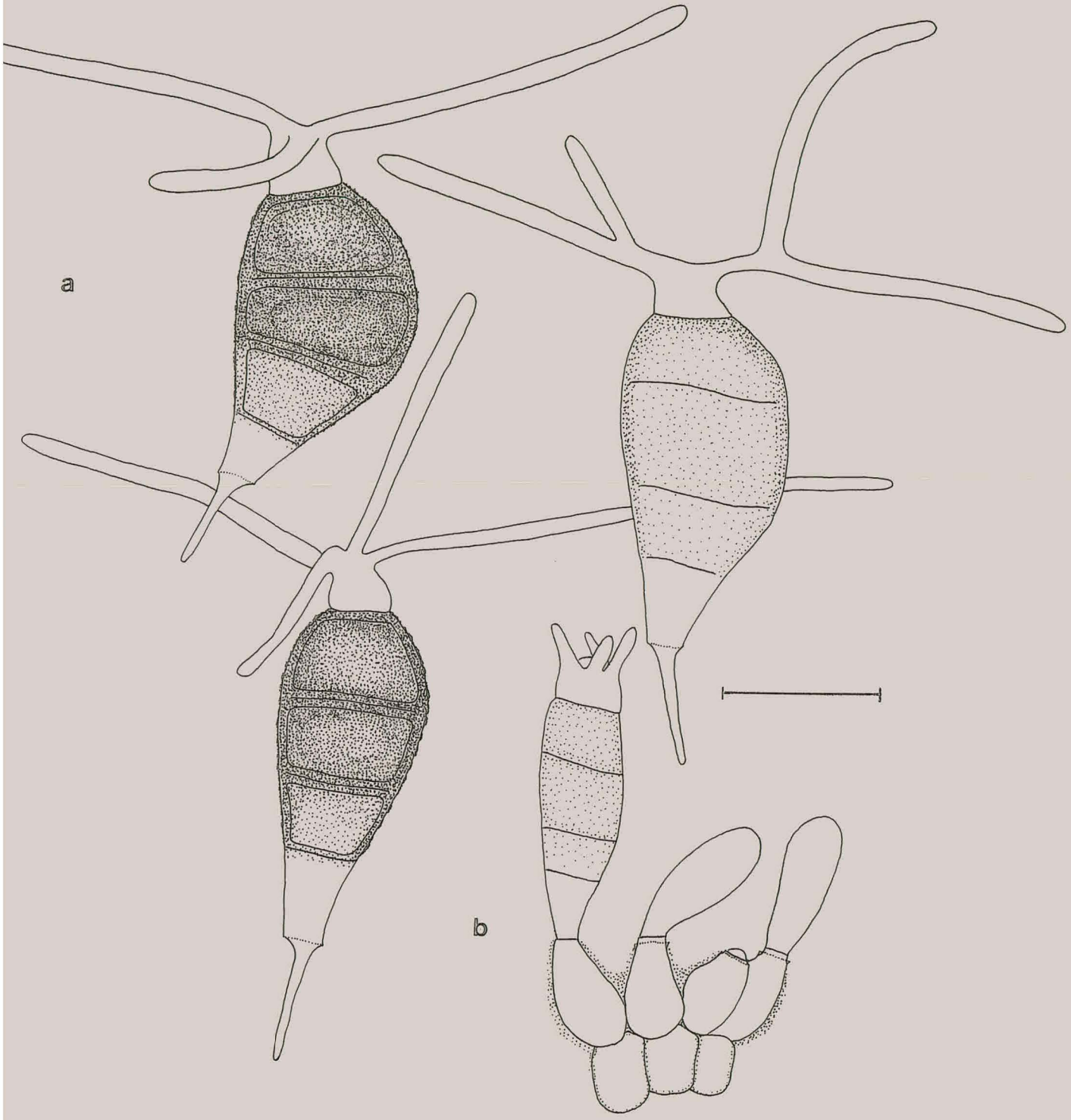

Fig. 4. Diagramatic representation of *Pestalotiopsis* sp. (PREM 56186). **a.** Conidia. **b.** Conidiogenous cells showing up to two enteroblastic, percurrent proliferations (Bar = 10µm).