Cultivation and Diseases of *Proteaceae*: *Leucadendron*, *Leucospermum* and *Protea*

Pedro W. Crous, Sandra Denman, Joanne E. Taylor, Lizet Swart and Mary E. Palm

Cultivation and Diseases of *Proteaceae*: *Leucadendron, Leucospermum* and *Protea*

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Cover: This photograph was taken on the slopes of Stellenbosch Mountain (Nov. 2003), clearly showing the Fynbos, and how the *Proteaceae* are being threatened by urbanization (note houses and roads extending up the mountain), agriculture (vineyards in the distance), forestry (pine and eucalypt stands), and other wild exotic plant species.

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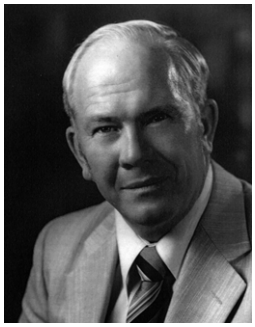
Dedication



This book is dedicated to *Peter Sidney Knox-Davies*. Prof. Knox-Davies passed away on 25 March 1999 in Stellenbosch, South Africa. During his impressive career, which spanned approximately 40 years, he made significant contributions to plant pathology as a science, as well as to the education of the many students who passed through his hands, including Pedro Crous and Sandra Denman. "Prof. Knox," as he was fondly referred to on campus, had a keen interest in the environment and a great love of the fynbos. He also initiated the fynbos pathology research programme, focusing not only on proteas, but also on rooibos tea and other crops indigenous to this Floral Kingdom.

Foreword

The *Proteaceae* is arguably the Southern Hemisphere's most charismatic plant family. A Gondwana relic, today it is best represented on nutrient-poor soils in Australia and southern Africa. *Proteaceae* are also important components of the Mediterranean floras of these countries, which are the richest non-tropical floras. The Cape Flora, with it's *Protea*-dominated Fynbos vegetation, is often considered a floral kingdom in its own right. A spin-off of being a dominant in nutrient-poor soils appears to be the production of large, robust, showy, long-lived flowerheads, adapted to pollination by birds and mammals. Little wonder then, that horticulturists have been enamoured with the *Proteaceae*.

By virtue of its prominence, *Proteaceae* have also enthralled ecologists. In the Cape Flora *Proteaceae* have taught us about fire ecology: the Blushing Bride hinted at a Phoenix-like existence, the Marsh Rose established fire as the central most-important ecological process in Fynbos, and the Mace Pagoda suggests that skipping fires may be an ecological strategy for coping with diseases and pathogens. *Proteaceae* have introduced to us canopy seed storage, ant seed dispersal, rodent pollination and a host of other fascinating strategies for surviving in Fynbos. An understanding of these, such as resprouting, ant and rodent seed dispersal, growth form and phenology are central in horticultural development and progress.

In conserving the Cape Flora, conservationists have grappled with the fact that, in this hotspot of biodiversity, we know about our plants, many of their larger pollinators and seed dispersers, but our knowledge of the less obvious flora and fauna is largely non-existent. Fortunately, it is here that horticulture can contribute to the conservation of Fynbos. Pests and pathogens are part of our proteoid legacy: they are part of the Fynbos ecosystem with which *Proteaceae* co-exist. Because commercially-cultivated *Proteaceae* are often planted near to natural stands of *Proteaceae*, indigenous diseases abound in *Protea* orchards. Some of these become commercially important, inviting attention from customs officials, pathologists and chemical companies. It is thus hardly surprising that our understanding of Fynbos plant pathogens is largely confined to those of *Proteaceae*. What their role might be in contributing to the biodiversity and ecology of Fynbos is still largely unknown, but the insights gained will aid in understanding how the Cape Flora evolved and is maintained.

Naturally, horticulturists are primarily interested in improving their crops. The cut flower industry is thriving, providing jobs, investment and spectacular flowers. Pathogens are thus a major focus of the industry. Whereas Cape *Proteaceae* grown outside of Africa have to acquire diseases from distant relatives and unrelated plants, in the Cape, horticulturists must contend with millions of year's worth of tightly co-evolved pathogens. In orchards, freed from the constraints of ecological checks, these pathogens may become of commercial significance. Quite apart from affecting the visual beauty of the cut flowers, the industry also faces intensive scrutiny from countries worried about the transfer of pathogens: including minor pathogens that might affect their fledgling and established *Proteaceae* cut-flower industries. The task facing our plant pathologists is thus a daunting one.

I am certain therefore that this book will be widely used. It is a milestone in our understanding and knowledge of pathogens affecting *Proteaceae*. It will also probably be quickly outdated, but then that is the plight facing all who tread in new arenas. What we have here is a solid foundation, a focal point with which to expand our horizons, a guidebook to start our exploration. This book is thus a welcome addition to the shelves of horticulturists, pathologists, customs officials, ecologists and conservationists. To some it is the culmination of decades of hard work. To others it is a state of art: a catalogue of what is known and what must be protected, monitored and kept under control. It is also a starting point: a window on an unexplored, exciting and unknown world - another insight into Fynbos ecology, brought to us courtesy of our *Proteaceae*. A journey has just begun.

Tony Rebelo
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December 2003

Preface

Because of their beauty, unique appearance, and relatively long shelf life, *Proteaceae* cut-flowers have become a highly desirable crop for the export market. The cultivation of *Proteaceae* is a thriving industry that supports thousands of jobs and other activities in countries where these flowers are grown. One of the limiting factors to the production of *Proteaceae* is the occurrence of a number of diseases. Some of these cause yield losses in the field. Additionally, the frequent occurrence of diseases on the cut-flowers often limits export of *Proteaceae* due to strict phytosanitary regulations in many countries. Knowledge of the fungi that occur on members of the *Proteaceae*, including host range, distribution and ability to cause disease, is essential in order to provide plant quarantine services with the information necessary for making accurate risk assessments. Further development and expansion of this industry depends on obtaining disease-free germplasm from countries where these plants are indigenous. Therefore it is important to document the fungi that occur on *Proteaceae*, and to establish the distribution of these fungi. Finally, because the *Proteaceae* are a major component of the South African Cape Floristic Region, which is the smallest, most diverse, yet most significantly threatened Floral Kingdom in the world, it is in the interest of conservationists and the *Proteaceae* industry to record all new diseases and potentially important pathogens.

A great deal of work on the diseases of *Proteaceae* has been conducted in South Africa. Prof. Peter S. Knox-Davies was the first to have a focused plant pathology research programme on this crop. Together with colleagues such as Walter F.O. Marasas and Sharon von Broembsen, he made a significant contribution towards our understanding of these diseases. Several students also received degrees under the guidance of Peter Knox-Davies while studying plant pathogens of *Proteaceae*, namely P.S. (Schalk) van Wyk, Suzelle Orffer (Serfontein), Lindi Benić and W.A. (Adriaan) Smit. In 1991 Pedro Crous joined the Department of Plant Pathology at the University of Stellenbosch, and took over the protea pathology research programme of the late P.S. Knox-Davies. Pedro was later joined in the department by Sandra Denman, who focused on the root disease and stem canker problems of *Proteaceae*. Joanne E. Taylor joined the group as post-doctoral candidate, with the specific task of studying and redescribing all the foliar fungal pathogens of *Leucadendron, Leucospermum* and, *Protea*, the three genera of major economic importance to South Africa. The group expanded further by obtaining post-graduate students, namely Lizeth Swart and Carien Lubbe (ARC-Fynbos, Elsenburg), and establishing international research ties with Mary E. Palm (USDA-APHIS, USA), Brett Summerell (Royal Botanic Gardens, Sydney, Australia), and many others. This led to an international network of researchers dedicated to collecting, describing, and determining the relative importance and distribution of *Proteaceae* plant pathogens.

In South Africa the most important commercially cultivated genera are *Leucadendron, Leucospermum* and *Protea*, which together formed the basis of the study. These genera have been successfully introduced elsewhere, such as in the USA (California and Hawaii) and Europe, where they are commercially cultivated. Australia has a much wider diversity of *Proteaceae* than South Africa, and commercially cultivates *Banksia, Grevillea,* and *Telopea*, among others. This reference treats a number of diseases of those genera but additional study is needed in order to document and better understand the host range and distribution of the fungal mycota of all genera of the *Proteaceae* worldwide.

Pedro W. Crous, Sandra Denman, Joanne E. Taylor, Lizeth Swart and Mary E. Palm
December 2003

About the authors

Pedro Crous did his B.Sc. (Forestry) and M.Sc.Agric. in plant pathology at the University of Stellenbosch, and obtained his Ph.D. (Agric.) in plant pathology at the University of the Orange Free State in 1992. His main research interests are the characterisation of fungal plant pathogens, and the integration of fungal morphology and DNA phylogeny. He heads an active research group focusing on the pathological, morphological and genetic characterisation of fungal pathogens in agriculture. He is Professor in Plant Pathology at the Universities of Stellenbosch and Pretoria (South Africa), and Wageningen (the Netherlands), where he is presently the Director of the Centraalbureau voor Schimmelcultures.

Sandra Denman obtained her B.Sc. (Botany and Environmental Sciences) degree at the University of Cape Town, and both M.Sc. Agric. and Ph.D. (Agric.) degrees in plant pathology at the University of Stellenbosch, South Africa. Sandra's main research interests are the epidemiology and ecology of plant pathogens on indigenous hosts. For several years she also headed a soil-borne disease programme on *Proteaceae* and other fynbos crops in South Africa. She is currently a researcher at the Forestry Commission in the U.K., where she leads research projects on *Phytophthora* diseases of forest trees.

Joanne Taylor undertook a B.Sc. (Biology) at Portsmouth University (U.K.), and M.Sc. (Ecology) at Durham University (U.K.), and a Ph.D. in fungal biodiversity and biogeography in Hong Kong in 1998. She then undertook post-doctoral research at the University of Stellenbosch (South Africa), a study aimed at a revision of the fungal leaf spot pathogens associated with *Proteaceae*. Her main research interests are in fungal taxonomy and ecology. She is currently a lecturer in mycology at the University of Botswana (Botswana).

Lizeth Swart did her B.Sc. (Agric.) and M.Sc. (Agric.) in plant pathology at the University of Stellenbosch, South Africa. She received her M.Sc. in 1999, entitled "Pathogens associated with diseases of *Protea*, *Leucospermum* and *Leucadendron*", while working as a researcher at the Fynbos Unit of the Agricultural Research Council. Currently, she works at the Department of Plant Pathology, University of Stellenbosch, partly in the Disease Clinic, but also rendering a diagnostic service to protea producers.

Mary E. Palm received her B.A. (Biology) from St. Olaf College in Northfield, Minnesota. She obtained her M.Sc. and Ph.D. in plant pathology (mycology emphasis) from the University of Minnesota in 1983. Her research interests include systematics of fungal plant pathogens, especially ascomycetes and their anamorphs. She is the National Mycologist with the USDA Animal and Plant Health Inspection Service and is responsible for identifying plant pathogenic fungi on imported plants and plant products. She is an adjunct Associate Professor in the Department of Plant Pathology, Pennsylvania State University and served as the President of the Mycological Society of America in 1997–1998.

SUMMARY

The *Proteaceae* represent one of the Southern Hemisphere's most prominent flowering plant families. Because of their beauty, unique appearance, and relatively long shelf life, *Proteaceae* cut-flowers have become a highly desirable crop for the export market. The cultivation of *Proteaceae* is a thriving industry that provides employment in countries where these flowers are grown, often in areas that are otherwise unproductive agriculturally. Diseases cause a loss in yield, and also limit the export of these flowers due to strict phytosanitary regulations. In this publication the fungi that cause leaf, stem and root diseases on *Leucadendron*, *Leucospermum* and *Protea* are treated. Data are provided pertaining to the taxonomy, identification, host range, distribution, pathogenicity, and control of these pathogens. Taxonomic descriptions and illustrations are provided and keys are included to distinguish species in genera where a number of species affect *Proteaceae*. Disease symptoms are described and colour photographs are included. Where known, factors that affect disease epidemiology are discussed. Disease management strategies are also presented that will assist growers and advisors in making appropriate choices for reducing disease in specific areas. Information is also provided relating to crop improvement, cultivation techniques, harvesting and export considerations. Further development and expansion of this industry depends on producing and obtaining disease-free germplasm from countries where these plants are indigenous. For that reason it is important to document the fungi that occur on *Proteaceae*, and to establish the distribution of these fungi. These data are essential for plant quarantine services for use in risk assessments. Taxonomic novelties introduced are: *Coniothyrium grandicipis* Joanne E. Taylor & Crous, sp. nov., *Pseudocladosporium proteae* (Viljoen & Crous) Crous, comb. nov., *Saccharata* Denman & Crous, gen. nov., and *Saccharata proteae* (Wakef.) Denman & Crous, comb. nov.

Key Words: canker pathogens, cultivation, cut-flower industry, disease control, foliicolous pathogens, harvesting, *Leucandendron*, *Leucospermum*, phytosanitary regulations, *Protea*, *Proteaceae*, root rot, wilt.

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SECTION 1

INTRODUCTION

PURPOSE AND CONTENT

This book is designed to serve several functions. It provides extensive background information on the importance, cultivation and harvesting of members of the family *Proteaceae*, especially *Protea, Leucadendron* and *Leucospermum*. Topics covered include history, crop improvement, propagation methods, cultivation and harvesting guides, post-harvest problems, and phytosanitary issues. This information will be very useful to protea growers and enthusiasts.

This book also provides in-depth information about more than 100 pathogens, mainly fungi, that cause diseases of *Proteaceae*. Included for each are descriptions of symptoms, detailed descriptions of the pathogen, and information on distribution, biology, and control measures. Additionally keys are included for genera with several species occurring on *Proteaceae*.

This book is intended to serve the needs of:

1. Persons that cultivate proteas who may use this book as a resource on cultivation practices, on diseases that may present problems, and on methods for controlling those diseases.
2. Plant pathologists, diagnosticians, extension agents, and others who can use this book to determine the cause of a disease and how to control the disease.
3. Regulatory officials who will find useful information for pest risk assessments, which are the basis for determining whether an organism is a quarantine pest.

ARRANGEMENT

The first section provides materials, methods and other information pertaining to how the data for the book were gathered. The second section covers the history, cultivation and harvesting of *Proteaceae*. The third section includes four chapters that treat the organisms and the diseases they cause based on the plant part affected. Chapter 1 treats foliar diseases, Chapter 2 stem, shoot, and flower diseases, and Chapter 3 root diseases. Chapter 4 covers diseases caused by bacteria and phytoplasmas. Finally, in the References, information is provided to all literature cited in the text. Three indexes are provided, namely a general index, a host index, and an index to nomenclatural novelties.

For each of the diseases or disease-complexes we provide an overview of the disease, including the common name. This is followed by a treatment of species involved in that disease complex. For each fungus we provide a description of symptoms, detailed morphological features of the pathogen on the host, and cultural characters. This information is based mainly on observations of the authors. When the information is from the literature a citation is provided.

The host range and geographic distribution are given for each species, with host cultivars cited as, e.g. *Protea* 'Lady Di.' Full details and authorities for all species are provided in the indexes at the back of the book. Distribution data are mostly limited to country, but provinces, where known, are indicated. The nine provinces of South Africa are abbreviated as follows: Western, Eastern and Northern Cape (WC, EC, NC), KwaZulu-Natal (KN), Free-State (FS), Gauteng (GT), Mpumalanga (MP), North-West (NW) and Northern Province (NP).

MATERIALS AND METHODS

Most samples examined by the authors were collected in Australia, South Africa or the U.S.A. (Hawaii and California). That diseased material was treated as follows:

Isolation from leaf and stem samples

Symptomatic leaf and stem samples of cultivated *Proteaceae* (*Leucadendron, Leucospermum*, and *Protea*) were collected and examined for the presence of fruiting structures or spores. Samples with mature fruiting bodies were processed immediately whereas those with immature or no fruiting bodies were stored dry, in plastic bags in a cold room at 4ºC for approximately 1 month. This method facilitated the production or maturation of fruiting structures. Cultures were obtained from single spores using one of two techniques. Generally, spores were liberated in a small amount of sterile water on the surface of a plate containing 2% malt extract agar (MEA; Biolab, Midrand, South Africa) supplemented with 0.04g/L streptomycin sulphate. After 1 h the plate was inverted to prevent the spread of yeasts and incubated at 25ºC. After a minimum of 24 h, germinating spores were located using a stereomicroscope with stage illumination and transferred to fresh MEA plates. Permanent slides were made of germinating spores, which are important identification features in some genera such as *Mycosphaerella*. For some ascomycetes, ascospore colonies were initiated using the spore shooting method described by Crous (1998).

Isolation from wilted or chlorotic plants

Declining plants with chlorotic leaves or symptoms of wilt or root rot were brought to the laboratory for analyses. A small section of the crown bark was removed to see whether there was evidence of white fungal mycelial fans beneath the bark. The crown also was split open longitudinally and roots and stems were cut in longitudinal- and cross-section to check for vascular discoloration. Isolations were made from the roots, crown and vascular tissue of plants. Diseased material was surface disinfected in 70% ethanol for 30 s, 1% NaOCl for 1 min, and again in 70% ethanol for 15 s. Pieces, approximately 5 mm long, then were taken from the margin between healthy and discoloured roots, crowns, and vascular tissue and were plated on PDA, *Phytophthora* selective medium (PH) (Solel & Pinkas, 1982) and Komada's medium (Komada, 1975). The plates were incubated at room temperature for 4–10 days. Hyphae growing out of the tissue were subcultured onto divided plates containing PDA in one half of the dish and carnation leaf agar (CLA; Fisher *et al*., 1982) in the other. Divided plates were incubated for 3–6 weeks at 25°C under near-ultraviolet and cool white fluorescent light with a 12 h light cycle.

Cultural and microscopic characters

To encourage sporulation *in vitro*, colonies were transferred to divided plates containing MEA in one half of the dish and CLA in the other. Cultures were incubated at 25ºC under continuous near-ultraviolet light. Isolates that did not sporulate under those conditions were inoculated onto plates containing 2% potato dextrose agar (PDA; Biolab, Midrand, South Africa) or potato carrot agar (PCA; Gams *et al*., 1998).

For microscopic examination, the fungi were mounted in clear lactophenol or water (where stated) and measurements made at 1000 × magnification. The 95% confidence intervals were determined from 25–50 observations. The minimum and maximum ranges are given in parentheses.

Colony growth and cultural characteristics were determined at different temperatures on MEA after different periods of incubation, depending on the genus (as stated). Where cultural characteristics were determined on PDA or PCA, this is noted in the text. Linear growth was measured on cultures on MEA grown from 5–35ºC at 5º intervals in the dark, as explained in Crous & Wingfield (1996). Colony colours (top and reverse) were determined for cultures incubated at 25ºC in the dark using the colour charts of Rayner (1970).

Voucher specimens

Herbarium specimens are lodged at the National Collection of Fungi, Pretoria, South Africa (PREM), with some specimens at HKU(M) in Hong Kong, and CABI BioScience (IMI) in the UK. Reference cultures are maintained at the Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands (including those formerly deposited at STE-U).

SECTION 2

PROTEACEAE – IMPORTANCE, CULTIVATION AND HARVESTING

INTRODUCTION

Proteus was the mythological Greek god who, when grasped tightly, changed into numerous animate or inanimate forms. The floral variability within the *Proteaceae* may thus explain why this family was named after Proteus by Linnaeus (Rebelo, 2001). The word 'protea' is commonly used to refer to any member of the *Proteaceae*, and will be used as such throughout this text (Paterson-Jones, 2000).

There are seven subfamilies in the *Proteaceae*, 82 genera, and approximately 1700 species (Criley, 1998), nearly all of which occur in the Southern Hemisphere. The *Proteaceae* always have been regarded as very different from other flowering plant families based largely on the distinctive structure of the flowers themselves, For example, the floral sheath (perianth) of each individual flower, which covers the style, is split into four equal parts. When the flower is ready to be fertilised, the sheaths peel back to reveal the pollen presenter near the end of the style. Other distinguishing features include the presence of four anthers that adhere to the perianth, and a single-chambered ovary where, after fertilisation, one or more seeds form (Rebelo, 2001).

The *Proteaceae* are one of the Southern Hemisphere's most prominent flowering plant families and are amongst the oldest groups of flowering plants. They existed in Gondwanaland long before it started to break up 300 million years ago (Vogts, 1982). The subfamily *Proteoideae* occurs in Australia and New Zealand, and is dominant in Africa. In contrast, the *Grevilleoideae* occurs mainly in Australia, and to a lesser degree in South America and the South-Western Pacific Islands, and only a single species of this subfamily occurs in Africa (Vogts, 1982; Rebelo, 2001). In general there is not a specific genus that occurs naturally in both South Africa and Australia, although more than half of the genera in South America are also indigenous to Australia (Vogts, 1982).

The richest areas, in terms of species concentration, are Eastern and Western Australia, and the Western Cape region of South Africa. Australia has the greatest diversity of *Proteaceae*, including representatives of all seven subfamilies. More than 800 species representing 45 genera are found in Australia, of which 550 species are found mainly in the South-Western parts. Africa, the second most species-rich region, has members of only two of the subfamilies (Paterson-Jones, 2000). Approximately 400 species occur in Africa, of which 360 species are found in southern Africa. Remarkably, 330 species (representing 14 genera) are confined to the Cape Floral Kingdom, making this area exceptional in its natural assortment of *Proteaceae*. The Fynbos is a major part of the Cape Floral Kingdom and the *Proteaceae* forms one of the main components of the Cape Floristic Region along with ericoids, restioids and geophytes (Cowling & Richardson, 1998).

The distribution of *Proteaceae* in the rest of the world is less impressive, with about 90 species found in Central and South America, 80 on the islands east of New Guinea, and 45 species in New Caledonia. A few species also occur in Borneo, Madagascar, the Malaysian Peninsula, New Guinea, New Zealand, South-East Asia, and southern India (Vogts, 1982; Rebelo, 2001). One species occurs as far north as southern Japan (Paterson-Jones, 2000).

The Cut-Flower Industry

AFRICA

TEMPERATE AFRICA

Protea (*P.*) is the largest genus in Africa with the widest distribution (Paterson-Jones, 2000). Currently, about 114 species of *Protea* are recognised, of which 82 occur in southern Africa south of the Limpopo River. Thirty-five species occur in tropical Africa north of the Limpopo River, and 3 species are common to both regions. Seventy of the 82 South African species are endemic to the Cape Floral Kingdom, while a group of 19 species occurs in the summer rainfall area of southern Africa (Beard, 1993; Paterson-Jones, 2000; Littlejohn, 2001b). Approximately 30 *Protea* species have been commercially harvested or cultivated for the production of cut-flowers since the 1960's in South Africa. Currently, only 10 species are commercially cultivated for export quality cut-flowers. The remaining species are sold in minor quantities due to problems with cultivation, poor quality or unfavourable flowering times (Littlejohn, 2001b).

Leucadendron (*Lcd.*) is the second largest genus of the *Proteaceae* in Africa, and only occurs in South Africa (Paterson-Jones, 2000). Eighty-nine species have been described to date (Williams, 1972; Rebelo, 2001), of which only two species are found outside the Cape Floral Kingdom, along the east coast (Littlejohn & Robyn, 2000; Paterson-Jones, 2000). They are dioecious, i.e. male and female flowers are produced on different plants. In some species, male and female flowerheads are so different in appearance that it is difficult to tell that they are of the same species (Paterson-Jones, 2000). Various products of *Leucadendron* are used commercially. Foliage is used in the fresh-cut-

flower industry, while the cones are used in the fresh and dried flower industry (Littlejohn, 1999). Very limited quantities of dried products are produced in other countries cultivating these plants (Littlejohn & Robyn, 2000). *Leucadendron* spp., marketed as 'Cape Greens' in South Africa, are largely picked from natural stands or from broadcast seed-sown stands (Coetzee & Littlejohn, 1995; Littlejohn & Robyn, 2000). The *Leucadendron* cultivar 'Jubilee Crown' is sold in the largest quantities, followed by *Lcd.* 'Safari Sunset', and *Lcd. platyspermum* (Anon., 2002).

The genus *Leucospermum* (*Lsp.*) consists of 48 species. Their natural occurrence is confined to southern Africa, mostly in the Cape Floral Kingdom (Rourke, 1972). Their natural distribution extends up the east coast and eastern highlands of South Africa to the Chimanimani mountains of Zimbabwe, and also up to the west coast into Namaqualand (Paterson-Jones, 2000). Species of *Leucospermum* are commonly referred to as pincushion proteas. Only a few species have been utilised as cut-flowers (*Lsp. conocarpodendron*, *Lsp. cordifolium, Lsp. glabrum, Lsp. lineare*, *Lsp. patersonii*, *Lsp. vestitum*), but natural and manmade interspecific hybrids (clonal selections), are also grown commercially (Jacobs, 1985; Littlejohn, 1999). *Lsp. truncatulum* is used for its foliage (Littlejohn, 1999).

Most cultivated proteas, namely *Protea*, *Leucospermum* and *Leucadendron* species, are indigenous to South Africa (Forsberg, 1993). South African germplasm was brought to other countries where it was utilised to develop a cut-flower industry. Countries that grow Cape Flora include Australia, the Azores (Leandro, 2002), Chile, El Salvador, France, Israel, Madeira, New Zealand, Portugal, South Africa, Spain (Canary Islands), the USA (California and Hawaii) and Zimbabwe (Regional reports, 1998; Leonhardt & Criley, 1999). The cultivation of proteas in many other countries developed along with the South African protea industry (Coetzee & Littlejohn, 2001). Because both the Northern and Southern Hemisphere countries presently cultivate proteas, they can be found on the international markets during most of the year.

Historically, the utilisation of proteas has been important to the socio-economic development in South Africa. *Protea* plants have had many uses such as in the manufacturing of furniture and wagon wheels (*P. nitida*), to tan leather, as a source of black ink (Rourke, 1980), and as traditional medicines (Van Wyk *et al*., 1997). The South African 'trekboere' (farmers) treated coughs and other chest complaints with the 'bossiestroop' (syrup) they made from the nectar of *Protea repens* (Cowling & Richardson, 1998). During the late 18th century, the Dutch settlers planted forests of *Leucadendron argenteum* (the silver tree) and *Leucospermum conocarpodendron* for firewood (Vogts, 1982). Later, street hawkers started to pick flowers in the wild and sold them on the markets and streets of Cape Town (Coetzee & Littlejohn, 1995). Seeds and plants were taken to Europe during the 18th and the 19th century, and in 1810 the first proteas were cultivated in Kew by Francis Mason and William Aiton (Vogts, 1982).

In the 1800's, mission stations were established in the rural areas of the Cape. The inhabitants of the Elim and Genadendal mission stations were the first to export dried, indigenous protea flowers to Europe in 1886 (Krüger & Schaberg, 1984). In 1910, *P. cynaroides* was cultivated commercially for the first time in South Africa on a farm outside Stellenbosch (Lighton, 1960). Since 1913, proteas have been cultivated in the National Botanical Gardens at Kirstenbosch (Vogts, 1962).

Commercialisation of the dried flower industry began in the mid 20th century, when large quantities of dried flowers were exported by ship to Europe (Coetzee & Middelmann, 1997). However, in the 1960's, air transport gave an important economic boost to the protea export industry because the more expensive fresh cut-flowers could now be shipped (G.M. Littlejohn, ARC, Fynbos Unit, Elsenburg, South Africa, pers. comm., 2002). By the 1980's, harvesters and exporters provided a semi-consistent supply of products harvested from the wild (Wessels *et al*., 1997).

SOUTH AFRICA

The South African *Proteaceae* comprise 14 genera, of which 7 genera are commercially utilised (Vogts, 1982; Littlejohn, 1999; Paterson-Jones, 2000; Rebelo, 2001). Three of these, *Leucadendron*, *Leucospermum* and *Protea* are of the greatest commercial value and are grown for their exotic, brightly coloured and textured flowers or bracts, which are in high demand on the world floriculture market (Coetzee & Littlejohn, 2001). The commercial trade in Fynbos in South Africa is composed of the dried flower industry (40%) and the fresh-cut-flower industry (60%). About 80% of dried flowers and 70% of fresh cut-flowers are exported (Coetzee & Middelmann, 1997; Wessels *et al*., 1997; Coetzee, 1999).

The dried flower industry markets about 64 products. Some products include foliage of *Leucadendron* and other species, cones of *Leucadendron*, *Protea* inflorescences (buds and open flowers) and *Protea* rosettes consisting of the receptacle of the flower only. Products are harvested from the wild during much of the year but the main harvesting period is during winter and spring.

The fresh cut-flower industry markets 137 species or cultivars, of which *Protea*, *Leucospermum* and *Leucadendron* are the most important. The fresh cut-flowers are mainly exported to Europe, but the American and Far East markets are also considered as export possibilities (Coetzee & Middelmann, 1997). During 1998, 3 666 tons of fresh-cut-flowers were exported of which 30% was represented by the genus *Protea*. Sales in South Africa and export of *Protea* flowers occur throughout the year, while export quantities peak during October (Coetzee & Littlejohn, 2001). During the 1999/2000 season, there was an overall increase of 1 million *Protea* stems exported compared to the previ-

ous period, as well as an overall increase of 250 000 *Leucadendron* stems (Anon., 2001).

A survey conducted in South Africa in 1998/99 indicated that 189 000 ha of natural production area was utilised, 2 795 ha supported broadcast seed-sown cultivation, 950 ha were seedling-based orchards, and 491 ha consisted of cultivar-based orchards (B. Baker, Secretary, SAPPEX, South Africa, pers. comm., 2002).

In 1996, the majority of the producers still harvested from the field (Coetzee & Littlejohn, 1995), although approximately 350 growers cultivated *Proteaceae* commercially (Wessels *et al*., 1997). Rural communities, such as Genadendal, Karwyderskraal, Sandfontein and Elim, have a history of harvesting from the wild (Heydenrych, 1997) and still rely on this. In 1999, the Fynbos industry directly employed 6 250 employees and together with their families, 25 000 people were dependent on the industry (Anon., 1999b). However, farmers are now attempting to cultivate proteas due to low returns on wild material, which is usually aesthetically inferior to cultivated material. Furthermore, concern for degradation of the natural veld and diminishing genetic resources is encouraging cultivation (Brinkhuis *et al*., 2001).

The economic yield of the dried and fresh-cut-flower industries in 1996 was about ZA R 37.2 million and ZA R 64.5 million, respectively. The monetary value of the industry in 1996 was approximately ZA R 81.7 million (Coetzee & Middelmann, 1997) (the exchange rate in 1996 was approximately 4.5 ZA R to 1 US $). In 1999, the export market was worth approximately US $ 15 million, with a further US $ 15 million generated from peripheral industries such as packaging and export agencies. Combined with the domestic sales of proteas (worth approximately US$ 3 million) the protea industry in South Africa was worth over US$ 30 million in 1999 (G.M. Littlejohn, pers. comm., 2001).

TROPICAL AFRICA

Thirty-one species of *Protea* occur naturally north of the Limpopo (Beard, 1993). The tropical species vary from being localised (e.g. *P. praticola* that only occurs in the highlands of southern Tanzania), to widespread (e.g. *P. madiensis*). The flower heads of tropical *Protea* spp. are generally not as exotic as the Cape species. Colour of flower heads is variable, but generally bracts and flowers are pinkish. *P. asymmetrica* of the Inyanga Highlands of Zimbabwe is probably the most beautiful species, while *P. rupestris* is the most bizarre. The bud is enclosed in smooth, red to purple bracts which completely fall away when the head opens, leaving the protruding flowers.

Some of the tropical species are the only real trees in the genus *Protea*, reaching 5–6 m in height (*P. petiolaris*) or up to 10 m (*P. rupestris*). The largest is *P. welwitschii* (Beard, 1963), which is found in the mountains of western Angola. The growth habit of some of the species is variable, ranging from trees to shrubs to miniature (suffrutescent) forms, in which the plant consists of numerous ephemeral stems arising from an underground root. Other tropical species are less variable and tend consistently to be either shrubs or miniature forms. It may be that the 15 suffrutescent species have evolved to suit the fire-prone habit of the tropical savanna. The tree habit is considered ancestral, evolving to the shrub habit, and into the suffrutescent form (Beard, 1993, 1995).

Tropical *Protea* species occur in two principal habitats. One habitat is mountainous and related to the Cape Fynbos, while the other is a distinct plateau woodland. This savanna woodland covers much of Africa south of the Sahara and extends between altitudes of 1000 and 2000 m above sea level.

The Chimanimani Mountains of Zimbabwe have the only tropical *Leucospermum* species, namely *Lsp. saxosum* (Beard, 1995). In the 1970's, commercial cultivation of proteas started on a large scale in Zimbabwe, with the first protea cut-flowers being exported in 1981 (Middelmann & Archer, 1999). Growth conditions in Zimbabwe are significantly different from the Mediterranean-type regions where most commercial *Proteaceae* are grown. The tropical climate in Zimbabwe is hot and wet in summer and dry in winter (Percival *et al*., 2001). Because indigenous, tropical *Protea* species do not have commercial characteristics such as the vigour and stem length needed for horticultural cultivation, the need for cultivars arose. Nearly all *Proteaceae* cultivars planted in Zimbabwe have been imported from Australia, New Zealand and South Africa. Thus, most cultivars obtained are indigenous to winter rainfall regions, and these Mediterranean-type species are highly susceptible to diseases that flourish during the hot, wet weather that prevails in tropical Zimbabwe (Archer, 1998). Commercial nurserymen are continually developing hybrid cultivars more suitable for sub-tropical conditions. About 13 cultivars have been developed in Zimbabwe, of which five have been registered with the International Protea Register (Sadie, 1998a). By 1998, 95% of the industry was cultivar-based (Archer, 1998).

Currently, there are 270 ha of intensively cultivated proteas in Zimbabwe, consisting of *Leucadendron* (37%), *Leucospermum* (30%), *Protea* (24%), and 9% other *Proteaceae* genera (Percival, 2000). Various species of *Protea* are being cultivated of which 'Pink Ice' is the primary cultivar (Middelmann & Archer, 1999). *P. cynaroides* grows well in most parts of the country and has been planted on a large scale, while there are also substantial plantations of *P. neriifolia* cultivars. There has been, however, only a 7% increase in *Protea* plantations since 1997 due to disease and nutritional problems (Percival *et al*., 2001). *Sphaceloma protearum*, for example, destroyed nearly 70% of the *Protea* 'Pink Ice' plantations in 1997.

Leucospermum varieties, except the *Lsp. cordifolium* types, grow vigorously in Zimbabwe and are

tolerant of different soils. There are large plantations of the indigenous *Lsp. saxosum*, *Lsp.* 'Tango', *Lsp.* 'High Gold', *Lsp*. 'Scarlet Ribbon' and *Lsp. cuneiforme* selections. Plantations of *Lcd.* 'Safari Sunset', *Lcd.* 'Inca Gold', *Lcd*. 'Joan Condy', *Lcd.* 'Jester' and *Lcd.* 'Silvan Red' have increased significantly (Percival, 2000; Percival *et al*., 2001). Since the last survey done in 1997, there has been a 40.8% increase in the total number of plants cultivated in Zimbabwe (Percival, 2000).

The 125 protea growers are mainly in the north-eastern part of the country, but five areas can be identified, namely Centenary, Central Watershed, Eastern Districts, Makonde and Karoi (Archer, 1998; Percival, 2000).

Seventy-five percent of the flowers are exported to the Netherlands. Other export markets include Belgium, Hong Kong, Japan, Kuwait, Switzerland, Taiwan, UK, United Arab Emirates and the USA. Only 4% of the crop is sold locally (Archer, 1998). Protea exports in 1998/99 massed 1923 metric tons and sold for an estimated US $ 8.84 million (Export Flower Grower's Association of Zimbabwe, pers. comm., 2001).

OTHER AREAS

AUSTRALIA

Southwestern Australia has the greatest genetic resource of *Proteaceae* in the world, with approximately 620 endemic species (Lamont *et al*., 1995). The Botanical Garden in Adelaide started cultivating South African Cape flora in 1871 (Lighton, 1960). It is estimated that approximately 800–1000 ha are now planted to South African *Proteaceae* (Middelmann, 2002). Proteas are also grown commercially in the southwestern part of Western Australia, the east coast of New South Wales, parts of southeastern Queensland, most of Victoria, Adelaide Hills, Fleurieu Peninsula, the southeast part of South Australia, and parts of Tasmania – mainly on the northern coast, but also around the Hobart area (K.L. Delaporte, Dept. of Horticulture, Viticulture and Oenology, Adelaide Univ., Australia, pers. comm., 2002).

Protea production in Australia has increased substantially since 1984, with over 200 ha planted in Western Australia (Webb, 1990). The first plantations contained a large number of species. In the early 1990's, species of *Leucadendron* in particular were cultivated. It has been estimated that approximately one third of the cut-flowers exported from Western Australia during 1996 (AUD $ 15 million) were proteas of South African origin. These flowers were chiefly destined for the eastern states of Australia, Canada, France, Germany, Japan, Netherlands, Singapore, Taiwan and the USA (Sedgley, 1998b). In 1998, approximately 81 ha were cultivated in Queensland and that produce was destined for markets in Canada, England, Germany, Hong Kong, Japan, Netherlands, Singapore, Taiwan and the USA. A large proportion of the Queensland domestic product also went to southern Australia (Moffatt, 1998).

The indigenous *Proteaceae* in Australia are declining due to a number of environmental changes. In some areas, land-clearing, increased fire frequency, and post-fire invasion of weeds threaten the native protea habitat. The spread of pests and pathogens as well as changing weather patterns also negatively affect survival of these plants. *Phytophthora cinnamomi*, to which 92% of *Proteaceae* from South-Western Australia are susceptible, has become so widespread that it has caused a major epidemic in the southern sandplains. Decreased annual rainfall also enhances the disease threat posed by canker-causing fungi. Furthermore, reduced population sizes and lower numbers of possible pollinators such as insects, birds and mammals, diminish the chances of flowering, seed production and seedling establishment (Lamont *et al*., 1995).

FRANCE

Growers in the south of France cultivate *Proteaceae* along the French Mediterranean coast. Many species of *Proteaceae* occur naturally in Mediterranean climates, thus it was expected that these plants would flourish there. The low winter temperatures and calcareous soils, however, resulted in the need to grow *Protea* under shelter and in soilless substrates to obtain optimum growing conditions (Montarone *et al*., 1997). Therefore the protea industry in France is very small and limited to controlled conditions.

ISRAEL

In Israel, the export floricultural industry started in the late 1960's and proteas were introduced to Israel in 1974. Commercial products are limited to *Banksia*, *Grevillea*, *Lcd. discolor*, *Lcd.* 'Safari Sunset', *Leucadendron* spp., *Lsp. patersonii* and *P. obtusifolia* (Ben-Jaacov, 1986a, 1992).

Currently in Israel there are 236 ha planted to *Protea*, *Leucospermum* and *Leucadendron* spp. and 40.1 million stems are harvested annually (J. Ben-Jaacov, ARO, Volcani Center, Israel, pers. comm., 2002). Generally, however, it is difficult to grow proteas in Israel. Factors that limit production include high soil pH, lack of irrigation water, high mineral content (soil phosphate), the presence of free lime in the soil, and an abundance of soil-borne diseases (Ben-Jaacov, 1986a, 1992; Ben-Jaacov *et al*., 1992). Furthermore, the climate in Israel is drier and hotter during summer than in other protea growing areas. Frost can cause extensive damage to proteas. Proteas are therefore grown under intensive conditions of fertilisation and irrigation, employing artificial growth substrates such as volcanic ash. A few species were selected from initial trials and have been successfully and economically grown on a larger commercial scale. *Protea obtusifolia* is tolerant to alkaline soil (BenJaacov, 1986a) and grows in limestone-derived soils in its natural habitat (Rebelo, 2001).

Cultivation of *Leucadendron* 'Safari Sunset' started in the late 1980's. This cultivar grows well in some locations, giving superb flowers and a high yield (Ben-Jaacov *et al.*, 1992). The strong market appeal and relative ease of cultivation has made 'Safari Sunset' the most important cultivar in the industry, and Israel is the largest producer of that cultivar in the world, producing 24 million stems in 1998–1999 (Silber *et al*., 2001). The present area under 'Safari Sunset' cultivation is about 163 ha and takes place under various climatic conditions throughout Israel (Ran *et al*., 2001; Wolfson *et al*., 2001).

NEW ZEALAND

How South African *Proteaceae* reached New Zealand is unclear. It is widely believed that plants or seed were brought to New Zealand by soldiers returning from the Anglo-Boer War (1899–1906) (Matthews & Carter, 1983). In 1922, a nursery began selling *P. repens* and in 1945 protea cut-flowers were sold for the first time, mainly to Japan and the Far East. Today, approximately 200 ha are planted with *Proteaceae* with the main export markets being Canada, Europe, Hong Kong, Japan, Korea, Taiwan and USA (Soar, 1998; Middelmann, 2002; G. Jewell, Te Horo Ornamentals, New Zealand, pers. comm., 2002).

PORTUGAL, MADEIRA ISLAND AND THE AZORES

The cultivation of *Proteaceae* on Madeira Island was initiated in the 1970's, with seed that was imported from South Africa. Currently, Madeira Island has 14–20 ha planted with proteas (Fernandes & Blandy, 1998; Rodrigues, 2001). *Protea cynaroides, P. neriifolia*, *Protea* 'Pink Ice', *Protea* 'Susara', *Lsp. cordifolium* and *Lcd.* 'Safari Sunset' are most commonly planted. Various microclimates suitable for different proteas occur on the island due to its topography, and for this reason, almost any species or variety can be produced on Madeira Island, including *P. magnifica*. The timing of flowering can be regulated by growing at different altitudes, thereby extending the marketing season. At higher elevations, the soil nutrient levels decrease rapidly, making the soil increasingly more suitable for proteas. Soil pH can vary from 4.5 to 6.5. The organic matter content of the soil is very high and soils are normally deep and the drainage acceptable. Madeira Island is close to the European markets and has direct flights to several countries. The Portuguese mainland is an important market. Furthermore, because Madeira is in the Northern Hemisphere, flowers can be produced at times of the year when the Southern Hemisphere producers cannot supply the markets. Land and farm input costs, as well as soil-borne diseases, are constraints to the protea industry's development on this island (Rodrigues, 2001).

In 1997 several varieties of *Protea*, *Leucospermum* and *Leucadendron* (15 ha) were established in coastal, southwest Portugal. The soils in this area are acidic, sandy and suitable for the cultivation of proteas (Leandro *et al*., 2001).

SPAIN (CANARY ISLANDS)

Commercial plantations of proteas in Tenerife were established in the early 1980's. The area under protea cultivation is increasing, but presently it is approximately 20 ha (Bethencourt Díaz *et al*., 2001). Commercially important proteas include *Leucadendron* 'Safari Sunset', *Lcd. discolor*, *Lsp. cordifolium*, *Lsp. patersonii*, *P. cynaroides, P. eximia, P. neriifolia, P. obtusifolia* and *P. repens* (Rodríguez-Pérez, 1992, 1995; Rodríguez-Pérez *et al*., 1997). *Leucadendron* 'Safari Sunset' is the most promising cultivar due to its fast growth and early production (Pérez-Francés *et al*., 1995). *Protea obtusifolia* plants in commercial plantations have been grown from seeds imported from South Africa (Rodríguez-Pérez, 1990). *Leucadendron discolor* was established in 1988 in Los Rodeos (La Laguna) in Tenerife, from rooted cuttings imported from Israel (Rodríguez-Pérez *et al*., 1997). Since 1998, various cultivars of the genera *Protea*, *Leucospermum* and *Leucadendron* have been evaluated at the University of La Laguna (Rodríguez-Pérez *et al*., 2001b).

Presently a total area of 43.5 ha is under cultivation (Tenerife, 23 ha; La Palma, 18 ha; Gran Canaria, 2 ha; La Gomera, 0.5 ha) (J.A. Rodríguez-Pérez, Dept. of Ciencias Agrarias, Univ. of La Laguna, Tenerife, Spain, pers. comm., 2002). Most of the produce is exported to the European Union (mainly to The Netherlands), while the rest is sold in mainland Spain. Only a small percentage of the production is marketed locally (Rodríguez-Pérez, 1995; Bethencourt Díaz *et al*., 2001; J.A. Rodríguez-Pérez, pers. comm., 2002).

THAILAND

Protea, *Leucospermum* and *Leucadendron* plants were planted in the northern highland of Thailand in 1997. The climatic environment at 1000 m elevation is similar to the production areas in Hawaii, with the exception of a rainy winter period. *Protea* and *Leucadendron* plants established quite well, while *Leucospermum* plants proved to be highly susceptible to disease (Kuanprasert *et al*., 2001).

USA (CALIFORNIA AND HAWAII)

A wide variety of propagation material was imported in 1964 into Hawaii. Since 1968 the protea cut-flower industry there has developed significantly. Today, approximately 60 ha of *Proteaceae* are cultivated (Wilson, 1998) in soils ranging from loam to volcanic cinders and ash (Parvin, 1986). Production on the Island of Maui is in the Kula/Olinda areas. Big Island locations are near the Kona area. The actual growing region stretches north to Waimea and to South Point. Flowers are marketed in Asia, Canada, Europe, Hawaii, and mainland USA (Wilson, 1998). Similarly to Zimbabwe,

the winter rainfall climate in Hawaii causes problems with disease proliferation, and cultivars of certain species, such as *P. magnifica,* are particularly susceptible.

Many species of *Proteaceae* were imported into California during the 1960's. Today, approximately 450 ha are planted with woody, Southern Hemisphere plants for cut-flower production, of which the genus *Protea* represents approximately 20% (Perry, 1998). In 1998 the official state income from *Proteaceae* was listed as US $3.6 million. A large portion of the crop is sold within California (< 35%), with 45% selling to the rest of the USA and Canada, and 10% exported (Perry, 1998).

CROP IMPROVEMENT

Genetic resources, the gene bank and sampling

Genetic improvement generally has six stages: harvesting in the wild, basic domestication, clonal selection, interspecific hybridisation, complete domestication and control of single genes (Littlejohn, 2001e). In the 1960's intraspecific variation was reported in the genus *Protea*. The need for identification, collection and management of South Africa's genetic resources of *Proteaceae* was recognised. As a result a gene bank project was initiated in 1973 and the South African Fynbos gene bank (Littlejohn & De Kock, 1997; Littlejohn, 2001c) was established. The gene bank conserves the genetic diversity of plant species with floricultural potential, or species that possess desirable genetic characteristics such as tolerance to Phytophthora root rot, thereby providing the basis for breeding, selection and evaluation of new *Proteaceae* cultivars (Brits, 1990b; Moss, 1994; Littlejohn & De Kock, 1997). The breeding research programme on *Proteaceae* was initiated in 1974 (Anon., 1999d) and is presently situated at Elsenburg, Stellenbosch.

FIELD GENE BANK

The field gene bank was developed with seed from superior localised species populations harvested from the wild (Robyn *et al*., 2001). Mass selections were made on the basis of adaptability to cultivation, and floral traits that would enhance the commercial value of the plants (Brits *et al*., 1983). Clonal selections were subsequently made of the best plants within the seed-derived populations, which were then established in the field gene bank (Robyn *et al*., 2001). Gene bank plants were evaluated for characteristics important for floricultural use and hybridisation (Littlejohn & De Kock, 1997; Littlejohn, 2001c). These evaluated plants were then used as parents in the production of hybrid seed. Hybrid seed may result from intra- or interspecific crosses (Littlejohn *et al*., 1995). Seed collections act as resources for species variation (Littlejohn & De Kock, 1997).

In addition to conserving genetic diversity obtained through breeding programmes, the field gene bank also conserves rare and endangered species and cultivars *ex situ* (conservation of plants away from their areas of natural occurrence) (Littlejohn & De Kock, 1997; Littlejohn, 2001c). The Fynbos flora is threatened by human activities and interference, as well as by alien shrubs (Rebelo, 1992; Littlejohn & De Kock, 1997) and needs to be conserved. Many species in the lowlands and mountains of the Cape Floral Kingdom are listed as rare and endangered (Hilton-Taylor, 1996). The IUCN Red Data species list of South African plants includes *Lcd. argenteum, Lcd. daphnoides, Lcd. discolor, Lcd. dregei, Lcd. galpinii, Lcd. laxum, Lcd. nervosum, Lcd. platyspermum, P. aristata, P. longifolia, P. odorata, P. pityphylla,* and *P. pudens* (Anon., 1999c).

Table 1. *Proteaceae* production areas in the world.

Country/State	Year: Hectarage	Areas
Australia	1998: 81 ha (Queensland) (Moffatt, 1998) 2002: 230 ha* (Western Australia) (Middelmann, 2002) 2002: 1000 ha* (K.L. Delaporte, pers. comm., 2002; Middelmann, 2002)	South-west of Western Australia, the east coast of New South Wales, parts of south-eastern Queensland, most of Victoria, Adelaide Hills, Fleurieu Peninsula, the south east of South Australia, and parts of Tasmania – mainly on the northern coast, but also some around the Hobart area (K.L. Delaporte, pers. comm., 2002)
Chile	2002: 17 ha* (Middelmann, 2002)	52-62° latitude (Schiappacasse, 2002)
El-Salvador	1998: 7.5 ha (Veltman, 1998)	
France	2002: 0.75 ha (P. Allemand, I.N.R.A. – U.R.I.H., France, pers. comm., 2002) 2002: 2.5 ha* (Middelmann, 2002)	South of France (near Toulouse, Toulon, Avignon) and French Mediterranean coast (P. Allemand, pers. comm., 2002)

Israel	2002: 236 ha (J. Ben-Jaacov, pers. comm., 2002) 2002: 1087 ha* (Middelmann, 2002)	Galilee and Northern Heights (Cohen, 2002)
Madeira Island	2001: 14 ha (Rodrigues, 2001)	Entire island (Rodrigues, 2001)
New Zealand	2002: 200 ha* (G. Jewell, pers. comm., 2002; Middelmann, 2002)	Southern parts of North Island and east coast of South Island (Jewell, 2002)
Portugal (incl. Azores)	2002: 24.3 ha* (Middelmann, 2002)	Southwest (Leandro *et al*., 2001)
South Africa	1999: 189 000 ha natural veld 2795 ha seed-sown 950 ha seedling-based orchards 491 ha cultivar-based orchards (B. Baker, pers. comm., 2002) 2002: 4236 ha* 1000 ha intensive cultivation (Middelmann, 2002)	Eastern Cape Province; Gauteng; Kwa-Zulu Natal; Mpumalanga; Northern Province; North-western Province; Western Cape Province (G.M. Littlejohn, pers. comm., 2002)
Spain (Canary Islands)	2001: Tenerife: 23 ha La Palma: 18 ha Gran Canaria: 2 ha La Gomera: 0.5 ha (J.A. Rodríguez-Pérez, pers. comm., 2002) 2002: 53.5 ha* (Middelmann, 2002)	Northern slope (Bethencourt Díaz *et al*., 2001)
Thailand		Northern highland (Kuanprasert *et al*., 2001)
USA (California)	2002: 405 ha* (Middelmann, 2002)	North San Diego County, Santa Barbara, Ventura Counties, Monterey, Santa Cruz (Perry, 1998)
USA (Hawaii)	2002: 71 ha* (Middelmann, 2002)	Island of Maui: Kula/Olinda; Big Island: Kona, north to Waimea and South Point (Wilson, 1998)
Zimbabwe	2000: 270 ha (Percival, 2000) 2002: 2887.6 ha* (Middelmann, 2002)	Centenary, Central Watershed, Eastern Districts, Makonde, Karoi (Archer, 1998)

**Proteaceae* includes genera other than *Leucadendron, Leucospermum* and *Protea*.

The basic technology necessary to conserve the *Proteaceae* plants in the field gene bank is well developed and documented (Littlejohn *et al*., 2000b). *Ex situ* conservation can, however, limit the genetic diversity relative to the gene pool due to size limitations. Furthermore, in a gene bank evolution is effectively stopped, although genetic drift may occur. Poor gene bank management and lack of information regarding cultivation, seed physiology, seed germination, dormancy and breeding systems, may negatively affect the gene bank, but the biggest threat is the cost of plant maintenance and lack of consistent long-term funding. The policies on cultivar distribution and royalty collection are attempts to enable the long-term sustainability of cultivar development (Littlejohn, 2001c; Robyn *et al*., 2001). For economical reasons, only the five most important cut-flower genera: *Protea*, *Leucospermum*, *Leucadendron*, *Serruria* and *Mimetes* can be conserved in any quantity in the field gene bank in South Africa. Commercially important and rare and endangered species, rehabilitation material, primitive cultivars and semi-domesticated plants should, how-

ever, all be included in the gene bank collection (Littlejohn, 2001c).

In 1997, the gene bank maintained 123 species accessions and 197 hybrid accessions of *Leucospermum* (Littlejohn & Blomerus, 1997). Currently, the field gene bank comprises over 2000 clonal accessions, consisting of 30 000 plants of more than 113 species and many interspecific hybrids (Littlejohn, 2001c).

SEED GENE BANK

The field gene bank needs to be supported by a representative seed bank so that it has a maximum economic impact on the crop (Littlejohn, 2001c). Seed banks can store larger and more diverse quantities of genetic resources than field banks since the infrastructure is cheaper (Given, 1994). Recently acquisitions have focused on economically important species from diverse habitats. The Fynbos seed gene bank comprises 200 accessions (Littlejohn, 2001c).

Genera such as *Leucospermum* have long-lived seeds, whereas serotinous (flower heads that stay closed for several years) *Protea* and some *Leucadendron* spp. have short-lived seeds. The exact requirements for maintenance of *Proteaceae* seed viability are, however, still unknown and more research needs to be conducted on this aspect (Robyn *et al*., 2001).

SAMPLING

Investigations into the genetic diversity in species are underway to provide the guidelines for effective sampling from wild populations (Littlejohn, 2001c). New techniques, such as genetic fingerprinting, can be used to assess the levels of genetic variability within and between species, thereby eliminating duplication (Littlejohn, 2001c; McGregor *et al.*, 2001). At the phenotypic level (the physical expression of the genes or genotype), varying amounts of genetic diversity have been observed in different *Protea* species, with the species with wide habitat ranges showing more diversity (Vogts, 1989). Morphologically there seems to be correlation between habitat range and genetic variability. Using RAPDs (Random Amplified Polymorphic DNA), it has been shown that the genetic variability observed in *P. neriifolia* (the species with the widest habitat range) was the largest both within and between populations sampled. In contrast, the genetic variability of *P. coronata* was intermediate, with greater variation between populations than within populations. *P. holosericea* was least variable. Genetic diversity is therefore correlated with habitat range and should be kept in mind when formulating sampling strategies for the different species (McGregor *et al.*, 2001).

Breeding programmes/cultivar development

The aim of a breeding programme is to develop cultivars suitable for commercial exploitation. Breeding superior selections is in many cases the only method of ensuring high quality cut-flowers. Development and breeding of *Proteaceae* cultivars started in the late 1960's in South Africa (Littlejohn *et al*., 1995). In 1973, Frank Batchelor was the first breeder to register five protea cultivars with plant breeder's rights (Brits, 1984). Fulfilling the industry requirements of high quality, low price, and consistent supply, is central to the development of fast growing, productive cultivars that exhibit high field resistance to diseases and pests. Wild genetic resources can be utilised as sources of disease and pest resistance early in the development of cultivars (Littlejohn, 2001c). The South African and American selection and breeding programmes for the African genera of *Proteaceae*, based on interspecific hybridisation, resulted in superior, unique cultivars which form the basis of the international industry (Brits *et al*., 1983; Littlejohn, 2001c). Interspecific hybridisation provides a floral product that is easily distinguishable from the wild product based on superior quality, and many of the seed-propagated natural species cannot compete commercially with the clonal and hybrid cultivars (Littlejohn, 2001c). The first distinct cultivar of *P. repens*, 'Guerna', was released in 1978 in South Africa.

The three most important selection criteria for proteaceous crops include quality (aesthetic appeal), yield and *Phytophthora* tolerance. However, novelty and reliability are also very important (Brits *et al*., 1983; Sedgley, 1995). Plants are evaluated for their flower traits, such as flowering time and duration, aesthetic characteristics of foliage and flowers, stability of traits, stem length, vigour after pruning, vase life of the flowers, how well they survive transport, and yield characteristics. In addition to these qualities, producers require attributes such as ease of cultivation and propagation, disease resistance and commercial worth (Brits *et al*., 1983; Brits, 1984; Littlejohn *et al*., 1995; Littlejohn & De Kock, 1997; Blomerus & Littlejohn, 2000; Coetzee & Littlejohn, 2001).

Genetic improvement and the development of proteas as a whole, tends to be relatively slow due to the long life cycle (3–4 years) of these plants. In 1992, the majority of protea cultivars grown in South Africa represented natural crosses. By 1998, 89 cultivars had been released from the breeding programme at the Agricultural Research Council in South Africa (Littlejohn *et al*., 1998). In South Africa, 70% of the *Leucospermum* plantings are cultivars, while only 50% of *Leucadendron* and *Protea* are cultivars (Littlejohn, 2001c).

The *Proteaceae* are difficult to grow and hybridise (Brits, 1984). Low seed set after inter- and intraspecific hybridisation (Horn, 1962; Brits, 1984; Brits & Van den Berg, 1990), poor germination (Brits, 1992b), and cross incompatibility between species (Littlejohn *et al*., 1995) are some constraints. Hybrid seed set will generally drastically decline with a slight increase in phylogenetic distance, e.g. in *Leucospermum* and *Leucadendron* (Van den Berg & Brits, 1995).

The breeding system in the *Proteaceae* is complex. Self-incompatibility, self-compatibility and different

pollen vectors are observed within and between different species (Van der Walt & Littlejohn, 1996a, b, c; Wright, 1994a). Pollen vectors are known, but the extent of pollen dispersal from a single plant source by the pollen vectors remains unknown (Wright, 1994a). Vogts (1989) documented much morphological variation within species. DNA technology has been used to identify genotypes of *Banksia* species and could be used for characterising other Proteaceous crops (Sedgley, 1995). However, the technology is not transferable since each genus requires different primers (McGregor *et al*., 2001).

The *Proteaceae* are open-pollinated plants in which seed populations produce a variety of seedlings with varying characteristics (Brits, 1984). The selection of a single plant from within the seed population, and clonal propagation by cuttings, can then result in a new cultivar (Littlejohn *et al*., 1995). Growing a new cross from seed and evaluating the hybrid could take up to 10 years. . Seed is collected during the year following the cross. Seed of *Leucospermum* and *Leucadendron* can be sown in the same year, but with *Protea*, it could take up to 18 months before seed is sown. Great losses are suffered in the first 3–4 months; and to counter this, seedlings are planted into pots for the first 12–14 months. It could therefore take up to two and a half years from the date of making a cross until the plant is in the ground. In *Leucadendron*, the first flowers can be expected after 2 years, 2–3 years in *Leucospermum*, and in *Protea* after 4–5 years. Cuttings are then made from promising seedlings and evaluation work is done for a further 3 years (Anon., 1999a).

Clear, strict selection criteria with long-term goals must be developed for breeding *Proteaceae*. Available germplasm needs to be exploited for a continuous supply of new and interesting cultivars (Sedgley, 1995). Within *Leucadendron*, *Leucospermum* and *Protea*, a number of species are widely used, while some are only used as breeding parents and others are of no commercial value. Different species and their characteristics are important in interspecific hybridisation, but intraspecific variation is a major resource for improving quantitative traits, disease and insect resistance, and adaptability (Littlejohn *et al*., 2000b). In vigorous interspecific crosses, yields are in general increased by 100–150%, e.g. in *Leucospermum* 'Scarlet Ribbon' and 'Sunrise' (Brits, 1985a, b).

LEUCADENDRON

The genus *Leucadendron* is chiefly marketed as foliage, which is used in flower arrangements. The desirable combination of large flower heads, long flowering stems and a high yield is very rare within a single species (Van den Berg & Brits, 1995). The main aim is to breed a product with a long marketing period (Littlejohn *et al*., 1995), but characteristics such as an increased range of colour forms, larger flowers and long flowering stems are also important. Crosses between different species must be used to combine good qualities from different species in order to make a single-stem cut-flower (Van den Berg & Brits, 1995).

Leucadendron is a complex crop to breed, since the plant is dioecious, with, in many cases, only the female type desirable for floriculture use (Littlejohn & Robyn, 2000). However, genetic variation available in *Leucadendron* is vast, and interspecific hybridisation within *Leucadendron* is easier to manipulate than in other genera of the *Proteaceae*, and gives excellent results (Van den Berg & Brits, 1995).

Although poor seed set results are indicated (Littlejohn, 1998), the hybridisation programme has thus far proven to be highly successful. Forty percent of the single stem cut-flowers exported represent improved plant material in the form of vegetatively propagated cultivars (Littlejohn & Robyn, 2000). The ARC Fynbos Unit began a hybridisation programme with *Leucadendron* in 1986. Initially only the closely related species such as *Lcd. discolor*, *Lcd. laureolum* and *Lcd. salignum* were hybridised. In the following years, more species were added to the parents, such as *Lcd. elimense*, *Lcd. eucalyptifolium*, *Lcd. gandogeri* and *Lcd. nervosum*. From these hybrids more recent cultivar releases have been made, including 'Chameleon', 'Rosette', 'Laurel Yellow', 'Flash' and 'Disco Date' (Littlejohn, 1998). The current range of *Leucadendron* cultivars in South Africa includes clonal selections from within species and interspecific hybrids (Littlejohn *et al*., 1998). Vigorous F_1 hybrids with exceptionally long stems and attractive large flower heads were obtained. High yields in these hybrids result from strong F_1 hybrid vigour, while these selections also flower over a relatively wide seasonal flowering/ marketable period (Van den Berg & Brits, 1995; Robyn & Littlejohn, 2001). Various *Leucadendron* species and cultivars are cultivated, of which products are marketed as single-stem foliage products (silver, red, green and yellow foliage) or foliage products with female cones (Littlejohn, 1999).

Interspecific hybrids selected from seedling populations resulted in the release of a number of cultivars from New Zealand (Bell, 1988) and Australia, such as the well-known 'Safari Sunset', 'Silvan Red' (Littlejohn & Robyn, 2000), 'Red Gem', 'Inca Gold', 'Pisa', 'Jubilee Crown' and 'Katie's Blush' (Littlejohn, 1998). Internationally, *Leucadendron* is the *Proteaceae* genus sold in the largest quantities, with approximately 30 million stems moving through the Dutch auction system annually (Anon., 2002). These large quantities are attributed to the widely cultivated 'Safari Sunset' (Littlejohn & Robyn, 2000).

In mid-1998, a programme was initiated at the University of Western Australia to develop *Leucadendron* varieties adapted to Australian growing conditions and that would have longer marketing periods. One of the aims was to extend the flowering time so that blooms would still be produced in November and December. Most of the species and varieties finish flowering by

November and December in Australia, leaving a gap of about 2 months when prices peak over the Christmas holidays. If the floricultural attributes from the present varieties could be combined with the flowering time from another group of species, the *Leucadendron* marketability could be extended for another 2 months and a higher price benefit would be obtained. More than 300 different interspecific cross combinations from 81 genotypes of 23 species were hand-pollinated in the 1998/1999 flowering seasons (Sedgley *et al*., 2001).

In *Leucadendron*, low seed set in taxonomically diverse, controlled pollinations instigated investigations into cross incongruency between species, pollen viability and stigma receptivity (Robyn & Littlejohn, 2001). The production of progeny between *Leucadendron* species of the section *Alatosperma* is favourable, but hybridisation between species not in the same section, nor sub-section, has thus far given poor results, as in the case of *Leucospermum* and *Protea*. Cross-compatibilty is positively correlated with relationship (Van den Berg & Brits, 1995; Littlejohn & Robyn, 2000). Controlled crosses (Littlejohn, 1998) executed between different commercial species of *Leucadendron* indicated that closely related species hybridise more easily than distantly related species.

When pollen was sown on an artificial pollen medium recommended for *Protea*, no pollen tubes were observed. In scanning electron microscopy observations, the stigma surface appeared papillate. It is speculated that the pollen tubes penetrate between the papillae gaps and grow between the cells in the style to reach the ovary. Further research is necessary to investigate the interior of the style for a stylar canal or tissue differences in species that could prevent pollen tubes from growing down the style (Robyn & Littlejohn, 2001).

Sedgley *et al.* (2001) used two methods to test the viability of *Leucadendron* pollen after storage. In the first set of tests, viability was assessed by observing pollen germination and pollen tube growth of pollen sown *in vitro* using 20% sucrose as the germination solution. In the second test, pollen was stained with fluorescein diacetate to determine the membrane integrity and presence of the enzyme esterase, so that pollen viability could be estimated (Heslop-Harrison & Heslop-Harrison, 1970; Sedgley *et al*., 2001). Results indicated that *Leucadendron* pollen could be stored for one year without losing much viability under suitable conditions. Desiccated pollen stored at -80°C maintained the highest viability during storage (up to 55.3% at day 365). Desiccated pollen stored at -20°C also maintained good viability (up to 46.3% at day 365). Non-desiccated pollen was not suitable for long-term storage. Room temperature (20°C) and ordinary refrigerator temperature (4°C) were not suitable for long-term storage of *Leucadendron* pollen (Sedgley *et al*., 2001).

The success of breeding programmes will depend on the ease of hybridisation within and between species, and specifically, the ability to overcome the barriers to interspecific hybridisation (Littlejohn *et al*., 1995). Breeding programmes must also be coordinated internationally in order to advance common breeding goals of *Proteaceae* (Brits, 1992b).

LEUCOSPERMUM

The genus *Leucospermum* consists of 48 species, providing an enormous amount of variation between species that can be utilised in breeding. Only a few species are utilised as cut-flowers, yet interspecific hybrids exist as clonal selections (Jacobs, 1985). Collection and introduction of natural interspecific hybrids has occurred (Brits & Van den Berg, 1991), and controlled crosses have been made between species in an attempt to produce later flowering cultivars with improved colour and shape, and enhanced tolerance to *Phytophthora cinnamomi* (Brits, 1992b).

Today, active breeding programmes are conducted at the Fynbos Unit, ARC, Stellenbosch, South Africa (Brits, 1992a, b; Littlejohn *et al*., 1995), at the Maui Agricultural Research Station of the University of Hawaii (Leonhardt *et al*., 1995; Leonhardt & Criley, 1999) and in Israel (Shchori *et al*., 1995). The fourth edition of the International Protea Register (Sadie, 1997), lists 30 registered cultivar names and another 58 have been noted but not registered for selections and interspecific hybrids (Criley, 1998).

Leucospermum breeding started in South Africa in 1974. Selections and hand pollination to produce intra- and interspecific hybrids has resulted in the release of 25 cut-flower cultivars since 1979. A hand pollination technique was developed after morphological studies of the stigma grove were carried out. Initially only pure species were used for hybridisation, but since 1986, hybrids have been introduced (Blomerus & Littlejohn, 2000).

Thirteen *Leucospermum* species are currently commercially utilised, of which *Lsp. conocarpodendron*, *Lsp. cordifolium*, *Lsp. cuneiforme*, *Lsp. erubescens, Lsp. glabrum*, *Lsp. lineare*, and *Lsp. patersonii* are more important species. The species are divided into four groups, namely orange, yellow, the glabrum-group, and novelty types (Littlejohn, 1999). *Leucospermum cordifolium* or *Lsp. cordifolium* hybrids have the most desirable flower head type, therefore breeding at the ARC is aimed at developing a series of cultivars in red, yellow, orange and pink that flower successively from as early to as late as possible in the season. Adaptability, vigour, yield and root disease tolerance are increased in cultivars as a result of interspecific hybrids (Littlejohn *et al.,* 1995).

The most widely grown species are floriferous, spreading shrubs on which relatively short-stemmed inflorescences are borne in the spring. Horticulturists have had to develop management practices to improve stem length and straightness so that these flowers could be used as cut-flowers (Leonhardt & Criley, 1999). Genetic improvement of these characteristics will, how-

ever, reduce costly management inputs (Leonhardt *et al*., 2001). The *Leucospermum* breeding programme at the University of Hawaii emphasises improved horticultural characteristics such as early flowering, extended flowering season, vertical plant growth habit with long, straight, and light-weight stems having small, narrow leaves and reduced leaf pubescence. Other important qualities include ease of propagation, high yields, good post-harvest characteristics, new and improved colours, market acceptance, as well as genetic resistance to root and foliar diseases (Leonhardt *et al*., 1995, 2001; Leonhardt & Criley, 1999).

Many commercial *Leucospermum* cultivars are bulky, heavy and cumbersome to pack due to large stem diameters and large heavy-textured leaves. In contrast, *Lsp. lineare* has slender, light-weight, yet strong stems with narrow, nearly needle-like foliage. Breeding programmes have demonstrated that these characteristics are heritable, and that *Lsp. lineare* hybrids have improved leaf and stem characteristics. This species is also free of foliar pubescence, which attracts and retains moisture, providing an environment for fungal spore germination and infection (Leonhardt *et al*., 1995). For these reasons, therefore, *Lsp. lineare* is the most widely used species in hybrids. *Lsp. reflexum* has slender, light-weight, long stems with small and somewhat erect leaves. *Lsp. reflexum* as a parent has produced hybrids with very long stems and flowers that are ovoid to globose when young, and as the styles reflex, the flower becomes a flat disk, larger in diameter than any other *Leucospermum*. The plants are also vigorous and high-yielding. These will be unusual new additions in the floral marketplace (Leonhardt *et al*., 2001).

Breeding for disease resistance is a high priority when sources of resistance can be found. In Hawaii, various species are evaluated for resistance to *Elsinoë* (Nagata *et al*., 1995), *Phytophthora* (Moffat & Turnbull, 1994a), *Botrytis* and *Drechslera* (Nagata *et al*., 1995; Leonhardt *et al*., 1995, 2001).

Commercial producers in Hawaii compete in North American markets with Californian producers. The flowering season for *Leucospermum* begins several weeks earlier in Hawaii than in California. Producers in Hawaii could enjoy this advantage for a longer period if earlier-flowering cultivars could be developed. A long flowering season is also an objective. Five of the new cultivars bloom over a period of approximately 8 months (Leonhardt *et al*., 2001).

The Hawaii breeding programme has utilised numerous species and F_1–F_3 hybrids to produce seedling populations that are evaluated for horticultural characteristics and disease resistance (Leonhardt *et al*., 1995; Leonhardt & Criley, 1999). Since 1994, over 3000 seedlings have been evaluated, of which seven new hybrids of the 11 species used as parents were introduced in 1999, and 19 in 2000. This includes the first-time release of *Lsp. reflexum* hybrids. Seven of the new cultivars flower in November, while eight extend the late season into June or July (Leonhardt *et al.*, 2001).

Fresh pollen remains viable for up to 6 days when stored at room temperature and up to 6 weeks when stored at 5°C (Brits & Van den Berg, 1991). Pollen viability can be tested by germinating in 12% sucrose plus 100 mg boron/L (Brits, 1992a). Shchori *et al*. (1992) reported that better germination was achieved using Taylor's medium in a hanging drop.

PROTEA

Protea breeding was initiated in South Africa in 1974, with the first cultivars released in the late 1970's (Littlejohn *et al*., 2000a). Cultivars in *Protea* are predominantly interspecific hybrids (Coetzee & Littlejohn, 2001). No interspecific hybrid South African cultivars currently originate from controlled pollination between species, due to the low seed set obtained (Littlejohn *et al*., 2000a). A few intraspecific South African cultivars are the result of controlled hand-pollination (Littlejohn, 2001b). Breeding methods that are used in *Protea* include mass selection from seed-regenerated populations, selection of superior clonal material from within seedling populations, selection of interspecific chance hybrids, production of controlled hand pollinations, and segregating material from interspecific hybrids (Littlejohn *et al*., 2000a). F_2 segregation should prove a worthwhile source of genetic variation in breeding *Protea,* because significant clustering of progeny with regard to some of the traits measured is evident. If the parental combination of traits is being inherited as a group, i.e. if there is poor recombination between the two parental genomes, clustering of the measured traits will occur. This indicates that fewer intermediate types occur resulting in most progeny being similar to the parental species. However, sufficient recombination does occur so that plants with new traits are produced. An example of this is the *P. pudens* type plant with a small, bright red small inflorescence (Littlejohn, 2001d).

Intraspecific crosses between different ecotypes of product lines such as *P. cynaroides*, which flower throughout the year (Brits *et al*., 1983), will result in having cultivars available in different colours and sizes that flower successively throughout the year. Interspecific hybridisation will, however, be necessary to extend the flowering time and overcome leaf blackening in product lines such as *P. compacta*, *P. eximia* and *P. repens*. Due to their close relationship, it will not be too difficult to use *P. magnifica* and *P. neriifolia* in this regard. Interspecific hybridisation is also necessary to develop small types of proteas (Littlejohn *et al*., 1995).

Proteaceae are normally exported under a species name or so-called trade or group name (based on size of the flower), and this is an important factor in developing different cultivars. The *Protea* cultivars are divided into three groups, namely the large proteas comprising *P. compacta*, *P. cynaroides*, *P. eximia,* and *P. mag-*

nifica; medium proteas, *P. grandiceps*, *P. lorifolia*, *P. laurifolia*, *P. neriifolia*, *P. obtusifolia*, *P. repens,* and *P. longifolia* hybrids; and small proteas, namely *P. laticolor*, *P. mundii*, *P. pityphylla,* and *P. scolymocephala* (Littlejohn, 1999).

Insufficient seed production seriously hampers breeding attempts in *Protea.* It is well documented that many of the *Proteaceae* produce few viable seeds per inflorescence in their natural habitat (Horn, 1962; Rebelo & Rourke, 1986; Esler & Cowling, 1990). This inherently low seed set is further reduced when interspecific hybridisation is attempted (Brits & Van den Berg, 1990).

Pollination is essential for seed development but is difficult in *Protea*. The *Protea* flowers are proterandrous, with the anthers dehiscing prior to the stigmatic groove becoming receptive (Vogts, 1971; Van der Walt & Littlejohn, 1996b). The anthers dehisce and deposit their pollen during flower opening on the pollen presenter, a modified region of the style directly below the stigma. Foraging fauna then collect the pollen from the pollen presenter and disperse it through foraging (active pollination), which results in fertilisation. Passive pollination (i.e. pollination in the absence of an active pollen vector) produces no seeds (Brits, 1984). The *Protea* spp. used as cut-flowers have large, conspicuous inflorescences that are predominantly bird-pollinated, but species differ in dependency on birds as pollinators, with insects being effective pollinators in *P. laurifolia*, *P. magnifica* and *P. neriifolia* (Coetzee & Giliomee, 1985; Wright *et al*., 1991). Once successful pollination has occurred, the viable seeds are clustered (grouped) on the involucral receptacle, which may be a mechanism to reduce insect predation (Wright, 1994b; Mustart *et al.*, 1995).

Different controlled hand-pollination strategies have been evaluated (Littlejohn *et al.*, 2001). One method implements the pollination of a continuous circle of florets (Van der Walt & Littlejohn, 1996b), while another method is a modification of the former method in which pollination of grouped florets on the involucral receptacle takes place (Littlejohn *et al*., 2001). No viable seed set was obtained using the continuous circle of florets pollination method in *Protea* interspecific hybridisation in the past (Brits, 1992b). However, intraspecific hybridisation using this pollination method in *P. repens* and *P. cynaroides* resulted in seed set above 20%. Seed set from interspecific hybridisation in related genera, using the continuous circle of florets method, ranges from 3.5% in *Banksia* (Sedgley, 1998a) to 11% in *Leucospermum* (Blomerus & Littlejohn, 1999).

Although using the grouped florets pollination method in combination with taxonomic relatedness does not provide a method for obtaining seed set from all possible cross combinations, the seed set obtained is sufficient to provide new genetic combinations for use in a breeding programme. It is proposed, therefore, that *Protea* species should be hand-pollinated 2–6 days after anthesis in order to obtain maximum seed set. However, scanning electron micrographs have indicated that the opening of the stigmatic groove may not occur as early as 2 days after anthesis in all proteas, as observed in *P. repens* and *P. eximia* (Van der Walt & Littlejohn, 1996b). This has implications for the optimal timing of application of the pollen to the stigma. It may be necessary to perform multiple applications of pollen to maximise seed set, rather than using a common time frame for all crosses, since differences between species exist (Van der Walt & Littlejohn, 1996b; Littlejohn *et al*., 2001).

The maintenance of pollen viability facilitates the crossing of plants that flower at different times of the year. It would therefore be useful if the pollen could be kept viable from one season to the next or for even longer periods. Storage of pollen is primarily influenced by temperature and relative humidity (Visser, 1955; Stanley & Linskens, 1974). The viability of fresh and stored pollen is best determined by germination tests (Stanley & Linskens, 1974). Van der Walt and Littlejohn (1995) used the hanging drop technique of Van Tieghem (1869), in an artificial sucrose medium, to test the capacity of pollen to germinate and to produce normal pollen tubes. An extremely high germinability of 90% was obtained with *Protea* pollen, although pollen tubes did not grow more than 120 µm long. *Protea* pollen grains are very small (± 30 µm diam), depending on species. It appears that the resources of the pollen grains used to produce and sustain pollen tube growth, are very limited, and that the pollen needs some nourishment from the style for it to produce longer tubes (Van der Walt & Littlejohn, 1995). Germinability does not correspond well to ovule penetration (Stanley & Linskens, 1974), which is the ultimate test of pollen viability. Pollen germination was maximal at a very wide incubation temperature range of 5–30°C, but a marked drop in germination occurred beyond 30°C. Pollen stored at -196°C in liquid nitrogen and at -14°C to -18°C in a freezer, retained a germination percentage as high as that of fresh pollen after 6 months. Pollen stored at 2–7°C maintained a germination percentage as high as that of fresh pollen for only 1 month, followed by a sharp decline in germinability. Storage at 30% relative humidity showed the best germinability after 3 months, compared to storage at 60% relative humidity. After 6 months, germinability was almost zero at 10, 30 and 60% relative humidity.

Van der Walt and Littlejohn (1995) indicated that for crossing purposes, freezer storage without any humidity control seems to be the most practical and economic means of storing *Protea* pollen for breeding requirements, and that long-term storage of *Protea* pollen is not feasible at temperatures above 0°C. Storage in liquid nitrogen is also successful. Humidity control is important at storage temperatures above 0°C, but humidity control below 0°C has no advantage for many *Protea* species (Van der Walt & Littlejohn, 1996c). It has, however, been shown that differences in pollen longevity between different *Protea* species occur after

long-term storage (Van der Walt & Littlejohn, 1995, 1996c). A small amount of boric acid is essential to obtain good pollen germination in *Protea* (Van der Walt & Littlejohn, 1995). Further work needs to be done to understand the plethora of factors affecting *Protea* pollen production, viability, storage and germination (Van der Walt & Littlejohn, 1995).

International protea cultivar registration

The International Code of Nomenclature for Cultivated Plants (in short the Cultivated Plant Code, CPC) is a precise, stable and internationally recognised system for naming cultivated plants. The CPC consists of 32 articles providing guidelines on the acceptability of cultivar names. The CPC accepts and allows precedence of names accepted by the legislation of a particular country, over the rules of the Code. If the name is acceptable, the cultivar name should be registered in a system for the registration of proteas (Sadie, 2001).

The International Protea Register (IPR) is a system that exists for the registration of protea names <www.nda.agric.za/docs/geneticresources/legislation.htm>. The aim of the IPR is 'to promote uniformity, accuracy and stability in naming cultivars and to avoid confusion and duplication of cultivar names'. Furthermore, cultivar names are protected and free promotion is given. Registration in the IPR is undertaken once and is valid internationally. All the registered names of commercially available cultivars in all the protea-producing countries are supposed to be contained in the IPR. Since the IPR officially started in 1980, the number of registered cultivars has increased from 19 to 86. The importance of registration of cultivar names for the international protea industry cannot be emphasised enough. The success of the system relies on the co-operation of the people who are serious about the development and healthy growth of the international protea industry (Sadie, 1995, 1998b, 2001).

The International Registration Authority (IRA) for *Proteaceae* also compiles and maintains an international checklist of validly published protea cultivar names. Cultivar names not yet registered in the IPR, can therefore be identified, as well as those that have been discontinued, thus avoiding duplication of valid names (Sadie, 1995). When a cultivar has ceased to exist, that name may not be re-used (Sadie, 2001). There are approximately 230 cultivar names in the checklist (Sadie, 1995).

The system of Plant Breeders' Rights (PBR) originated in the International Convention for the Protection of New Varieties of Plants. The Union for the Protection of New Varieties of Plants (UPOV) was also founded, and protea countries such as Australia, Israel, New Zealand, South Africa, Spain and the USA (California and Hawaii) are members. The Convention protects new varieties of plants that are uniform and stable, by means of legislation. Member countries of the UPOV have national laws in agreement with those of the Convention. Since breeding new cultivars requires a lot of time and investment, plant breeders' rights are necessary to reward the breeders and enable them to receive royalty payment on all the propagation material sold of the particular cultivar. Plant breeders' rights are granted for a limited period of time, but for a minimum of 20 years, and are only legal in the country in which they are applied for and granted (Sadie, 1995).

Plant Breeders' Rights therefore protect the propagation material of the particular cultivar, as well as the cultivar name attached to the material. The cultivar name, however, is only protected when registered in the International Protea Register. Since 1973, the Department of Agriculture of South Africa is the International Registration Authority for protea cultivars representative of South African genera (Brits, 1980; Vogts, 1982; Brits *et al*., 1983; Sadie, 1995). The system for application of PBR has recently been linked to the system for the registration in the IPR, thereby synchronising the two registration systems (G.M. Littlejohn, pers. comm., 2002). In Australia, Plant Variety Rights legislation was introduced in 1987 (Sedgley, 1995).

A start has been made with the collection of herbarium specimens for the registered cultivars. These specimens are housed in the National Herbarium in Pretoria (PRE) (Sadie, 1998b).

PROPAGATION OF PROTEA PRODUCTS

Proteas are considered inherently difficult plants to propagate due to the low success rate of the various propagation methods (Malan, 1992). There are four basic methods of propagating protea plants: sexual reproduction (seed), vegetative propagation (cuttings), grafting and budding, and micropropagation (tissue culture). Only seed and vegetative cuttings are used commercially (Coetzee & Littlejohn, 2001).

Sexual reproduction (seed)

Prior to 1974, most proteaceous plants were commercially propagated by seed (Brits *et al.*, 1983). Seed is widely used for the establishment of varieties, and increasingly for the establishment of foliage plantations (Malan, 1995a, b). The dried flower industry almost exclusively makes use of seed reproduction, and most of the pure *Proteaceae* species are still propagated from seed. Seed propagation is less expensive and seedlings also establish easily. Seedlings grow better in dry conditions than cuttings, since they possess a very strong tap-root system. Seed propagation, however, has several disadvantages including poor seed germination (Blomerus & Robyn, 1999).

Seed of *Proteaceae* can be classified into two main types, *viz.* myrmecochorous and serotinous seeds. Myrmecochorous seeds, which are dispersed annually after the flowering season, are hard, nut-like seeds, which are round or ovoid in shape. They are hairless, with an oxygen-impermeable testa (Rourke, 1972; Van Staden &

Staden & Brown, 1973a, b, 1977; Brits, 1986b; Rebelo, 2001), and become dormant once released. These seeds are typical of *Leucospermum* species, but 38 species and three subspecies of *Leucadendron* (Rebelo & Rourke, 1986; Blomerus & Robyn, 1999) also have myrmecochorous seed. These nut-like seeds are disseminated by ants, except for some of the *Leucadendron* species (Blomerus & Robyn, 1999).

The serotinous seeds are found on species that have evolved flowerheads which become dried out after flowering. These retain their seeds long after maturity (serotiny), until the heat of a fire opens them and the seeds fall, germinating after the first substantial rainfall (Bond, 1985; Paterson-Jones, 2000). Serotinous seeds are relatively soft-shelled nutlets, which are flat or cylindrical in shape, often with wings or hairs, and are produced by species of *Protea* and *Leucadendron* species in the section '*Alatosperma*' (Kruger, 1983; Rebelo & Rourke, 1986; Blomerus & Robyn, 1999; Rebelo, 2001). The seed of *Leucadendron* are black, flattened and winged, while the seed of *P. magnifica* and *P. holosericea* are bigger and almost hairless compared to the other *Protea* species (Blomerus & Robyn, 1999).

A third less common type is cached fruits, which are large, rounded nuts produced by many *Leucadendron* species in the subsections 'Arid Conebushes' (*Membranacea*) and 'Sun Conebushes' (*Nucifera*) (Rebelo, 2001). The seed of these are thick, solid, round, heavy and wingless (Blomerus & Robyn, 1999). Some of these fruits are eaten, but most are buried by rodents (Rebelo, 2001).

TIME OF HARVESTING SEED

The number of viable seeds stored in the persistent infructescences of many proteas declines with increasing age (Bond, 1985). However, in order to obtain optimum germination, it is imperative that seed be left on the mother plant until maturity (Blomerus & Robyn, 1999).

Leucadendron

Harvesting of *Leucadendron* fruit should take place as the cones mature and before seeds are released (Sedgley *et al*., 2001). Seed of section '*Leucadendron*' are released 3–6 months after flowering, while seed of section '*Alatosperma*' stay in the cones for one or more years. The seeds are mature after about 4 months, and can then be harvested (Blomerus & Robyn, 1999), although if you are not sure about maturity it is better to harvest 6–7 months after anthesis (G.M. Littlejohn, pers. comm., 2002).

Leucospermum

In *Leucospermum*, mature seeds fall from inflorescences as they begin to dry out after flowering. Seeds mature at different rates, so release takes place for several weeks. In order to prevent seeds from falling out of inflorescences, they often are harvested when as many as 50% of them are under-developed, resulting in poor germination. Covering the inflorescences with gauze bags as soon as they show the first signs of desiccation will ensure that all seed in the inflorescence are mature and will be released naturally (Blomerus & Robyn, 1999).

Protea

The development of *Protea* seed is relatively slow and the time to maturity is not the same for each species. Most of the commercially important species need at least 9 months to become mature, but if the requirements for a certain species are unknown, it is safe to harvest one-year-old seed heads during flowering. Once mature, there is no need to leave seed heads on the mother plants. In fact, viability decreases due to fluctuating temperatures and moisture, and seeds are lost by insect predation and fire (Blomerus & Robyn, 1999). Van Staden (1978a) found that the viability of seeds of *P. neriifolia* in inflorescences declined very rapidly and suggested a maximum longevity of 18 months.

SORTING SEED

It is more difficult to sort *Protea* seed (except for *P. magnifica* and *P. holosericea*) than seed of *Leucospermum* or *Leucadendron*. Three types of seed can be found inside the seed head of *Protea*, namely: well-developed, viable seed; brittle, dry, empty seed; and big, woody seed. The only effective way to sort *Protea* seed is to feel each seed through the hairy exterior. Plump seed with a slight spongy feeling is generally viable. Seed sorting of *Leucospermum* and *Leucadendron* is easier, but must also be done carefully. Fresh, plump, shiny *Leucospermum* seed are viable, while brittle, papery or wrinkled seed must be discarded. The floating method is not reliable (Vogts, 1982; Blomerus & Robyn, 1999). The highly significant difference between sorted and unsorted seed with regard to germination was thought to be the result of separating fertile from infertile seed. Sound-looking but light seed of *Leucospermum* germinated poorly in comparison to heavy seed. However, the identical appearance of sound and unsound seeds makes sorting unreliable for ensuring high germination rates (Horn, 1962). Van Staden and Brown (1973a) found that as many as half of the plump, apparently healthy seed of *Leucospermum cordifolium* contained shrivelled embryos. Improved seed sorting to eliminate such seeds resulted in 37% germination compared with 25% in unsorted seed. Brown *et al*. (1986) furthermore indicated that "medium" weight seeds (91–110 mg) gave the highest pericarp germination. This indicates that seed sorting treatments involving flotation (Van Staden & Brown, 1973a) are not necessarily a satisfactory means of eliminating non-germinable seeds (Brown *et al*., 1986).

SEED STORAGE

Appropriate storage conditions are important so that viability is not lost during storage. Some species remain viable for many years, especially the nut-like seeds that

are naturally stored in the ground. High temperatures and atmospheric oxygen are detrimental to seed viability, and the lifetime of the seed is also shortened. Seed will stay viable for at least a year in a cool, dry place (Blomerus & Robyn, 1999; Robyn *et al*., 2001). Dry, mature seed of *Protea* must be stored at a low temperature in a sealed container. The period required for *P. neriifolia* seeds to begin germinating was longer for seeds stored at room temperature for 24 months than for freshly collected seeds. Seeds kept in liquid nitrogen or at low temperatures maintained their viability, and there was no increase in the time required to initiate germination (Van Staden, 1978b).

SEED TREATMENT BEFORE GERMINATION

Seed of proteaceous species germinate poorly (Vogts, 1960; Horn, 1962; Van Staden, 1966). Vogts (1960) postulated that endogenous inhibitors were responsible for poor germination, while Horn (1962) regarded inefficient seed sorting as one of the main factors. However, improved seed sorting did not enhance germination to the level expected of non-dormant seed, and neither did the removal of possible inhibitory compounds (Brown & Van Staden, 1973c, 1975a; Van Staden & Brown, 1977, 1982).

Dormancy in proteaceous seeds is due to a lack of growth promoters, particularly cytokinin, but also gibberellin (Brown & Van Staden, 1975a; Van Staden & Brown, 1977, 1982). Increased availability of oxygen was found to be the most important environmental stimulus for the germination of many proteaceous species (Van Staden & Brown, 1977). Brown & Van Staden (1975a) also observed that when oxygen was absent, germination did not occur and cytokinin levels were also low, suggesting that the synthesis of these compounds is oxygen-dependent.

The outer and inner pericarps probably also play a role in imposing seed dormancy, because removing them increases germination. The embryos that are enclosed in a pericarp consist of two layers. The outer layer is thin and brittle, and can easily be removed. The inner layer is hard and woody (approx. 0.5 mm thick). The inner layer shows no visible change upon imbibition, while the outer layer becomes soft and gelatinous, and probably regulates gas exchange to the embryo. Removal of the outer layer results in improved germination due to high oxygen tensions, since the impermeable pericarp (seed coat) acts as a barrier to oxygen diffusion to the embryos (Brown & Van Staden, 1973a; Van Staden & Brown, 1973a). Non-domesticated or wild *Leucospermum* species have highly impermeable seed coats on intact, mature seeds (Brits, 1990c).

Nut-like seeds: Some species of *Leucospermum* and *Leucadendron* produce hard, nut-like seeds that require alternating temperatures for germination (Brits, 1986a, 1987; Brits & Van Niekerk, 1986). Intact *Lsp. cordifolium* seeds require oxygenation (Van Staden & Brown, 1973b; Brits, 1986b). Brits et al. (1995) indicated that dormancy in intact *Leucospermum* seeds is enforced by embryo anoxia, regulated by the impermeable testa. Germination of hybrid seeds of *Leucospermum* resulting from poorly compatible parental species poses a serious problem because, apart from severe dormancy factors, a significant proportion of embryos in seed batches are normally underdeveloped or aborted (Brits, 1990c).

Enhanced germination can be obtained by mechanical scarification (Van Staden & Brown, 1973a), acid scarification in concentrated sulphuric acid (H_2SO_4) (Brits & Van Niekerk, 1976), oxygen incubation, or soaking in 1% hydrogen peroxide (H_2O_2) (Brits & Van Niekerk, 1976; Brits, 1986b) and incubation at 8–9°C alternated with 24°C (Brits, 1986a, b, 1990c).

Scarification can improve the seed coat permeability to oxygen and also relieve the mechanical constraint imposed by the hard seed coat, which facilitates germination of embryos with a low viability (Brown & Van Staden, 1973a; Van Staden & Brown, 1977; Brits & Van Niekerk, 1986; Brits, 1990c). Mechanical scarification is labour-intensive and therefore not recommended for commercial nurserymen. Acid scarification by soaking seeds in concentrated sulphuric acid is just as effective and easier to implement. Acid scarification for 7.5 min in concentrated sulphuric acid increases germination of *Lsp. cordifolium* from 37–67%. Nut-like seeds must be treated with acid for a period of 5–10 min. After treatment, the seed must be thoroughly washed in distilled water (Vogts, 1982; Blomerus & Robyn, 1999).

Oxygen incubation increases the germination of seeds with intact seed coats. It also enhances the germination of acid scarified seeds. Scarification alone is therefore not a good substitute for oxygen incubation when maximal germination is required (Brits, 1990c). Premature commercial harvesting of *Leucospermum* seed is a common practice. Prematurely harvested seeds can result in incompletely developed, more oxygen-permeable seed coats, and a poor response to oxygenation (Brits & Van Niekerk, 1986).

Incubation in pure oxygen or under moist, cold conditions (5°C) enhanced germination by stimulating the production of growth promoters, particularly cytokinins. It is suggested that the primary effect of oxygen is to increase the rate of respiration and thus provide the energy required for the synthesis of butanol-soluble cytokinin, which leads to cotyledon expansion and subsequent radicle elongation. It is also possible that these higher levels of growth promoters are involved in the mobilisation of food reserves in seed (Van Staden & Brown, 1973b, 1977). Germination of seed of *Lcd. daphnoides* was increased 50% by chilling at 5°C, and 400% by incubation in high oxygen concentrations (Brown & Van Staden, 1975b). Chilling the seed for 30 days increased germination significantly, and resulted in a simultaneous increase in the butanol-soluble cyto-

kinins of *Protea compacta* and *Lcd. daphnoides*. The results for *Lcd. daphnoides* suggest that the effect of chilling on coat-imposed dormant seed is to bring about an interconversion of cytokinins, rather than a *de novo* synthesis as apparently occurs in dormant seed of *P. compacta* (Brown & Van Staden, 1973b). Acidic gibberellin-like substances were also recorded during incubation in oxygen. However, stratification does apparently not affect gibberellin levels of *Lcd. daphnoides* seeds. Gibberellins do not play a primary role in breaking dormancy, but appear to be effective in promoting germination in the presence of an elevated level of cytokinins (Brown & Van Staden, 1975b). Incubation in oxygen gas is also beneficial for poorly developed embryos. The technique, however, requires specialised equipment and is therefore not recommended for commercial use (Blomerus & Robyn, 1999).

Hydrogen peroxide has an oxygen-generating effect when taken up by *Proteaceae* seed (Brown & Dix, 1985), similar to that of other oxygenating treatments, such as scarification (Brits & Van Niekerk, 1976). A treatment of 1% H_2O_2 solution for a period of 24–48 h is recommended as a commercial treatment for *Leucospermum* seed (Vogts, 1982; Brits, 1986b; Blomerus & Robyn, 1999). Increasing the oxygen tension with H_2O_2 in *Lsp. cordifolium* seed strongly improved germination, independent of air temperature (Brits & Van Niekerk, 1986). This treatment is practical and economical to obtain 100% germination of all viable seed of *Lsp. cordifolium* sown in an open seedbed. H_2O_2 treatment has no effect on the germination percentage of seeds of serotinous species (Brits, 1986a).

Fluctuating daily temperatures seem to improve germination of nut-like seeds more than a constant temperature. Seed must therefore be sown during the cooler periods of the year, while seedbeds must be positioned in a sunny area. It seems that 10 and 20°C are, respectively, the low and high optimum temperatures for the nut-like *Proteaceae* seed (Brits, 1986b; Blomerus & Robyn, 1999). The seed of nut-like *Leucadendron* species germinated optimally when diurnal temperature fluctuations between 20°C in the daytime and 10°C at night were used. Many species of the section '*Alatosperma*' only required a low temperature to germinate optimally (11°C) (Sedgley *et al*., 2001). Brits (1986a) found that in *Leucospermum* species, optimal germination temperature regimes correlated with the general temperature regimes of their natural habitats.

Brits *et al*. (1995) concluded that gibberellins are necessary to induce seed germination, while cytokinins control the key processes during germination. Synthesis of both hormones depends on a moderately low temperature as the primary environmental requirement. For gibberellin synthesis, a secondary, daily pulse of high temperature is required.

Germination can furthermore be improved by a combined treatment with gibberellic acid and cytokinin following the germination criteria mentioned above (Brown & Van Staden, 1975a; Brits, 1990c). Soaking *Lsp. cordifolium* seeds in a solution of Promalin (GA_4, GA_7 and benzyladenine) significantly increased the germination percentage of seeds. Treatment of seeds with similar concentrations of benzyladenine alone decreased germination. This suggests that it is not the benzyladenine but the gibberellins or possibly a combination of gibberellins and the cytokinins that are responsible for promoting germination (Brown *et al*., 1986). Presoaking seeds in Promalin provides essential hormone(s) that act as promoters of germination. The level(s) of endogenous hormones are therefore increased or complemented by applied hormones, especially where suboptimal environmental factors prevent synthesis or interconversion of endogenous hormones (Brits, 1990c).

Brown *et al*. (1986) also indicated an improvement in germination of *Lsp. cordifolium* seeds when they were soaked in respectively ethrel, ethelene gas, solutions of polyethylene glycol, or gibberellic acid (GA_3).

All these above-mentioned factors along with seed disinfection, maximise *in vitro* seed germination and the germination rate in *Lsp. cordifolium*. Cumulative treatments of oxygen, plus Promalin, plus acid scarification, resulted in an increase in germination percentage and rate in six *Leucospermum* species (Brits, 1990c). Dormancy factors are apparently variable in their expression in seed populations. For example, a seed population does not require one threshold low-temperature level or duration of temperature to break low-temperature dormancy of all seeds (Brits & Van Niekerk, 1986). This could explain why the progressive fulfillment of environmental requirements gives stepwise increases in the germination percentage (Brits, 1990c).

Serotinous seeds (*Protea* and *Leucadendron*): Seed of South African *Protea* and *Leucadendron* spp. exhibit dormancy. Germination percentages are normally low, and germination takes place over extended periods of time (Rodríguez-Pérez, 1995). Dormancy seems to be imposed by a low temperature requirement and by the constraints of the pericarp, which prevents simultaneous germination of all seeds (Deall & Brown, 1981). The low temperature requirement can be satisfied by incubating seed at approximately 8°C (Brown & Van Staden, 1975a; Deall & Brown, 1981; Brits, 1986b; Mitchell et al., 1986). About 80% of viable seed will germinate within 90 days if kept sufficiently moist and at temperatures ranging from 5°C (stratification) to 25°C (Van Staden, 1966; Brown & Van Staden, 1973c). The optimum cues for maximum germination seem to differ between the *Protea* species, as has been observed in *Leucospermum* (Brits, 1990c).

Stratification for 60 days at 5°C and subsequent transfer to the optimum temperature for germination at 10°C, scarification, incubation in pure oxygen, and

treatment of seed with Promalin, improved the germination of *P. compacta* (Brown & Van Staden, 1973c; Mitchell *et al*., 1986). However, Brits (1986a) suggested that in serotinous seeds such as *Protea* spp., there is only a low-incubation temperature requirement and no need for an increase in the partial pressure of oxygen during germination.

It is significant that in many instances, applied cytokinins can substitute for the chilling requirement (Webb & Wareing, 1972). In *P. compacta* seeds, an increase in cytokinin levels has been recorded during stratification (Brown & Van Staden, 1973c). Many of the seeds actually germinate during the stratification process itself, and it has been established that this species germinates to varying degrees over a range of temperatures below 25°C (Horn, 1962; Brown & Van Staden, 1973a).

A threshold level of cytokinin may be required to break dormancy, resulting in germination (Brown & Van Staden, 1973b). Changes in endogenous cytokinin levels, both during stratification and under optimum germination conditions, are of significance in the germination process of *P. compacta* seeds (Mitchell *et al*., 1986). Brown and Van Staden (1973c) suggested that the increase in cytokinin activity was due to a conversion from "storage" to "free" cytokinins. The effect of applied cytokinin on the germination of *P. compacta* seed was tested. Promalin increased germination significantly. Cytokinin alone could not stimulate germination, suggesting that changes in endogenous cytokinins within the seeds are probably not related to the breaking of dormancy, but rather to radicle elongation. Stimulation of germination could only be done by gibberellic acid (GA_4 and GA_7). It seems that it is the gibberellin levels that are enhanced as a result of stratification, which indicates that the endogenous levels of these hormones require further investigation (Mitchell *et al*., 1986).

The use of gibberellins can often substitute for the low-temperature germination requirement (Taylorson & Hendricks, 1977). However, treatment with GA_3 depressed germination in *P. compacta* (Mitchell *et al.*, 1986). In contrast, an improved germination was observed after seeds of *P. eximia* and *P. neriifolia* had imbibed a GA_3 solution (100 ppm), although no significant effect was observed in *P. cynaroides* (Rodríguez-Pérez, 1995).

Improved germination can be obtained both for nut-like and serotinous seeds when seeds are soaked in warm water at a constant temperature of 50–60°C for 15–30 min before they are sown. Benic and Knox-Davies (1983) improved germination of *P. compacta* by hot-water treatment. Seed-borne fungi and bacteria are then controlled during germination. Warm-water treatment followed by fungicide treatment (2 g thiram/1 L water for 30 min or 0.5 g thiram/1000 seeds) is particularly effective for *Protea* seed (Blomerus & Robyn, 1999). This treatment is especially of use for germination in seedbeds. Heat-sensitive seed such as *Lcd. platyspermum* should not be treated with warm water (Blomerus & Robyn, 1999).

Sowing of seed

Autumn is the best time for sowing seed of proteas in both the winter and summer rainfall areas of South Africa. Untreated seed of *Leucospermum* can be sown from the end of February in the Southern Hemisphere, a month earlier than in the Northern Hemisphere, which is the best sowing time for the other proteas (April–May). Roots of seedlings therefore have enough time to establish before the summer. Seeds are sown on a large scale in a seedbed (Vogts, 1982; Blomerus & Robyn, 1999). Smaller amounts of seed can be sown between hessian sacks or on filter paper in Petri dishes. The Petri dishes are maintained at approximately 8°C for 6–10 weeks for germination to occur (Littlejohn *et al*., 2001).

Seedbeds for nut-like seed must be positioned in an open, sunny area, while shade nets are necessary for other seed types. The seedbed medium must be acidic (pH < 5.5), well-drained and sterilised. The medium must consist of either coarse, acidic, washed river sand, or three parts sand mixed with one part fine peat moss. The water quality must be good since high pH and/or salt content can be very detrimental to seedlings (Blomerus & Robyn, 1999).

Seed must be uniformly and sufficiently spaced. Seed sown too densely, will result in weak, long and thin seedlings. Seed must be sown very shallowly (1 cm deep). *Leucospermum* seed can be sown deeper to a depth of 2 cm. The seedbed medium must never be left to dry out, while water-logged conditions can permanently damage seedlings (Blomerus & Robyn, 1999).

Protea seed normally germinates within 2–6 weeks (Littlejohn, 2001b). Some species with an inherent dormancy period (e.g. *Lsp. cordifolium* and *Lcd. argenteum*) take up to 4 months to germinate. The average germination percentage for *Leucadendron* species of the section '*Alatosperma*' is higher than that of other proteas, and may be 90% or more (Blomerus & Robyn, 1999).

As soon as the cotyledons appear between the two seed lobes, all proteas can be planted out in individual containers and grown under shade (Blomerus & Robyn, 1999). Propagation of cut-flower species by seed will probably in the future be restricted to plant breeders attempting to breed material with improved characteristics. The need to obtain good germination of all fertile seed is thus even more important than before, especially in the case of hybrid seed artificially fertilised at great cost (Malan, 1992).

Vegetative propagation (cuttings)

Vegetative propagation provides the only method of obtaining large quantities of plants of the same genotype. This allows for the propagation of hybrid plants without losing their unique properties, as would happen if seed were used. Furthermore, the growing cycle is

synchronised and plants from cuttings often come into production one year sooner than those grown from seed (Brinkhuis & Goliath, 1999).

Members of the *Proteaceae* can be propagated by means of shoot cuttings. Terminal (Jacobs & Steenkamp, 1975; Jacobs, 1982a; Vogts, 1982; Brits, 1986c; Malan, 1992), sub-terminal and basal cuttings (Harré, 1988, 1995) form roots and are used commercially.

Although a 20 cm cutting is commonly used, a shorter cutting, down to the node type, can also be rooted effectively. Leaf bud cuttings consist of a complete leaf and a little portion of stem (from the terminal shoot section) supporting the corresponding axillary bud (Hartman & Kester, 1974). Rooting leaf bud cuttings in propagation beds with bottom heating (± 25°C), a mist system, and different treatments with indole butyric acid (IBA) is possible in *Leucadendron* 'Safari Sunset', *Lsp. patersonii* and *P. obtusifolia* (Rodríguez-Pérez, 1992).

Without damaging the cutting, leaves are cut from the lower half or third of the cutting directly against the stem (Jacobs, 1982a; Vogts, 1982). In general, a 5 s basal dip in IBA at 4000 ppm is followed by planting the cuttings in well-aerated sterilised medium (consisting of sand or polystyrene and peat moss), in perforated plastic bags or peat cups. The rooting house must have intermittent mist and bottom heating (22–25°C) (Rousseau, 1967; Blommaert & Rosseau, 1971; Jacobs & Steenkamp, 1975; Jacobs, 1982; Vogts, 1982; Harré, 1988; Malan, 1988; Brinkhuis & Goliath, 1999). Rooting in *Protea* generally occurs within 6–16 weeks (Coetzee & Littlejohn, 2001). Cuttings are put in a shade house for 3–4 weeks, after which they are put in direct sunlight (Brinkhuis & Goliath, 1999).

Great differences in the rooting ability of stem cuttings exist between protea species (Malan, 1992) and between genotypes (Brits, 1986c). According to Chadwick (1955), 'the successful vegetative propagation by cuttings depends on internal, anatomical and physiological factors as well as external and environmental factors'. Large differences in rooting capacity between species, cultivars and clones may be linked to propagation bed temperature (Brits, 1986c), physiological status of the cutting (Harré, 1987), growth regulator treatment (Montarone *et al*., 1997), type of cutting or the position of the stems or cuttings on the mother plant (Brits, 1986c; Montarone *et al*., 1997), light intensity (Harré, 1988), rooting medium aeration (Harré, 1988; Malan, 1988), frequency of misting (Brits, 1986c), the age of the mother plant, the substrate, the climatic conditions, and damage to plants or phytosanitary problems (Montarone *et al*., 1997). Specific requirements have to be adapted for each cultivar for optimal results (Brits, 1986c; Harré, 1988, 1995).

Studies in France indicated that for the best rooting of cuttings in soilless media, the mother plants must be young, and cuttings should be taken at median positions on flower bud stems (Montarone *et al*., 1997). Mother plants need to be manipulated in order to keep them vigorously growing, in order that wood of increasing physiological maturity is not used for cuttings (Harré, 1987; Malan, 1992). Faruchi *et al*. (1997) found that semi-hardwood cuttings of *P. obtusifolia* taken from juvenile plants rooted at a higher percentage than cuttings taken from mature plants, while cuttings taken from shaded plants rooted at a higher percentage than those from sun-grown plants. Cuttings grown under 50% shade showed a higher percentage of rooting than cuttings produced under 30% shade, but both rooted better than the unshaded control cuttings (Faruchi *et al*., 1997).

The time of harvesting cuttings is important. The time is dependent on the maturity of the wood at the end of the growing cycle and the required time for the cutting to develop roots (Jacobs, 1982a; Vogts, 1982). In *Leucospermum* and *Leucadendron*, all species terminate shoot growth during autumn. Semi-hardwood cuttings are harvested shortly thereafter (March–April) (Southern Hemisphere) (Jacobs, 1982; Vogts, 1982; Malan, 1992; Brinkhuis & Goliath, 1999). Rooting of *Leucospermum* cuttings in autumn allows field establishment of cuttings of the current season during the favourable climate of autumn to late winter (Brits, 1986c). Cuttings are supposed to be rooted and ready for planting before August (Vogts, 1982).

In the Western Cape Province of South Africa, growth cycles in *Protea*, however, are less synchronised within the period from November to the end of April (Vogts, 1982; McKay, 1985; De Swardt, 1989). Cuttings are therefore not all at the optimal stage for rooting if harvested at the same time (D.G. Malan, Fynflor, Stellenbosch, South Africa, pers. Observ.). G.M. Littlejohn (pers. comm., 2002), however, found this observation not to be true on pruned bushes, except for *Protea* 'Cardinal'. Mother plants must, however, be manipulated and prepared and cutting material must be carefully selected in order to ensure cuttings of a high rooting potential (Harré, 1987, 1989). *Protea* cuttings are harvested from November to April after hardening of the current season's growth (Brinkhuis & Goliath, 1999).

The procedure used by most growers in South Africa for rooting of protea is to apply indolebutyric acid (IBA) (4000–8000 ppm) dissolved in 50% ethyl alcohol as a quick dip to the basal few millimeters of the cuttings for 5–10 s. Certain *Protea* species were found to be very sensitive to naphthaleneacetic acid (NAA) (Jacobs, 1982). The addition of auxin (Harré, 1989) and other growth regulators such as gibberellic acid, ethephon and daminozide (Criley & Parvin, 1979; Brits, 1986c; Gouws *et al*., 1990), in addition to IBA, can also promote rooting (Malan, 1992). Especially at low temperatures, auxin should be applied to ensure acceptable rooting percentages (Brits, 1986c). Increased IBA concentration improved the rooting percentage, whereas the increase of dipping time or the depth of dipping had little effect on the auxin absorption rate or rooting (Ja-

cobs, 1982; Montarone *et al*., 1997). Lack of hormonal treatment significantly delayed rooting in unwounded and wounded basal and terminal cuttings of *Lsp.* 'California Sunshine' (Rodríguez-Pérez *et al*., 2001a). Increased IBA concentration (4000 ppm) favoured root formation in unwounded basal cuttings more than in wounded cuttings (Rodríguez-Pérez *et al*., 1997).

Wounding of the base of cuttings is effective in promoting rooting of some *Protea* cultivars, such as *P. obtusifolia*, up to 60% (Rodríguez-Pérez, 1990), as well as in *Leucadendron* 'Safari Sunset' (Rodríguez-Pérez *et al*., 1993), *Leucospermum* 'Sunrise' (Rodríguez-Pérez *et al*., 1999) and *Lsp.* 'California Sunshine' (Rodríguez-Pérez *et al*., 2001a). Wounded, terminal cuttings of *Lcd. discolor* treated with 4000 ppm IBA rooted better and faster than unwounded ones. Cuttings are wounded by making two to four shallow and opposite longitudinal incisions with a sharp blade in the basal bark, penetrating as far as the outer cortex and extending upwards for about 2 cm (Rodríguez-Pérez, 1990; Rodríguez-Pérez *et al*., 1997). Wounding of cuttings, in combination with IBA (4000 ppm), enhanced rooting (Rodríguez-Pérez, 1990; Rodríguez-Pérez *et al*., 1993, 1997, 1999, 2001a; Pérez-Francés *et al*., 2001a).

Temperature, together with the interaction of genotype, has a greater effect on rooting than the application of auxin and the terminality of cuttings (Brits, 1986c). Most protea species and hybrids root satisfactorily with bottom heating (22–25°C), but some will root better at lower temperatures (McKay, 1985; Brits, 1986c). Root formation is normally very slow at low temperatures. Rooting frames should, therefore, be fitted with bottom heating for the cooler months during autumn and winter (Brits, 1986c). The current rooting system of cuttings is, however, rather expensive. A cheaper system, e.g. one without a requirement for bottom heating, needs to be developed to simplify the rooting of cuttings (Malan, 1995). *Leucospermum* cultivars that root readily could be rooted without bottom heating if cuttings were taken as soon as extension growth stopped during February (Southern Hemisphere) (Brits, 1986c).

Leaves and buds play an important role in rooting. The stimulus originating from leaves or buds increases the number of rooted cuttings (Favre, 1977). Montarone *et al*. (1997) indicated that the branching ratio of rooted cuttings is especially affected by cutting vigour. Lastly, but very importantly, strict sanitation in the rooting frames must also be implemented to control diseases and limit losses (Benic, 1986).

Grafting and budding

Proteas have very strict nutritional requirements (Brits, 1990a). They grow well in soils with low pH, low conductivity (Vogts, 1980), and low levels of certain elements such as potassium and phosphorus In many species there is a strong preference for ammonium rather than nitrate nitrogen (Claassens, 1986). Soil-borne diseases and pests, such as *Phytophthora cinnamomi* (Von Broembsen & Brits, 1985) and nematodes (Rohrbach, 1984) pose risks and limitations in the cultivation of proteas (Brits, 1990a). Since some of the protea species are very slow to root, prolific rooting ability in clonal rootstocks is a very important characteristic (Brits, 1990b).

Rousseau (1966) suggested the idea of using rootstocks in *Proteaceae* as early as 1966, but rootstocks have been used commercially only during the last decade (Ben-Jaacov *et al*., 1992). Grafting of *Protea* has focused on using alkaline tolerant *P. obtusifolia*, which grafts compatibly with many other *Protea* species, as a rootstock (Brits, 1990a, b; Moffatt & Turnbull, 1994b).

Although *P. obtusifolia* is a useful rootstock, a better rootstock is still sorely needed. Characteristics to consider when selecting a rootstock include growth vigour (Brits, 1990a, b), a broad range of compatibility with the scion (Brits, 1990b), fast rooting ability (Malan, 1989; Brits, 1990b), mature main stem diameter suitable to accommodate thick scion stems and rootstock longevity, as well as tolerance to drought, cold, waterlogged conditions, to pH (H_2O) values in the order of 6.5–8.5, to relatively high salt content of the soil solutions (specifically high P, K and NO_3) (Brits, 1990b), and to soil-borne disease (McCredie *et al*., 1985; Brits, 1990b; Von Broembsen & Brits, 1990) and nematodes (Rohrbach, 1984; Ben-Jaacov, 1986a).

An extensive search for rootstocks within *Proteaceae* resistant or tolerant to root rot caused by *Phytophthora cinnamomi* indicated a slight degree of genetic tolerance against *Phytophthora* in the genus *Leucospermum* (Von Broembsen & Brits, 1985), as well as successful scion and rootstock combinations within the different genera. Combinations where graft incompatibility occurred were also observed (Brits, 1990b; Moffat & Turnbull, 1994b). The same situation applies to tolerance to relatively high pH values and concentrations of certain salts of the soil solution in both *Protea* and *Leucospermum.* Claassens (A.S. Claassens, Dept. of Soil Science and Plant Nutrition, Univ. of Pretoria, unpublished) concluded that information on the natural substrate of a species population could provide clues to its adaptability under specific cultural conditions. A wide range of useful compatible scions and rootstocks exists between species and sections in the genus *Protea*, such as *P. aristata* on *P. compacta* and *P. magnifica* on *P. eximia.* A number of generally useful *Protea* and *Leucospermum* rootstock qualitites are found amongst the members of these groups. However, they are not combined in a single species. Good rootstock characteristics could be combined by means of interspecific hybridisation, backcrossing and selections (Brits, 1990b).

In Israel, soil problems such as high pH, high phosphate, and soil-borne diseases result in the need for daily irrigation and fertilisation. Additionally, the lifespan of plantations is limited (Ben-Jaacov *et al*., 1992; Ackerman *et al*., 1995a, b). Some species, native to

high-pH soils in South Africa, were studied as potential rootstocks for use in Israel in the late 1980's. The best results were obtained using a clonal selection of *Lcd. coniferum* 'Orot'. 'Orot' is currently widely used as the rootstock for 'Safari Sunset' (Silber & Ben-Jaacov, 2001). Another rootstock used for grafting is *Lcd. galpinii* (Wolfson *et al*., 2001). Several studies have shown that the growth of grafted plants was significantly superior to that of ungrafted plants and that this advantage was more significant under conditions of nutrient deficiency and non-optimal pH (Silber & Ben-Jaacov, 2001). This technology makes it possible to grow a much wider range of *Leucadendron* cultivars in soils in Israel (Ben-Jaacov *et al*., 1992; Ackerman *et al*., 1995a, b).

There is a need for using rootstocks and grafted plants of other proteaceous genera under Israeli conditions, in addition to *Leucadendron*. Some species of *Protea* and *Leucospermum* can be used as lime-tolerant rootstocks, and this forms the key for successful protea cultivation on a large commercial scale in Israel (Ben-Jaacov *et al*., 1992, 1994; Ackerman *et al*., 1995a, b). Grafting is relatively easy in the genus *Leucospermum. Leucospermum patersonii* is probably the most suitable rootstock since it is very lime-tolerant. However, it has a relatively short life-span under Israeli conditions and is sensitive to nematodes. In the genus *Protea*, *P. obtusifolia* gave the best grafting take and growth performance when used as rootstock. No incompatibility problems were encountered, even when grafting *P. cynaroides* on *P. obtusifolia*. Further testing of scion/rootstock combinations may lead to specifically selected scion/rootstock/soil combinations (Ben-Jaacov *et al*., 1992).

Crop science evaluation of rootstock effects on yield and cut-flower quality needs to be conducted and tested on a regional basis prior to commercial introduction (Brits, 1990b; Malan, 1992). Qualities required for any region need to be combined if a rootstock is to succeed (Brits, 1990b).

Grafting and budding techniques have been evaluated in cuttings (Malan, 1989; Brits, 1990a), nursery plants, rooted cuttings and seedlings (Rousseau, 1966; Vogts *et al*., 1976), micrografting (A. Ackerman, ARO, Volcani Center, Israel, pers. comm., 2002), and topworking of young, established plants (Rousseau, 1968; Harrington, 1988; Brits, 1990a). The best method is the grafting or budding onto cuttings, rooted or unrooted, since this is the fastest production method, reasonably successful and the easiest to apply (Brits, 1990a). Wedge grafting of pot-held rootstocks with semi-hardwood scions gave good results with *Leucospermum* and *Protea* (Brits, 1990a, b; Turnbull & Moffatt, 1994; Ackerman *et al*., 1995a). Both grafting and budding can be applied within and between proteaceous genera (McCredie *et al*., 1985; Van der Merwe, 1985).

Grafting has been reported to be easy, but the commercial use of the available techniques has been found to not always be successful, or to be very expensive (Ben-Jaacov *et al*., 1989; Malan, 1989, 1995a, b) (Fig. 1). Commercially acceptable techniques, therefore, need to be developed (Malan, 1995a, b).

Brits (1990a) found that elimination of an intermittent misting system for large-scale commercial grafting required a much smaller total leaf area of the scion than in conventional grafting. Limiting scion size to only one or two prominent axillary buds, plus a small piece of their subtending leaf, effectively controls desiccation. Furthermore, a *Leucospermum* scion must be covered with PVC-tape and a plastic bag to restrict moisture loss (Brits, 1990a). Vogts (1982) as well as Brinkhuis & Goliath (1999) provide detailed descriptions of the wedge-grafting technique.

Wedge-grafting was not very successful in the genus *Protea*. Furthermore, a serious problem with grafting *Protea* is wound blackening in both the scion and rootstock due to the accumulation of phenolics (Brits, 1990a). The poor grafting performance of *Protea* in comparison with *Leucospermum* is, however, not due to incompatibility of scions and rootstocks. Poor grafting in *Protea* is associated with the inherent difficulty of rooting in this genus, and with the blackening of the rootstock and scion cut surfaces. In *Protea* grafting, water stress and contamination must be minimised, as well as the period of exposure to the air of cut surfaces. *Protea* rootstocks must therefore be developed that root easily and that do not blacken excessively. More successful grafting techniques and pre-treatments of scion material need to be developed for the genus *Protea*, while scion/rootstock interspecific compatibility also needs to be investigated (Brits, 1990a; Malan, 1992).

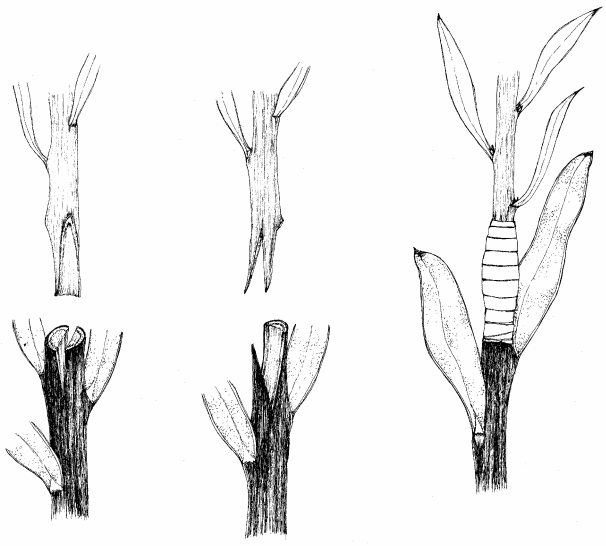


Fig. 1. The cutting-graft technique is one method used in cultivating species of *Leucadendron, Leucospermum* and *Protea*. Scions are bound to unrooted rootstocks that are placed in mist-beds to stimulate quick rooting and union of the scion to the rootstock.

The cutting-graft technique, used with unrooted cuttings, is therefore more successful with *Leucadendron* (Ackerman *et al*., 1997) and *Leucospermum* than in *Protea* (Brinkhuis & Goliath, 1999). Rooting and graft union are achieved simultaneously in a mist propagation facility. The scions and unrooted rootstocks are tied together and placed into a mist propagation bed that stimulates concurrent, quick rooting of the rootstock and a union of the scion and the rootstock to produce new grafted plants (Ackerman *et al.*, 1997).

In Israel, the cutting-graft technique is used commercially. Complete grafted *Leucospermum* plants are produced in 4–8 weeks, and in 6–8 weeks for *Leucadendron*. The use of cutting-grafts has various advantages. Cultivars grafted onto tolerant rootstocks give more vigorous growth, higher production and better quality flowers. Cutting-graft is a quick propagation technique that can be applied year-round, and is suitable for rapid large-scale commercial production. Plants transplanted into small containers are ready for field planting in 3–4 months. Time, growing-space requirements and costs are greatly reduced by this technique (Ackerman *et al.*, 1997). Brinkhuis & Goliath (1999), however, found that in the case of *Protea*, grafting must rather be carried out on rooted cuttings due to the poor rooting ability of most *Protea* species.

For large-scale commercial plantations, it is important to use rapid and efficient grafting methods. However, cuttings root well only if thin, young wood is used, and tying such delicate grafts is very difficult and inaccurate on a commercial scale. A technique was therefore developed to use flexible, transparent microtubing to bind together splice-grafted cuttings of the woody shrub, *Chamaelaucium* (Ben-Jaacov & Ackerman, 2001).

Budding makes more economical use of propagating material than grafting, and can be especially effective in the propagation of difficult to root and slow-growing cultivars of *Protea*. It can be done on rooted and unrooted cuttings (Harrington, 1988; Brinkhuis & Goliath, 1999). The budding of unrooted rootstock cuttings appears to be the most suitable method for effective large-scale commercial budding of proteas. The bud is inserted at a position approximately ⅔ up the cutting, while retaining a number of leaves distal to the bud (Fig. 2). The rootstock is cut above the bud only after the cutting has rooted before transplanting, the bud union has healed, and the vegetative growing season has begun (Brits, 1990a). Vogts (1982) and Brinkhuis & Goliath (1999) describe this technique in detail.

Grafting and budding can be carried out successfully from autumn to spring. Autumn to late winter is the best period, in order for the wound to heal during the winter before transplantation in containers, and the resumption of shoot growth in spring. Shoot growth must first resume before grafted plants can be established in the following autumn or winter in the field. Autumn to late winter is also the most favourable period for cutting grafting, since it is the best for rooting. Vigorous plants can be grafted or budded in early spring, followed by growth resumption in early summer, or cutting grafting can take place in autumn, and growing scions on rooted cuttings can be transplanted directly to the field in the late spring. Scions on plants which are grafted or budded too late or have poor vigour tend to remain dormant until the following shoot growth period. Early survival rates of established *Leucospermum* plants are variable. It seems that proteas that root and establish with difficulty from cuttings also propagate with relative difficulty when grafted (Vogts, 1982; Brits, 1990a).

Micropropagation (tissue culture)

Micropropagation of proteas is used not only for the rapid multiplication of new selections, but also to overcome quarantine requirements for international exchange of material, to overcome problems experienced with conventional propagation techniques, for rejuvenation of physiologically mature material, and for *in vitro* physiological, nutritional and other studies (Ben-Jaacov & Jacobs, 1986; Malan, 1992). However, the technique also has some disadvantages. The development of the technique is costly, *in vitro* produced plants have a longer pre-production period, and there is a relatively small (1 000 to 20 000) annual requirement for *Proteaceae* plants per selection/hybrid.

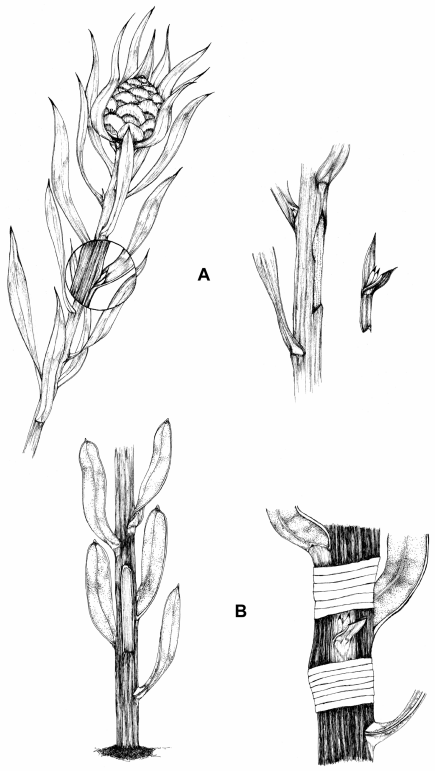


Fig. 2A–B. A. Budding is a grafting method that can be done on rooted and unrooted cuttings. A bud attached to a small piece of parent bark with a thin layer of wood below it is harvested from a parent shoot bearing several buds. B. The bud is inserted approximately ⅔ up into the rootstock cutting, which retains a number of leaves distal to the inserted bud.

Although disadvantages exist, they do not outweight the above-mentioned advantages of micropropagation (Malan, 1995a, b), especially when other propagation techniques are unsuccessful. For example, species of the genus *Leucospermum* are considered easy to root in South Africa (Jacobs & Steenkamp, 1975), but in Israel rooting success varies from 50–90% with no clear explanation. Also, reliability in the supply of propagation material is a prerequisite in the pot plant industry. Micropropagation can therefore be very useful for supplying a reliable quantity of *Leucospermum* material for the industry (Tal *et al*., 1992a). The use of lime-tolerant *P. obtusifolia* as rootstock in Israel is limited due to the fact that vegetative propagation of the species by cutting is difficult, slow and unreliable (Rodríguez-Pérez, 1990). Micropropagation was therefore investigated (Watad *et al*., 1992). In Tenerife, where the demand for 'Safari Sunset' is high in comparison to its availability, micropropagation was initiated to address this issue (Pérez-Francés *et al*., 1995).

LEUCADENDRON

Micropropagation was implemented because vegetative propagation of *Lcd. discolor* cuttings presented some difficulties due to the relatively weak rooting capacity of some clones (Pérez-Francés *et al*., 2001b). Pérez-Francés *et al*. (2001b) reported that the best explants were partially lignified nodal segments (10–15 mm in length bearing one or two nodes), collected from growing branches of field-grown plants. PVP was applied to avoid oxidation. A seasonal effect was observed in explant viability with the best responses observed during spring. For the establishment phase, nodal explants were cultured on Murashige & Skoog (1962) (MS) medium at half strength with 3% sucrose, 0.7% agar and 0.5 mg/L benzyladenine. The addition of IBA resulted in more cultures having axillary buds. Proliferation ability was maintained by transferring shoots to fresh medium. The addition of auxin to the medium during the multiplication phase did not significantly improve explant response. Further research is needed to improve the multiplication phase in order for micropropagation for it to be commercially feasible (Pérez-Francés *et al*., 2001b). Axillary bud formation and growth was also increased *in vitro* in 'Safari Sunset' when half-strength MS medium was supplemented with the cytokinin 6-benzyladenine, while the use of phytogel as an agar substitute gelling agent significantly increased bud proliferation (Pérez-Francés *et al*., 1995).

LEUCOSPERMUM

Tissue culture techniques for the propagation of *Leucospermum* (Ben-Jaacov & Jacobs, 1986; Kunisaki, 1989, 1990; Rugge *et al*., 1989; Tal *et al*., 1992a, b) have been developed. These consist mainly of induced axillary bud sprouting of multi-nodal explants in culture on modified MS medium with cytokinin (Malan, 1992). Van Staden and Bornman (1976) initiated callus from the cotyledons and hypocotyls of *Leucospermum cordifolium* seedlings. They found that Miller's (1965) medium was the best medium to support the growth of the callus. Pérez-Francés *et al*. (2001c) reported that the best medium for establishment of cultures was half strength MS medium supplemented with 1 mg/l benzyladenine, 3% sucrose and 0.7% agar. Furthermore, the response of explants during the establishment phase depended on the collection period. Explants collected in spring resulted in cultures with the most viable axillary buds. The medium used for the multiplication phase was half strength MS, 0.2 mg/L benzyladenine (BA), 3% sucrose and 0.7% agar. Microshoots rooted in the MS medium containing indolebutyric acid (IBA), but only a low rooting percentage could be obtained.

Gibberellic acid is essential for rapid shoot proliferation and elongation (Tal *et al.*, 1992a), since long shoots are necessary for the weaning stage (Tal *et al*., 1992a). IBA added to the medium ensures the best rooting (Tal *et al*., 1992a). Pérez-Francés *et al*. (2001c) pre-treated mother plants weekly with benzyladenine at 200 mg/L for a month. This treatment considerably increased the number of buds per branch, and consequently the number of cuttings as a source for *in vivo* and *in vitro* vegetative propagation. However, no significant differences in growth and multiplication between pretreated and non-pretreated explants were observed during *in vitro* establishment and multiplication. The most suitable primary explants for the establishment of cultures were nodal segments (0.5–1 cm) bearing one or two nodes excised from actively growing branches. Young explants from the apical zone most frequently exhibited browning. Nodal shoot segments were treated with polyvinylpolypyrrolidone (PVP) to prevent phenolic browning of the explant. The explant type influenced the formation of axillary buds (Pérez-Francés *et al*., 2001c).

Relatively high light intensities promote *in vitro* rooting. The need for high light intensity for rooting and growth of *Lsp. cordifolium* (Tal *et al*., 1992a) may be the main reason for failing to establish this species in the conventional low-light, high humidity facilities (Tal *et al*., 1992a). Relatively high light intensity in the weaning facility improves the success rate of weaning, as does the combination of high humidity and a relatively dry medium. Longer plantlets with three to five nodes (rooted or unrooted) and *in vitro* rooted material established more successfully (Tal *et al*., 1992a). Kunisaki (1990) also stressed the need for high humidity conditions in the greenhouse after rooting of the cuttings *in vitro*, in order for the roots to become established in the potting medium.

The 'feeder leaf' system for *Leucospermum* resulted in substantially improved axillary bud sprouting (Rugge *et al*., 1989), and the establishment of single buds in liquid medium resulted in direct proliferation (Kunisaki, 1990).

Further research is needed for hardening and acclimatisation of *in vitro* plants and to improve the micropropagation protocol so that it will be commercially significant (Pérez-Francés *et al*., 2001c).

PROTEA

Protea material is probably the most difficult and slowest of all the *Proteaceae* to propagate (Malan, 1992). Tissue culture techniques for propagation of *Protea* have been developed (Rugge, 1995), but successful transplanting of rooted shoots to soil has not been achieved (Coetzee & Littlejohn, 2001). Originally, the major problem in the micropropagation of the genus *Protea* was the browning of the tissue due to phenolic compounds (George & Sherrington, 1984; Ben-Jaacov, 1986b). Obtaining sterile explants from field-grown plants and difficulties in getting axillary buds on explants to sprout were further obstacles (Rugge *et al*., 1989). Etiolation treatment of the actively growing shoots on the mother plants may be helpful to overcome browning and promote sprouting of axillary buds in *P. obtusifolia*. Furthermore, addition of gibberellic acid and benzylaminopurine (BAP) to the medium promoted bud sprouting and elongation (Ben-Jaacov & Jacobs, 1986; Watad *et al*., 1992). A similar effect of gibberellic acid was reported in *P. cynaroides* (Ben-Jaacov & Jacobs, 1986). Treating actively growing distal axillary shoots on field grown mother plants of *P. repens* 'Embers' with benzyladenine, and supplementing half-strength MS medium with benzyladenine, significantly reduced browning and promoted axillary bud sprouting *in vitro* (Rugge, 1995). The application of benzyladenine to mother plants of *Protea* 'Ivy' and 'Carnival' (De Swardt, 1989) also increased axillary bud sprouting (Malan, 1992). Ben-Jaacov & Jacobs (1986) increased sprouting of shoots on mother plants sprayed with Promalin compared to the control plants.

Gibberellic acid further increased bud break *in vitro*, while somatic embryos developed from the base of the shootlet throughout the multiplication stage (Rugge, 1995). Callus, proteoid roots (Van Staden *et al*., 1981), and embryos have been raised from mature cotelydons of *Protea*.

Watad *et al*. (1992) reported the *in vitro* establishment of *P. obtusifolia* from multinodal shoot explants where leaves were cut off. They concluded that a "feeder leaf" system seems to be important for axillary bud development in this species.

Selection of explant material is very important to the successful establishment of proteaceous plants *in vitro*. Shoots sprouting from the lignotuber are the best source for explants for *P. cynaroides*. Semi-hardwood shoots of explants must be used, since shoots from hairy, softwood explants fail to grow (Ben-Jaacov & Jacobs, 1986).

CULTIVATION

The use of effective cultivation practices is essential in order to produce high quality cut-flowers (Littlejohn *et al*., 1995). Many proteas can be grown with relative ease but if improved cultivation practices are not utilised the resultant product is generally of poor quality and orchard life is limited (Malan, 1995a, b). Fortunately, cultivation methods for a number of *Proteaceae* have been developed and well-documented (Matthews, 1993; Malan, 1995a, b; Harré, 1995, Eigenhuis, 1999a, b). However, horticultural requirements differ and may be problematic for some proteaceous types, species or cultivars (Malan, 1995a, b).

Establishment requirements

PHYSICAL PROPERTIES OF THE SOIL

The cultivation of *Protea* is limited by the availability of suitable soils and climatic conditions (Vogts, 1989). Most South African *Proteaceae* grow on sandy-clay soils that are well-drained, nutrient-deficient and acidic, except in the case of lime-tolerant species such as *P. obtusifolia*. A soil suitable for *Proteaceae* must have less than 20% clay and less than 40% silt. However, many species will also grow very successfully on soils containing a clay/loam fraction of 30–50% as long as it is well drained. Some proteas, such as *P. magnifica*, *P. compacta* and *Lsp. cordifolium*, will not grow in soil with a high clay content. Others, such as *Lcd. salicifolium*, *Lsp. formosum*, *Lsp. glabrum*, *P. cynaroides*, *P. lepidocarpodendron*, and *P. mundii*, are adapted to, or may even prefer slightly wetter or shallower soils. The adaptability of varieties to heavier and poorly drained soils should be quantified to allow for better farm planning (Malan, 1995a; Eigenhuis, 1999a; Coetzee & Littlejohn, 2001; Montarone, 2001a; Rebelo, 2001).

In South Africa, particularly the Western Cape Province, agricultural land suitable for cultivation of proteas is limited. High-quality agricultural land with irrigation is a relatively expensive component of protea production (Coetzee & Middelmann, 1997). Two forms of cultivation are practiced, namely intense cultivation of clonal and seed material in rows, and broadcast seed sowing. The latter is used primarily for species used in the dried flower industry (Coetzee & Littlejohn, 1995).

In Western Australia, proteas grow well in the infertile, acid soils. The majority of proteas are planted in rows under drip irrigation and are regularly fertilised (Webb, 1992).

In Israel, 'Safari Sunset' is grown in volcanic ash (tuff) to avoid soil restrictions caused by a high soil P content. Tuff is a volcanic material, characterised by high porosity and high-saturated hydraulic conductivity (Silber *et al*., 1997).

Although the climate in the south of France is favourable for protea production, most soils in that area are calcareous clay soils which are unfavourable for Proteaceae which are not tolerant of limestone (Allemand *et al*., 1995). Cultivation under glass in soilless media is therefore necessary (Montarone & Allemand, 1995). Cultivation of plants on a substrate that is not

derived from soil is termed soilless cultivation. The substrate can be sand, perlite, gravel or volcanic lava and the nutrient solution is supplied by drip or recycled. An ideal substrate must ensure a good distribution between water and air (50% minimum for air), avoid compaction, be non-degradable, avoid damaging the roots, be as inert and porous as possible, free of toxic elements and plant pathogens, and easy to disinfect (Montarone, 2001a).

Montarone (2001b) discussed the advantages and disadvantages of soilless culture. Advantages include reducing problems related to soil-borne pathogens, ensuring a high, long-lasting yield with a high level of quality, decreasing the working time, and providing the possibility to optimise the fertilisation, irrigation and production schedule. Disadvantages include the high costs to set up the system, adaptation to a new technique, and constraints such as analytical controls and regular pH and conductivity measurements. Lemaire *et al*. (1989) pointed out some constraints associated with containerised cultivation. These include the limited volume available for the root system to grow and the need to provide the roots with water and minerals in order to balance supply and demand while optimising production (Montarone & Ziegler, 1997). Montarone & Allemand (1995) established a nutrient solution that is recycled in a closed-cycle watering system.

Soilless cultivation is expensive, but an increase in production and quality compensates for the higher costs incurred by soilless greenhouse cultivation (Allemand *et al*., 1995). Soilless cultivation of proteas is therefore considered economically viable in the south of France (Coetzee & Littlejohn, 2001).

AIR TEMPERATURE

Many proteas can endure a wide temperature range from as low as -5°C to as high as 45°C. The amount of frost damage depends on the species and age of the plant. Older plants produce inflorescences higher in the plant and can therefore escape the frost damage that would affect young and developing inflorescences. Mild frost will damage only the inflorescences and leaves, but the flowers will be unmarketable. In the south of France severe frost can occur (Montarone & Ziegler, 1997). Unfortunately, the *Proteaceae* indigenous to southern Africa are not sufficiently frost-resistant to tolerate the winter climatic conditions of southern France. Some proteas grow well in snow, such as *P. magnifica*, but at certain developmental stages the inflorescence can be damaged by snow.

High temperatures also can damage plants. Plants can survive temperatures as high as 45°C as long as a wind is blowing that can cool the plants. Windless days with temperatures reaching 35°C can, however, damage young growth (Eigenhuis, 1999a).

SOIL DEPTH

Soil preparation prior to planting depends on the soil type and depth (Coetzee & Littlejohn, 2001). Soils are generally ploughed to a depth of at least 60 cm (Malan, 1995a, b), but in some cases a depth of 1 m is recommended. The water table must be lower than 60 cm. It is advisable to plant proteas against a slope in order to prevent drainage problems (Eigenhuis, 1999a). In very shallow soils, ridging is recommended to improve the depth of soil available for plant growth. Ridging is also used to improve the drainage of heavy soils. In rocky soil or on steep slopes, no soil preparation is carried out.

Soil chemistry

Proteaceae originating from Australia and South Africa mostly grow on low pH (4.0–6.0), nutrient-poor, leached soils (Montarone & Allemand, 1995; Rebelo, 2001). Some members of the *Proteaceae* grow well on soils with a pH lower than 3.0 and others at a pH higher than 9.0, but the majority prefer soils with a pH range of 4.0–6.0 (Vogts, 1982; Montarone, 2001a). Soil pH has a direct effect on growth and root elongation (Yan *et al*., 1992). Growth response of plants in high pH solutions (pH 7) is significantly lower compared to plants grown in low pH solutions (pH 5.5). A high pH inhibits root growth and thus also affects shoot growth. Roots in high pH soils are poorly branched, and some roots are black and dead (Ganmore-Neumann *et al.*, 1997).

Root hair size and development is influenced by various environmental factors. Root hair development is inhibited in plants grown at high pH. This decreases the potential root surface area, which may lead to a decrease in mineral uptake (direct effect of pH), and decreases in exuding of organic acids to the rhizosphere (indirect effect of pH). Reduction of root growth at pH 7.0 could be attributed to the inhibition of cell elongation without affecting cell division.

'Safari Sunset' requires low pH in its rhizosphere for adequate growth, as root hairs develop only at pH lower than 6 (Ganmore-Neumann *et al.*, 1997). This cultivar was bred in New Zealand for the local acid soils. Growers in Israel, therefore, have encountered problems due to the high pH soils, high free-lime content, and in many cases, too high levels of phosphate (Ganmore-Neumann *et al*., 1997; Silber & Ben-Jaacov, 2001). Silber *et al*. (2001) found that fertilisation management affected the rhizosphere pH of 'Safari Sunset' plants grown in pots filled with tuff, controlled the rhizosphere pH and consequently improved growth. At high NH_4 application, the pH declined below pH 5, while at low NH_4 application, the pH rose above 7. Maximum yield was obtained when the rhizosphere pH was 6.0. Impairment of plant growth at low pH may result from H^+ toxicity or from the release of high concentrations of Al (aluminium) and Mn (manganese) from the substrate. At a high pH (> pH 7), plant growth was restricted by low availability of micronutrients. Despite the use of chelates, leaf-Fe, -Zn and -Mn concentrations were lower in shoots of NO_3-N-fed plants, than those in NH_4-N-fed plants grown at a lower pH (Silber *et al*., 2001).

The conclusion that the rhizosphere pH may be the most important factor affecting the development of 'Safari Sunset' plants is supported by the results of an experiment conducted in hydroponic systems. Plants grown in an aero-hydroponics system at pH 5.5 developed normally, while those at pH 7.5 did poorly. Root development of plants grown at pH 7.5 was restricted, resulting in inhibited development of root hairs, poor branching and root death. Whether pH affects plant development directly through physiological mechanisms that influence root hair formation, or indirectly through nutrient availability mechanisms, is not clear (Silber *et al*., 2001). To overcome these soil limitations, the rhizosphere conditions must be improved, and sensitive cultivars must be grafted onto resistant rootstocks (Silber & Ben-Jaacov, 2001). The breakthrough of culturing 'Safari Sunset' in Israel after many years of stagnation was mainly due to the ability to grow the plants in a trench filled with tuff, either in direct contact with the soil, or not (Avidan, 1996), together with the use of rootstocks, and/or employing nutritional management that reduces the pH (Silber & Ben-Jaacov, 2001).

The availability of some of the micro-elements in the growth medium is pH-dependent. High levels of Mn and Fe in the leaves are correlated with low pH levels in the growth medium (Ran *et al*., 2001). Silber *et al*. (2000) found that N, P, K, Zn and Mn concentrations were higher in a aero-hydroponic system, while that of Fe was lower in 'Safari Sunset' plants grown at low pH. Liming and adjustment of the macro- and micro-nutrient levels by fertilisation prior to soil preparation, to a depth of 1 m, is recommended (Coetzee & Littlejohn, 2001).

ESTABLISHMENT

The decision as to which establishment technique or system is to be followed, is purely a financial one, depending on the commercial value of the products being established. Furthermore, the success rate in the establishment of proteas in the field is variable (Malan, 1995a, b). Plants are planted from May until October (Southern Hemisphere). Earlier is usually better, and when plants are planted late in the year, irrigation must be available (Eigenhuis, 1999a).

Cuttings are generally transplanted within 4–8 months from setting, depending on the season of propagation and rainfall. The rooting bag is removed with as little root disturbance as possible, and the rooting medium is buried in the soil at planting. Polyethylene or organic mulches may be used to prevent burning the cuttings at the soil surface, to control weeds and to conserve water under dryland conditions. Success rates of 0–100% have been attained (Malan, 1995a, b).

Higher income products that are not yet cultivars or selections, and do not warrant high input costs, are still grown commercially from seed. Furthermore, large amounts of seeds of lower income dried-flower, foliage and cut-flower products, exhibiting little natural variation, are sown directly into prepared rows or lanes (Malan, 1995a, b). Seedlings may be transplanted directly from germination beds to the field at the 2–6-leaf stage, but are often transplanted earlier to nursery bags and grown for a further 6–9 months under nursery conditions. They are then established the same way as cuttings (Malan, 1995a, b).

PLANTING SPACE/DENSITY

Appropriate planting density is influenced by various factors, such as species type or cultivar, whether the land is dry or will be irrigated, soil type and type of machinery that will be utilised (Eigenhuis, 1999a). Hot, humid conditions are not well tolerated by *Protea* spp., so sufficient space for air movement between plants is important. High light intensity is required for healthy growth, so plants should not be overcrowded. The general recommendation is to use a between-row spacing of 3.5–4.0 m and a within-row spacing of 0.8–1.0 m, giving a plant density of 2 500 to 3 560 plants/ha. In practice, a much closer spacing, with plant densities of up to 6 000 plants/ha, is used by many farmers (Coetzee & Littlejohn, 2001). Standard plantations can be used for *Leucospermum* and single-stem *Leucadendron* spp., wide plantations for medium-size *Protea* and some *Leucadendron* spp., high-density plantations for *P. cynaroides*, and double-row plantations for *Leucadendron* spp. with a lignotuber, such as 'Safari Sunset' or *Lsp. cordifolium* 'Vlam', 'Goldie' and *Lsp. cuneiforme* (Eigenhuis, 1999a).

Fertilisation

It is assumed that the *Proteaceae* can grow almost without fertilisation (Buining & Cresswell, 1993), since most proteas grow in nutrient-poor soils in their native habitats (Rebelo, 2001). The South-Western Cape as well as South-Western and South-Eastern Australia are characterised by extremely low levels of total soil nutrients (Van Daalen, 1984). Those soils are characterised by pH < 6, N < 0.1% (w/w) and P < 0.02% (Specht & Moll, 1983).

Although proteas generally do not remove a large amount of nutrients from soils, after-harvest plants need to compensate for lost biomass and replenish their reserves. Harvesting stems for cut-flowers results in significant losses in biomass and consequently of mineral nutrients from the plants (Low & Lamont, 1986). Na, N, Ca, K and Mg were found to be the major elements removed by harvesting of *Leucadendron* stems (Cecil *et al*., 1995). Under certain circumstances, the nutrient capacity of the soil is not sufficient to replenish these nutrients, especially in highly leached, acidic, sandy soils. In the 1980's, Claassens (1986) demonstrated that yield increases occurred with increased nutrient supply. Therefore, supplementation of mineral nutrients is important to maintain production. *Proteaceae* are adapted to nutrient-poor soils (Montarone & Allemand, 1995) and have a narrow range of optimal concentrations of

mineral elements (Cramer, 1999). Fertigation techniques must be developed specifically for protea cultivation, based on an understanding of the nutritional requirements of the crop, in order to produce the highest quality cut-flower possible (Parvin, 1986).

Although extensive research on nutrition of *Proteaceae* has been conducted since the pioneering work of Van Staden (1967), the nutritional requirements for *Proteaceae* plants remains a complex problem, and much still needs to be clarified (Vorster, 1984; Claassens, 1986; Prasad & Dennis, 1986; Cramer, 1999; Montarone, 2001a).

PROTEOID ROOT SYSTEM

The most striking adaptation of the *Proteaceae* to the nutrient-poor soils on which they grow is the presence of proteoid roots, first described by Purnell (1960). Every species thus far examined in the genera *Protea*, *Leucospermum* and *Leucadendron* has a double root system consisting of proteoid and normal roots (Lamont, 1982, 1983a). Either one of these root systems may contribute 80% of the total root dry weight at any one time, but this varies between individual plants and different plant species, and with plant age, season, soil type and location of parent roots in the soil profile (Lamont, 1983a). Proteoid, short life roots have a vital role in nutrient and water uptake by the plant, especially during the growing season (Lamont, 1986). They enable the plant to extract soil phosphorus (Lamont, 1982), nitrogen and potassium efficiently (Vorster & Jooste, 1986a, b).

Proteoid roots are dense clusters of rootlets. Individual roots vary in length from a few mm to over 10 cm in length and may consist of hundreds or even thousands of hairy rootlets (Lamont, 1986). They increase the root surface, thereby increasing the absorption and exudation area of the root system (Hofer, 1995). The surface area to weight ratio of proteoid roots is almost 15 times that of the normal roots in *Lcd. laureolum* (Lamont *et al*., 1984). This structure provides greater access to soil particles and shortens the diffusion path for nutrients (Lamont, 1983b; Lamont *et al.*, 1984).

Seedlings usually possess some proteoid roots by the end of their first growing season (Lamont, 1972), and as the plant ages, they make up an increasing proportion of the annual growth of the surface root system. They tend to be concentrated in a thick band at the soil surface (Lamont, 1973; Lamont *et al*., 1984). When decomposing litter is present, they are even more concentrated in that substrate than in the humus-containing layer beneath. The use of black plastic as a soil covering in cultivated proteas has the disadvantage that it stops the proteoid roots from growing up into the litter as it accumulates on the surface. Furthermore, rotavating the soil, especially close to the plant, could be lethal since the most important part of the root system is in danger of destruction. On the other hand, control of weeds is important, since their shallow roots will actively compete with proteoid roots.

Proteoid roots are more concentrated underneath the plant's canopy as compared to outside the canopy (Lamont, 1973), but can extend for much greater distances (Lamont, 1986). In seasonal climates, proteoid root production is seasonal, as it is tied to growth of the surface parent roots (Lamont, 1976, 1983a). Their formation is enhanced by the presence of organic matter (Lamont, 1973; Lamont *et al*., 1984), soil bacteria (which do not invade the root) and moisture (Lamont, 1986). They readily form in localised wet pockets of soil, even during summer when the rest of the surface root system is dry (Lamont, 1976). Greater amounts of water will induce new root growth, including proteoid roots, although waterlogging conditions and drought will suppress formation (Lamont, 1986). Absence of proteoid roots implies increased susceptibility to drought, for a given shoot weight is supported by a much weaker root system than in plants with proteiod roots (Lamont, 1986).

Lamont (1986), Heinsohn & Pammenter (1986) and Claassens & Folscher (1980) have shown correlations between levels of nitrogen, potassium and phosphorus and the maintenance of a proteiod root system that is responsible for good plant growth. High nutrient levels in the soil, especially phosphates, inhibit the formation of proteoid roots in many of the *Proteaceae* (Grose, 1989; Silber *et al*., 1997). Lamont (1986) showed a negative effect of high phosphorus at any level of nitrogen on proteiod root development. In the presence of sufficient ion concentration, no proteoid roots developed in 'Safari Sunset' (Silber *et al*., 1997). Proteoid root production also decreases as the proportion of clay to sand is increased (Lamont, 1986), and there is no doubt that the presence of abundant proteiod roots is a sign of a healthy plant, whether in a pot or in the field. Proteoid roots develop where nutrient supply is highest within the soil profile, but they are absent altogether when the total nutrients exceed some critical level (Lamont, 1972).

In contrast, Silber *et al.* (1997), found that proteiod roots were abundant only in undeveloped plants exposed to a poor nutrient solution, and were absent in properly developed plants provided with sufficient nutrients.

PHOSPHORUS

The general belief is that proteas are sensitive to high levels of phosphorus (P) (Nichols *et al*., 1979; Claassens, 1981; Malan, 1995a, b; Montarone & Ziegler, 1997). In Israel, various growth disorders were attributed to high soil P content and therefore, cultivation was restricted to soils having low P concentrations (Silber *et al*., 1997). It was demonstrated that by increasing P concentration in the nutrient solution, a decrease of total and root fresh weight of *Leucadendron* 'Safari Sunset' occurred. The effect was stronger in plants grown at a low pH (Ganmore-Neumann, *et al*., 1997). Montarone (2001a) also reported phosphorus toxicity in

very young plants grown in soilless conditions at concentrations above 4 ppm. Growth reduction at high P levels was reported for *Proteaceae* plants by Nichols *et al.* (1979), but extremely high soluble P levels were used (Silber *et al.*, 1997).

In contrast, Silber *et al*. (1997, 2001) found that at very low initial levels of P, increases in P concentration improved plant growth of 'Safari Sunset' significantly, and this indicated that 'Safari Sunset' was not sensitive to a high P level in the nutrient solution. Increasing the concentration of P in irrigation water to 20 mg/L significantly increased the leaf-P concentration and improved the yield of plants grown in pots filled with tuff. The same trend was observed in an open-field experiment. At low levels of available P, growth was inhibited (Silber *et al*., 2001). These results are in agreement with those of Prasad & Dennis (1986) for 'Safari Sunset'. Prasad & Dennis (1986) reported that values of available P > 25 ppm were satisfactory for 'Safari Sunset' grown in containers. In Israel too, an irrigation solution with 20 ppm of P resulted in growth of 'Safari Sunset' without any indication of toxic symptoms attributed to high phosphorus (Montarone, 2001a).

The above findings contradict the general view that species of *Proteaceae* are sensitive to high P concentrations (Silber *et al*., 1997). Silber *et al.* (1997), however, did find that *Leucadendron* plants were sensitive to phosphate levels in excess of 40 ppm in the soil, especially if in combination with a soil pH higher than 6.5. Amendments added to the soil reduced soil pH and phosphorus uptake, but no yield responses attributable to this change in pH occurred (Prasad & Dennis, 1986). Although the general recommendation has been to refrain from applying large amounts of phosphates (Coetzee & Littlejohn, 2001), it is clear that sufficient P must be available. It is advisable to send soil samples to a laboratory to determine soil P levels so that levels can be amended appropriately.

Vorster & Jooste (1986a) showed that potassium and phosphate are more effectively absorbed when proteoid roots are present. Phosphate absorption by proteoid roots peaks at a pH between 4.0 and 5.5. However, Vorster and Jooste (1986b) showed that proteoid roots might cause a temporary sink for potassium and phosphate, since these elements are more readily translocated from ordinary roots. It is possible that an energy-dependent mechanism might be involved in the translocation of ions from roots to the shoot.

NITROGEN

Nitrogen (N) appears to be a nutrient of particular importance in protea cultivation. More favourable growth is always associated with increasing N supply, particularly NH_4. However, considerable differences can be expected in growth responses to nitrogen levels between species and even within the same species collected from different sites.

Nitrogen is available in several forms, mainly as NH_4 and NO_3. Relatively high concentrations of NH_4 can be tolerated and the addition of sodium (Na) to the nutrient solution allows for toleration of even higher NH_4 concentrations. Some species can use NO_3 to some extent, but NH_4 is in most cases preferable. In one study, plants receiving only NO_3 were chlorotic and had very little flower bud development, suggesting that NO_3 is not an ideal source of N. Generally, yields are higher with a combination of NH_4 and NO_3 than with NO_3 alone (Claassens, 1986). *Leucadendron* 'Safari Sunset' grows best with a high NH_4/NO_3 ratio in the solution (Ganmore-Neumann *et al*., 1997) as was reported also for *Lcd. salignum* in water culture (Heinsohn & Pammenter, 1986). The general recommendation is to use fertilisers in which less than 50% of the nitrogen is bound in nitrates (Coetzee & Littlejohn, 2001).

Silber *et al*. (2001) found that lowering the NH_4 : NO_3 ratio in irrigation water, increased the pH and reduced the number of marketable branches of 'Safari Sunset'. The phosphorus concentration in the shoots increased significantly as a result of reduction of the NH_4 : NO_3 ratio. The addition of P to the irrigation water resulted in increases in both the N and P concentration in the shoot tissue, indicating that P deficiency probably restricted N uptake by the plants.

NUTRIENT BALANCES

Claassens (1986) pointed out that, under natural conditions, plants could tolerate relatively high levels of nutrient elements as long as one element was not present in an excessive quantity. Excessive availability of nutrients can lead to either luxurious production of vegetative growth with no flower production, or, in severe cases, death (Malan, 1995a, b). Plants die in high salt concentrations, but relatively high total salt concentrations can be tolerated if the P concentration is within acceptable limits (Claassens, 1986).

Accessibility of nutrients is related to soil-physical characteristics such as the adsorption/absorption ability of soil particles, quantity of the absorbing particles, organic content of the soil, and inherent status of the soil nutrients (Malan, 1995a, b). Because of all these variables and the fact that there is variation in uptake ability in different species, it is difficult to understand and generalise about nutritional requirements and balances in production of *Proteaceae* (Montarone, 2001a).

SOILLESS CULTURE AND NUTRIENT REQUIREMENTS

Montarone (2001b) defined a basic nutrient solution that seems to have appropriate levels of macro-elemen for soilless *Proteaceae* culture, although the quantities of the micro-elements supplied are empirical. Montarone & Ziegler (1997) clearly established the water and mineral requirements of *P. eximia* and *P. cynaroides* grown in soilless culture by analysing nutrient samples regularly in order to establish absorption kinetics. Nitrogen, phosphorus, potassium, calcium and magnesium

were analysed. A period of low absorption corresponded partly to the settling down of the plant, as well as to a period of low seasonal activity (very little light and heat). The absorption concentration of each element confirmed the existence of two distinct periods. The first corresponded to a growing period with a relatively high absorption concentration, and the second coincided with a pre-flowering period during which absorption concentration was lower. Growth rates mainly affected the volume of intakes and not the relative composition of the intake. The quantities of elements absorbed by a plant increased during its vegetative cycle, and varied not only according to the plant developmental stage, but also depending on the species and the required growth form (cut-flowers, mother plants or pot plants). Furthermore, it is well-known that there is a close link between the amount of water and minerals absorbed by a plant. The dry-matter produced has a relatively constant mineral concentration (Montarone & Ziegler, 1997).

These results enabled the establishment of the N, P, K fertilisation balance, as well as optimising the K, Ca and Mg proportions for several species. Great differences in fertilisation balance requirements between species were observed during the various developmental stages, due to species having different growth kinetics. Results indicated that the nutrient solution did not correspond to the growth requirements of the plants. Differences were observed for cultivars belonging to the same genus. In view of the number of species and varieties, no single solution can be recommended, but one must avoid limiting conditions for water and mineral elements. Keeping soilless nutrition at the optimum therefore requires a careful monitoring of drainage rates and mineral drifts in the leachates of each species (Montarone & Ziegler, 1997; Montarone, 2001a).

The solution naturally present in the soil is very dilute in comparison with artificial nutrient solutions, but it is constantly renewed by the exchanges with solid particles such as clay. Mineral elements for *Proteaceae* must therefore be supplied in large quantities but in a diluted solution. Since plants selectively absorb, at low concentrations, the essential elements from a medium, plants should be supplied with sufficient mineral elements (Montarone, 2001a).

Lastly, high salinity in the medium and an increase of pH from 5.5 to 9.0 resulted in chlorosis and leaf reddening of *P. eximia*, which could result in a complete drying of the plant. The undesirable effects of salinity could be eliminated by successive leachings in a well-drained medium (Montarone, 2001a).

LEAF AND SOIL SAMPLE ANALYSIS

It is important to be able to determine at which nutritional level the plant will give optimal productive growth. Standards, therefore, need to be established, to monitor these levels through leaf analysis. Once the optimum leaf nutritional levels (physiological age, shoot position) have been established, fertilisation requirements related to soil and fertiliser types, can be investigated. Annual leaf and soil sampling can be used together to maintain optimum nutrient availability (Malan, 1995a, b; Barth *et al*., 1996).

Some workers tried to correlate the levels of the elements in the leaves to the plant's general status and yield (Ran *et al*., 2001), but the use of tissue and soil samples to determine the seasonal nutritional requirements, has not been entirely successful. No consistent trends were apparent for green and chlorotic leaves, except for calcium and manganese that showed a significant decrease in levels from green to chlorotic. Analysis of protea material does not always reflect deficiencies or toxicities, even when symptoms are evident on the leaves (Parvin, 1986). Between type/variant/seedling variation is often higher than variation leading to nutritional abnormalities within types, making optimum level determination extremely difficult when working with non-clonal material (Malan, 1995a, b). Furthermore, seasonal differences, interplant differences in the cycle of growth flushes, differences in leaf position and between flowering stems, leaves, axillary shoots and blooms, make interpretation of leaf samples difficult (Maier *et al*., 1995; Barth *et al*., 1996). Possible synergistic and antagonistic interactions between nutrients also need to be considered when interpreting plant nutrient data (Coetzee & Littlejohn, 2001).

Since 'Safari Sunset' is a woody perennial bush, which needs fertilisation during most of the year (Avidan, 1996), it can be compared to other orchard crops, in which leaf analysis is used as a tool to determine fertilisation regimes. Ran *et al*. (2001) tried to encourage growers to use leaf analysis, together with soil sampling, to plan their fertilisation regime and to correct deficiencies during the growing season. However, Ran *et al*. (2001) did not find any positive correlation between levels of either macro- or micro-elements in the leaves, and the yield. Furthermore, Ran *et al*. (2001) found that an estimate of the desired levels of macro-elements could be used as a tool to verify the fertilisation regime for 'Safari Sunset'. The general status of the plants and leaf colour were in good agreement with the level of elements in the leaves. The pH of the growth medium is an important factor in the availability of micro-elements. Leaf samples submitted for analysis should be accompanied by growth medium samples. While planning the fertigation regime, the age of plants and the estimated yield should also be taken into account (Ran *et al*., 2001).

NUTRIENT RESERVES AND MOUNTAIN CATCHMENT MANAGEMENT

Proteaceae in the winter rainfall Fynbos areas of South Africa and Australia exist under extremely low nutrient conditions. There is concern that intense wild flower picking and frequent veld burning may severely deplete community nutrient reserves, since *Proteaceae* make up

a large percentage of the community mass and nutrient pools (Low & Lamont, 1986). Near Kleinmond in the Western Cape of South Africa, Slingsby & Bond (1980) have shown that post-fire regeneration of *P. compacta* was severely reduced where intensive picking of live heads had previously occurred. Low & Lamont (1986) argued that heathland systems may be partly 'leaky' due to an extremely poor colloidal status, and that leaching of nutrients after fire is exacerbated when heavy rainfall follows the fire (Low & Lamont, 1986).

Mountain catchment areas must be managed to ensure an optimum sustained yield of high-quality water through maintenance of the vegetation. Wild flower picking and unplanned fires are negatively affecting sustained yields. Unplanned fires burn 60% of the mountain areas, and heavy picking followed by unplanned fires can result in very poor regeneration. Further possible detrimental effects of flower picking in natural vegetation in catchment areas include serious depletion of seed stores, trampling and erosion, mortality through serious damage to individual plants, the spread of diseases such as *Phytophthora*, and a drain on nutrient pools from the ecosystem. Wild-flower picking should therefore be discouraged in favour of cultivation (Van Wilgen & Lamb, 1986).

Fires are common in the Cape Mountains of South Africa, and many species of *Proteaceae* rely on canopy-stored seed reserves to regenerate after fire. Picking intensities should therefore be at a level that will leave sufficient seed to provide for adequate regeneration. The problem of determining the level of seed necessary to ensure adequate regeneration following fire is complicated by season or fire effects. There can be more than a three-fold difference in the number of viable canopy-stored seed depending on the time of year (Coetzee, 1984; Bond, 1985), but there are often 10–100 times more seedlings per parent plant following autumn fires when compared to spring fires (Bond *et al*., 1984). *Protea* species are highly variable with regards to the development time of seeds (Vogts, 1982). Data showed that 50–75% or more of available seed comes from the current crop of flowers, depending on the species. An unplanned fire following heavy picking will result in low seed numbers despite some flowers being left from previous years. Fire season may, therefore, have more effect on populations of serotinous *Proteaceae* than the level of flower picking. The period of maximum seed availability and of maximum seedling recruitment coincides with the period when most fires occur in South Africa (January–March) (Van Wilgen & Lamb, 1986).

Since post-fire regeneration levels of Cape *Proteaceae* depend on the size of pre-fire seed stores, Midgley & Vlok (1986) suggested the most favourable fire season for *Leucadendron* species to be based on seed maturation data. Flowering time is mainly spring–summer in the east and winter–spring in the west (South Africa) (Williams, 1972), so the seed maturity period is also earlier westward. The study by Van Wilgen (1984), who used a climate model (Midgley & Vlok, 1986) for his predictions, indicated that the season of burn will not necessarily occur at the time most favourable for Proteaceae. Adequate fire control for natural areas must be implemented, but sustained yields in the long term will be best achieved from cultivated plantations of proteas (Van Wilgen & Lamb, 1986).

Irrigation

RAINFALL

Proteaceae grow naturally in areas receiving 250 mm of rain per season to as high as 2000 mm per year. The prevailing rainfall in an area influences the different types of proteas that can be cultivated (Eigenhuis, 1999a). Some protea species grow in both summer and winter rainfall areas, while others such as *P. magnifica* will only grow well in the winter rainfall areas (Eigenhuis & Eigenhuis, 1999). The main root growth period of *Protea* is in winter. *Protea* species and hybrids used in cultivation will, however, tolerate dry summer periods. Although sensitivity to a lack of water during the winter varies, this winter sensitivity partly explains why winter rainfall variants have difficulties adapting to summer rainfall cultivation (Malan, 1995a, b; Coetzee & Littlejohn, 2001).

WATER REQUIREMENTS

Water requirements for most *Proteaceae* have not been determined (Criley, 1998). Although originating in semi-arid habitats, the water needs of *Proteaceae* are far from insignificant. Furthermore, water plays an important role in *Proteaceae* nutrition. By increasing the leaching rate through more frequent irrigations, the salinity is corrected and the risks of toxicity are reduced. Solution transfer also allows the mineral content in the soil solution to be renewed, and avoids local depletion of some minerals in the medium when the plant demand exceeds the nutrient availability.

General guidelines are almost impossible since such large variation exists between plants, soil types and environmental conditions utilised in the cultivation of proteas, as well as the enormous natural inherent variation that exists between and within species. Guidelines were in the past primarily based on information obtained from other crops. Seasonal water consumption (crop factor) should be determined for each cultivar (Malan, 1995a, b) and irrigation recommendations should be cultivar-specific.

Montarone & Ziegler (1997) indicated that the water requirements of the different protea species grown under soilless conditions differ. Water requirements can be established by knowledge of where species grow naturally. Species growing in wet areas or near water sources have, for example, higher water requirements than species preferring dry areas (Manders & Smith, 1992). For example *P. cynaroides* has a water requirement 2.5 times that of *P. eximia* in soilless conditions

(Montarone & Ziegler, 1997). In nature, *P. cynaroides* grows in moist conditions whereas *P. eximia* grows on drier, well-drained slopes.

Environmental temperatures and the occurrence of mist in the area also influence the water requirements of cultivated proteas. High temperatures result in high evaporation. Proteas are therefore found naturally on cooler slopes. Where proteas occur at high altitude on mountain slopes it is possible for the plants to utilise moisture from the mist. Furthermore, the water-holding capacity of soil where proteas are found varies. Some proteas are only found in soils with a good water-holding capacity (Eigenhuis & Eigenhuis, 1999). However, species of *Protea* will not grow under water-logged conditions (Vogts, 1989).

Van Zyl *et al*. (1999) proved that maintenance of high soil water capacity is essential to the field survival of rooted cuttings of *Protea* 'Cardinal'. Active consumption of water continued throughout the year and maintaining a high level of soil moisture increased the shoot lengths and biomass production on 'Cardinal' in comparison with lower soil water levels. *Leucadendron* plants in cultivation use water throughout the year (Van Zyl *et al*., 1999), although the soil water depletion levels appear not to influence the shoot length (Littlejohn & Robyn, 2000).

Trials were conducted at Stellenbosch in South Africa to determine the water requirements and to observe the effect of irrigation on different types of *Proteaceae*. Each cultivar was subjected to four different irrigation treatments. Leaf water potential values showed that plants under dry land cultivaton and irrigation at 60% depletion of plant-available water (PAW) experienced higher water stress from February until April. Irrigation of *Protea* at 20% depletion of PAW resulted in significantly longer shoots compared to drier treatments. Irrigation at 20% PAW depletion, furthermore, resulted in the highest percentage success in the establishing of young *Protea* plants. Irrigation at 20% PAW also resulted in the longest shoots in the case of *Leucospermum*, and vase life of *Leucospermum* was significantly enhanced. Different soil water depletion levels did not have a significant effect on the shoot growth of young *Leucadendron* plants. Both *Lcd. laureolum* and *Lcd. salignum* typically grow in sandy soil. A strong sclerophyllic nature (hard thickened leaves) that prevents excessive water loss, may therefore explain the insensitivity to soil water depletion (Eigenhuis & Eigenhuis, 1999). Thus *Protea* and *Leucospermum* respond very positively to a sufficient supply of water, producing better quality blooms, while *Leucadendron* appears to be less responsive to soil moisture levels.

Commercial cultivation has shown that irrigation of proteas does, under certain conditions, significantly improve establishment, and increase the quality and quantity of production of many varieties (Malan, 1995a, b). The correct irrigation scheduling will ensure longer stem lengths and improved vase life. Plants will have a lower stress factor, will be less prone to diseases, and will establish better in soil. High-value cultivars will benefit from irrigation to maximise income per ha. However, irrigation could be uneconomical on low-income species or cultivars in full production when water availability is limited (Eigenhuis & Eigenhuis, 1999).

Care must, however, be taken not to over-irrigate since increased growth often causes axillary shoots to grow beyond the flower in some varieties such as *Lcd. salignum* and *P. repens*. These flowers are much more expensive to prepare for export or may be totally unmarketable (Malan, 1995a, b). Furthermore, excessive irrigation increases the risk of root diseases, especially those caused by *Phytophthora*.

IRRIGATION SYSTEMS

Factors such as crop potential, type of production system, the availability of water, the extent of weed infestation and type of soil, determine the irrigation system that is utilised (Malan, 1995a, b). Drip irrigation is the preferred method of supplying water to proteas. Water is used more effectively, leaf and stem diseases are not promoted, weed growth is partially limited and the application of dissolved fertilisers can be controlled more accurately (Eigenhuis & Eigenhuis, 1999).

Overhead irrigation is not suitable as it increases the possibility of foliar and flower diseases, and large droplets can cause physical damage to the flower heads and leaves (Eigenhuis & Eigenhuis, 1999; Coetzee & Littlejohn, 2001). Furthermore, much water is wasted during overhead application, and weed growth is stimulated. Micro-irrigation also promotes disease incidence, and loss through evaporation is significant (Eigenhuis & Eigenhuis, 1999).

IRRIGATION SCHEDULING

Scheduling of drip irrigation will be determined by the soil type. On clay soils, longer periods of irrigation are recommended in contrast to sandy soils where short, frequent pulses of water are more effective. Soil testing must be done prior to determining the irrigation period, since different soil types have different water-holding capacities. Soil moisture content can be determined using neutron probes, tensiometers or by gravimetric methods (Eigenhuis & Eigenhuis, 1999).

The highest leaf diffusive conductance, net photosynthesis and production of dry and fresh matter of *P. eximia* seedlings in soilless culture were correlated with the highest watering frequency, and *vice versa*. However, not even the highest watering frequency could prevent a gradual decrease in leaf diffusive conductance throughout the day. This can be attributed to the porphyry's low water retention capacity or the insufficient frequency of irrigation (Urban *et al*., 1995).

Optimum irrigation scheduling delivers the desired stem growth and quality, while water is saved, making effective irrigation scheduling economically viable (Eigenhuis & Eigenhuis, 1999).

Training and pruning

In the past it was believed that proteas should not be pruned during their pre-production years, to allow the plant time to establish itself. This may be applicable to plants growing in very dry, poor, deep, sandy soils, where it may take up to two years for significant above-ground growth (Malan, 1995a, b). However, training and pruning can greatly reduce the period to first production, improve plant architecture, and lengthen the period of plant productivity. Training and pruning can also balance the quality and quantity of saleable flowers produced per plant (Brits *et al.*, 1986; Malan, 1995a, b). The aim of pre-flower production pruning is to develop a complex structure of branches with sufficient nutrient reserves to support the growth of the flowering stems. Pinching (removal of apical buds) is done to increase the complexity of the plant.

The high cost of establishing proteas under intensive cultivation has necessitated young plant management to attain an economically viable crop as soon as possible after planting. Plants should be pinched and topped to increase the vegetative complexity of the plants during the first growing season (Hettasch *et al*., 1997). Adequate complexity is achieved within 1–2 years, depending on the cultivar or species (G. Jacobs, Department of Horticulture, University of Stellenbosch, South Africa, pers. comm., 2002).

Under conditions where the plants grow slowly, annual pruning is sufficient, but in warmer areas where plants grow faster, pruning will be required two to three times a year during the first two years (Coetzee & Littlejohn, 2001). The harvesting of flowers also represents a pruning action. Quantity of shoots is controlled during annual harvesting through planning the number of subsequent shoot production sites available per plant. The quantity and quality of shoots produced on every bearer is dependent on inherent plant characteristics, plant vigour, time of pruning, and the quality of the bearer. Pruning recommendations, therefore, should be species- or cultivar-specific (Malan, 1995a, b). Bushes in production must be pruned to leave bearers of sufficient length for the following crop during the harvest of flowering stems. It is important to leave a long enough bearer on the bush but the exact length of the bearer required differs from cultivar to cultivar; in general, 15–20 cm is a good length. New shoots developing from the lateral buds of the leaf axils on the bearers provide the next harvest of flowering stems. The time from harvesting flowers in one season, leaving a bearer, to the following harvest from lateral shoots that have developed from that bearer, varies. It can be as short as 11 months to as long as 34 months depending on the cultivar, the time of pruning (or harvesting the flowering stem), the light intensity, water supply, nutrients available and temperature during the growing season (Anon., 2000c). Additional pruning to remove unwanted vegetative stems is also required (Coetzee & Littlejohn, 2001).

LEUCADENDRON

Growth habit

The plant growth form ranges from sprawling ground-hugging types, such as *Lcd. salignum*, to large trees such as *Lcd. argenteum*. Male plants are normally more branched, slightly larger and bear smaller leaves and inflorescences than female plants (Littlejohn & Robyn, 2000). The commercial types can be divided into multi-branched foliage and single stem cut-flower products, but most growth habits in between are also represented. Training is essential to shorten the pre-production period and to control the quality vs. quantity of production. The very complex *Leucadendron* plants (*Lcd. conicum*, *Lcd. coniferum*, *Lcd. floridum*) do not respond well to extreme pruning practices controlling their architecture. This may be due to their axillary sites being spent as a result of poor apical dominance and weak sylleptic shoots present on retained bearers taking more than one season to assume dominance (Malan, 1995a).

Single-stem products exhibit a simplistic growth habit with strong apical dominance, and mostly also strong apical control. Training is mostly only designed to prepare an acceptable production structure for the plant. During harvesting, sufficiently long flowering shoots are all cut back, leaving a 10–15 cm stump with shorter flowering shoots harvested with thinning cuts. Later in the plant's productive life, thinning cuts may also be introduced with longer shoots to increase average shoot length per plant. Weak non-flowering shoots are retained, as they will produce the subsequent season's early crop (Malan, 1995a).

Flower initiation

During autumn, sprouting, but not elongation, of axillary buds can be induced by pruning *Leucadendron* spp. The ability of the apical meristem to initiate an inflorescence depends on the date when elongation stops and/or the shoot length at that time (Wallerstein & Nissim, 1992). This is probably the key to the control of the flowering period. The fact that axillary buds of *Leucadendron* can sprout, but cannot elongate, during the short days of autumn is probably an indication to the positive effect of long days on shoot elongation, as is the case in *Leucospermum* (Wallerstein, 1989). However, *Leucadendron* species flower from May to November, indicating different mechanisms for flower initiation (G.M. Littlejohn, pers. comm., 2002).

The earlier the time of growth cessation, the longer was the maximal shoot length reached and the higher was the stage of the inflorescence development at the beginning of February. *Leucadendron* 'Safari Sunset' plants that were pruned later than June failed to flower during the following spring (Wallerstein & Nissim, 1992). Different species of *Leucadendron* flower at different times of the year (Rebelo, 2001), but the most widely used cultivars flower during the late winter and

early spring (Littlejohn, 1997). Flowering time is not influenced by pruning manipulation and appears to be a genetically fixed characteristic (Littlejohn & Robyn, 2000).

Control of flowering

In many plants, including *Protea* (Hettasch *et al*., 1997), soft pinching is commonly used in order to increase the number of shoots on a stem. While the normal result of pinching is more shoots per branch, sometimes those shoots are of low quality. Soft pinching of 'Safari Sunset' enhanced branching and increased the number of export quality stems per plant. Pruning, however, yielded a much lower number of stems. The pinching treatment was carried out by removing the top 3–5 cm of the shoots with 2–3 pairs of fully spread leaves. The timing of pinching was crucial for obtaining large numbers of high-quality stems. In Israel, pinching in April–May (spring) under mild temperatures produced the best results, yielding 2–4 more branches per pinched stem. Later pinching decreased the quality by increasing spray branches, short branches and branches showing a 'witches broom-like' syndrome. The diameter of the branch affected the quality of the new stem. It is important to pinch branches that are at least 10 mm in diameter. This technique has increased production from 200 000 stems per hectare per year to 400 000–700 000 stems per hectare per year. Pinching at the right time increased the yield by 45%. Pinching can also be used to lengthen the marketing season. If done on part of the plot, the crop from pinching is obtained later than the unpinched part of the plot (Wolfson *et al*., 2001).

LEUCOSPERMUM

Growth habit

The genus *Leucospermum* with its 48 species is confined to southern Africa. They are evergreen, woody, perennial plants and their size and habit ranges from mat-like prostrate plants with trailing stems, to 50 cm tall, erect shrubs, to small trees (Rourke, 1972; Littlejohn, 2001a).

Shoot length can be manipulated by cultural practice. A single spray with gibberellic acid (GA_3) at 500 mg/L, 10–12 weeks after pruning results in a marked increase in the number of shoots longer than 30 cm. Longer shoots can be obtained with higher and/or more GA_3 applications. The increase in shoot length results primarily from the increase in node number (Malan & Jacobs, 1992). Napier *et al*. (1986b) reported that both internode length and number of internodes are increased. Shoot diameter appears to be unaffected by GA_3 applications, while the dry weight/cm shoot, and dry weight and area of individual leaves are reduced. GA_3 is effective only when the plants are in the vegetative phase and when conditions for shoot growth are favourable (Napier *et al*., 1986b). It has been demonstrated that one application of benzyladenine, applied early in the flower induction phase, increased both reproductive shoot diameter and the number of florets per capitulum, thereby increasing the flower quality (Napier *et al*., 1986a). Both BA and GA_3 can be used to advance the sprouting of new shoots in early spring, but neither BA nor GA_3 can be used to accelerate the development of the inflorescence in *Leucospermum* (after inflorescence initiation), since applications result in abortion of inflorescence buds and poor quality flowers (Wallerstein, 1989; Wallerstein & Nissim, 1992).

Flower initiation

Leucospermum plants are short-day flowering plants, and flowering can be prevented by exposure to artificial light to extend natural day-length or as a night-break treatment (Malan & Jacobs, 1990; Gerber *et al*., 2001). Apparently, flower initiation in *Leucospermum* takes place in response to high light intensities during the vegetative phase and in conjunction with short days (Jacobs *et al.*, 1986; Wallerstein & Nissim, 1992). Long days influence the rate of vegetative growth before inflorescence initiation and activate the axillary buds, so that further initiation can occur under short-day conditions (Wallerstein, 1989; Wallerstein & Nissim, 1992). Heavy shading during the high light intensity period of summer prevents flower initiation (Jacobs, 1983, 1985). Shade and late removal of the primary inflorescences adversely affect the quality of secondary inflorescences of *Leucospermum* 'Red Sunset'. It is possible that the higher temperatures of early summer, in conjunction with shading, are more detrimental to flower quality than shading applied in winter to primary inflorescences when temperatures are low (Jacobs, 1983).

Shoots of *Leucospermum* grow during summer and elongation stops in late summer (Gerber, 1999). Inflorescence initiation takes place during autumn, inactive axillary buds are present during winter, and flowering takes place in early spring. Control of the flowering period could be divided roughly into two major processes: control of inflorescence initiation and control of further flower development (Jacobs, 1983; Wallerstein & Nissim, 1992). Malan and Jacobs (1987) concluded that shoot growth cessation is not a reliable indicator that the plants had reached an induced state for reproductive development. Cessation of shoot elongation certainly seems implicated, but the question of whether it is a necessary condition is not clear (Criley, 1998).

In *Leucospermum* 'Red Sunset', the induced state for flower initiation has been attained by the first half of April (Jacobs, 1983; Napier, 1985). Applying growth regulators as a single spray did not advance or delay the onset of the induced state (Napier, 1985). Flowers are initiated only on shoots that have stopped elongation growth, a factor brought on by stress, unfavourable shoot/root ratio, or plant maturation (Jacobs, 1985). Long days delay the onset of the induced state (Jacobs *et al.*, 1986). After shoot growth stops, the upper axillary buds enlarge, producing bracts without floret initials. These bracts make up the involucral bracts, which

cover the peduncle (Criley *et al.*, 1990). In response to the shortening days of late summer/autumn and high light intensities, flowers are formed on these shoots at about the same time each year, although the exact time of flower initiation is species/cultivar-dependent (Jacobs *et al.*, 1986; Malan & Jacobs, 1987; Gerber, 1999). Floret differentiation is normally completed by early winter, and a rapid enlargement phase follows, leading to anthesis in late winter or early spring (Criley *et al.*, 1990). The time needed to complete flower development, which occurs through winter, varies between cultivars, and the rate of development depends on the climatic area in which the plants are grown. Both cultivar and area, therefore, affect the harvest time (Gerber, 1999). Flower initiation in *Leucospermum* (Malan & Jacobs, 1990) can therefore be manipulated through daylength control. For example, flower initiation is induced by short days in *Leucospermum* 'Red Sunset' (Malan & Jacobs, 1990).

According to Jacobs (1980), the change from the vegetative to the reproductive phase growth takes place during a relatively short period for long shoots. Disbudded plants lose their capacity to re-initiate an inflorescence during the short days of winter, a condition which may be more related to light energy relationships than photoperiod (Jacobs, 1983, 1985; Criley *et al*., 1990). Jacob's (1985) concept that inflorescence initiation was not a response to photoperiod, as it does not occur during the long days of summer, and the induced state is lost during short days of winter, changed as new evidence mounted. Since the transition from vegetative to induced state occurred at the same time every year, Malan and Jacobs (1987, 1990) suggested that the photoperiod might play a key role in the induction of *Leucospermum*. The low level of light energy needed to prevent initiation also argued the case for the participation of photoperiod in the process. Jacobs and Minnaar's (1980) observations of simultaneous reproductive development also supported the idea of a photoperiod switch.

Furthermore, a linear relationship was found between mean daily temperature and rate of secondary flower development in *Leucospermum* (Jacobs & Honeyborne, 1979; Wallerstein, 1989). The rate of flower development in *Leucospermum* is hastened by elevated temperatures (Jacobs & Honeyborne, 1979). In *Lsp. cordifolium* 'Vlam', only involucral bracts were found at a bud size of 7 mm. Floret development began in mid-October (Northern Hemisphere) as the bud length reached 10 mm. Floret development proceeded slowly through December and more rapidly in January until anthesis was attained in February (Criley *et al*., 1990).

Control of flowering

The short natural flowering period of *Leucospermum* plants causes a major cut-flower marketing problem (Brits *et al*., 1983). Marketing prospects of non-European cut-flowers to Europe fall essentially in the European winter months (November to March) (Hörmann, 1987). The flowering time of *Lsp*. *cordifolium* and hybrid clones of *Leucospermum* grown commercially is restricted to a short period during spring. In the Southern Hemisphere (South Africa) the *Leucospermum* cut-flower crop thus comes into market too early (September/October) while in the Northern Hemisphere, the crop is too late (March/April) for the period of peak demand in Europe. In South Africa, the overproduction of *Leucospermum* during September and October results in low prices on the European market. Between November and March, the supply of *Leucospermum* does not meet the demand (Vogts, 1982; Jacobs *et al*., 1986). Flowering can be successfully delayed, but an understanding of flower induction, initiation and development is still necessary to determine if advanced flowering can be achieved (Criley *et al.*, 1990). Treatments with gibberellic acid and cytokinins applied in July (Southern Hemisphere) promoted early flowering by 1–3 weeks in *Lsp. cordifolium* (Dupee & Goodwin, 1990).

A number of axillary inflorescence buds develop distally on *Leucospermum* flowering branches. They are borne solitarily in species having large inflorescences (e.g. *Lsp. cordifolium*) or in groups of up to 10 in species having small inflorescences (e.g. *Lsp. oleifolium*) (Rourke, 1972). Many buds form at the tip of the shoot, but only the primary inflorescence bud in *Lsp. cordifolium*, and other species with large inflorescences, normally develops into a marketable primary inflorescence (Brits, 1977; Gerber, 1999). Further development of the axillary flower buds is inhibited by the primary inflorescence (Jacobs *et al*., 1986).

The degree of inhibition of the axillary buds depends on their position on the shoot. The first 6–10 axillary buds immediately below the developing inflorescence are only partially inhibited since they develop to about 5 mm in diameter. Floret differentiation in these buds has progressed to the stage where the perianth initials are visible. They are buds referred to as secondary flower buds. Axillary buds lower down on the flowering shoot show no signs of development and are apparently completely inhibited. These buds can develop into inflorescences if they are released from correlative inhibition from April to the end of June (Jacobs *et al*., 1986).

It is this physiological phenomenon by which the lower flower buds are initiated, but remain undeveloped, that enables the technique of disbudding to be effective. When the uppermost flower bud is removed, one or more of the uppermost secondary flower buds begins to develop. The delay in harvest time is due to the delayed start of development of the secondary bud (Brits, 1977; Brits *et al*., 1983; Jacobs *et al.*, 1986; Gerber, 1999).

Both hand disbudding and spraying plants with ethephon (e.g. ethrel) are effective treatments in extending the harvesting period of pincushions by deactivation of the primary inflorescence bud (Brits, 1977; Jacobs & Honeyborne, 1978; Halevy, 1983; Brits, 1986d; Jacobs *et al*., 1986). A number of sequential treatments of dif-

ferent plantings will improve the overall harvest distribution even more (Brits, 1986d).

The later disbudding is done, the longer the delay in harvest time will be, up to a point which flowers will no longer form (Gerber, 1999). The later the primary inflorescence is removed, the more the developmental period of the secondary flower buds becomes shorter due to the rising temperatures of spring and summer (Jacobs & Honeyborne, 1979). The most vigorous shoots must always be left to flower at the normal time and are harvested leaving bearers. This is essential to ensure adequate time for regrowth of shoots for the next season's harvest (Gerber, 1999).

Late treatment can cause loss of yield through poor resumption of secondary bud development (Brits, 1977; Jacobs & Honeyborne, 1978). Shoot growth resumes since the secondary flower buds have aborted (Jacobs, 1980). The secondary flower buds subsequently abscise (Jacobs *et al*., 1986). Yield loss increases progressively with later treatments. Yield loss is due to non-resumption of inflorescence bud development, mostly on weaker, thinner and shorter flowering branches. Cut-flower quality can also decrease (Brits, 1986d). Some cultivars respond poorly to disbudding, therefore, cultivars reacting favourably to treatment will have to be selected (Brits, 1977; Gerber, 1999).

Disbudding can be used to spread the harvest peak, to delay the harvest time for optimum prices, and to prevent multiple-headed flowers. In some species, such as *Lsp. erubescens* and *Lsp. saxosum*, multiple-headed flowers are acceptable, but in the majority of large-flowered *Leucospermum* spp., it is a disadvantage and should be discouraged (Gerber, 1999).

Flowering time in *Leucospermum* can also be delayed by cutting back shoots to an inhibited bud. This effect can be obtained until the end of June (Jacobs *et al.*, 1986).

PROTEA

Growth habit

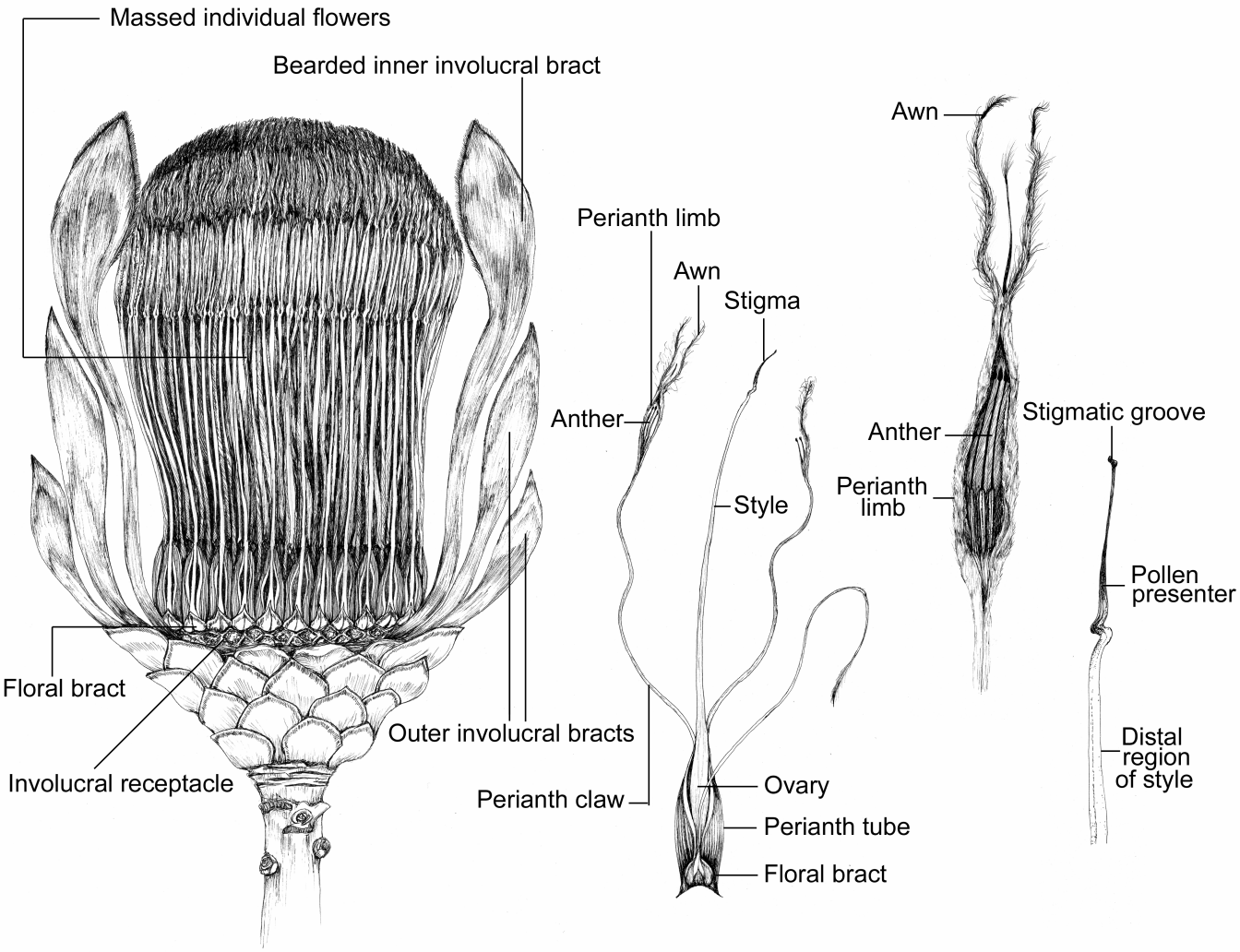


Fig. 3. A *Protea* flowerhead, with finer details pertaining to the floral bracts, anthers, styles and stigmata.

Species of *Protea* range from small, spreading shrubs such as *P. pudens*, to lignotuberous *P. cynaroides*, to the large tree species such as *P. caffra* (Littlejohn, 2001b). Shoot growth, flower initiation and development cycles of *Protea* are not as predictable as in *Leucospermum* and *Leucadendron*. The *Protea* inflorescence of commercial types is borne terminally on a shoot that consists of 2–5 growth flushes. Flushes arise in succession from a distal axillary bud after pruning. Actively growing shoots exhibit strong apical dominance (Malan & Le Roux, 1995). The differences in growth habit between types, which determine pruning severity needed, are the length of individual flushes and the ability of the apical meristems to retain apical dominance and control. Branching occurs distally on flushes at the intercalation (transitional area between flushes) (Malan & Le Roux, 1995). In these plants, branching complexity needs to be limited through training. In others (*P. compacta*, *P. eximia*) dominance is retained even to the extent that only one shoot will develop. In these plants, complexity needs to be increased by pinching the tip of the shoot (Malan, 1995a, b).

Flower initiation

The time when the flowers of the first crop will be initiated (which is not necessarily the first period of flower initiation) will also play a very important role in determining the timing and number of training treatments (Malan, 1995). Little is understood regarding the mechanisms controlling flowering initiation in *Protea*. Flower initiation does not occur at the same time in different genotypes. The wide variety in flowering times suggests that it is unlikely that a single mechanism will explain flowering in all *Protea* spp. (Malan, 1995; Gerber *et al*., 2001). Enormous differences exist in shoot growth and flower development patterns within the genus *Protea*. The collective marketing period of the product lines therefore also varies. This very often inhibits consistent, predictable production of high-quality long-stemmed *Protea* cut-flowers (Malan & Le Roux, 1995). Furthermore, the same species (*P. neriifolia*) has a different flowering period and yield at different locations (Dupee & Goodwin, 1990, 1992).

The period during which the transition from shoot growth to flower initiation takes place is the flower initiation window (Gerber *et al.*, 1995; Hettasch *et al*., 1997). It is not known what factor(s) control flower initiation in *Protea* 'Carnival' (Gerber *et al.*, 1995). A minimum requirement of at least two flushes is needed before the shoot can initiate a flower (Greenfield *et al*., 1994). Furthermore, the length and thickness of stems of *Protea* 'Carnival' contribute significantly to the ability of the stem to produce a flower (De Swardt, 1989). *Protea* 'Carnival' initiates flowers predominantly on the spring flush, and to a lesser extent, on the first summer flush, but not on the second summer flush or the autumn flush (Greenfield *et al.*, 1994).

Anatomical studies indicated that the spring flush is preformed and the apical bud contains all the necessary appendages of the spring flush before elongation begins. Growth of the spring flush therefore consists of extension of pre-formed internodes and pre-formed leaves. During elongation of the spring flush, meristematic activity in the apex of the shoot produces floral appendages, starting with the involucral bracts. When elongation of the spring flush is complete, the apical bud contains the full complement of involucral bracts, and the production of floral bracts, which subtend individual florets, begins. Flower development continues through summer and anthesis is reached in mid- to late-summer (Gerber *et al*., 2000b) (Fig. 3).

De Swardt (1989) found that no shoot growth or reproductive development occurred in *Protea* 'Ivy' under short day conditions, although shoot growth continued in winter when long days were simulated with artificial lighting. It is unlikely that flower initiation in *Protea* 'Carnival' is affected by day-length alone, since flowers are initiated on the first summer flush, produced in mid-summer (December) but not the second summer flush, produced in late summer (Gerber *et al.*, 1995).

Defoliation and shading at critical times inhibited flower initiation in many plants, indicating that leaves play a role in the conversion of a meristem from the vegetative to the floral state. Mature, overwintering leaves must be retained for a period of time for successful inflorescence initiation to occur. Growth and development of the 'Carnival' inflorescence is supported by the spring flush. Defoliation treatments that result in a weak spring flush retard inflorescence development and result in later anthesis. Defoliation treatments applied after completion of spring flush elongation have no effect on either vegetative or reproductive spring growth. In 'Lady Di', mature overwintering leaves are essential for shoots to achieve the induced state for flowering, and are also crucial to the early stages of inflorescence initiation. Current photosynthates from the overwintering leaves therefore support early growth and development of both the spring flush and inflorescence. Further growth is supported by the spring leaves once they are mature (Gerber *et al*., 2001).

Unlike 'Carnival' and 'Lady Di', which have a narrow window for flowering, 'Sylvia' has an open window and can produce flowers at any time of the year (Gerber *et al*., 2000a). Although 'Sylvia' can initiate inflorescences on any flush, they are more readily formed on the spring flush, provided it is subtended by one or more overwintering flushes. This is probably the expression of a facultative response to inductive conditions to which 'Carnival' and 'Lady Di' have obligate requirements; other *Protea* species and cultivars may be identified that have flower induction more similar to 'Sylvia' (Gerber *et al*., 2001).

Control of flowering

Generally, in cut-flower production, the number of flowers per hectare or per square meter is important, as are flower quality and time of harvest. The protea cut-flower industry must depend on a sufficient amount of flowers with good quality blooms. Stem length is an important quality criterion in cut-flowers, with higher prices being paid for flowers with longer stems. Time of harvest is important, as there are certain times when specific products are in low supply while the demand is high, and times when the supply of the product is high or competition from other products makes that product less attractive to the buyer. In the case of proteas produced for the European market, competition from European summer flower production is high during March to August and thus proteas command lower prices. During the European winter months from September to February (Southern Hemisphere), proteas are in high demand, but supply is generally low in January and February, since few species flower at that time (Gerber *et al*., 1995; Hettasch *et al*., 1997).

Protea species growing in their natural habitat flower at distinct times of the year. The majority of commercially used *Protea* spp. normally flower during autumn to spring (May to October in the Southern Hemisphere). Demand from the European market thus prompted studies aimed at elucidating how flowering is inititated and how flowering time can be manipulated (Coetzee & Littlejohn, 2001) so that market demands can be met. Pruning studies on *Protea* 'Carnival' showed that flowering time, stem length, and production of mature bushes can be manipulated by altering the pruning time (Gerber *et al*., 1995; Hettasch *et al*., 1997).

Protea cultivars that form flowers predominantly on spring growth flushes are usually harvested from February to May. 'Carnival' and 'Pink Ice' fall in this category. Shoot growth continues following picking in summer/autumn until flowers are formed terminally on the spring growth flush – a period of about 6 months (Gerber & Jacobs, 1998). Fifty percent of the flowers are initiated on spring flushes, at which stage stems generally consist of only two or three growth flushes (Hettasch *et al*., 1997). This time period is too short to produce export quality shoots of 50–60 cm, and the yield is low. This means that the annual cropping system where shoot growth and flower development must take place in one year is not a viable option. To overcome the production of low yields and short-stemmed flowers, a pruning procedure was developed in which there were high yields of long-stemmed flowers every second year. This biennial cropping system was developed for 'Carnival' and should be useful on all cultivars with a flowering habit similar to 'Carnival', such as *P. compacta* and *P. neriifolia* hybrids and selections (Gerber *et al.*, 1995; Gerber & Jacobs, 1998).

The pruning date also influenced the biomass allocation, and determined whether flowers were produced annually or biennially. Pruning in spring induced a biennial bearing pattern, while pruning in autumn induced an annual bearing pattern. The biennial harvest of one year was more than the cumulative annual harvest of two years. The stem length of the biennial harvest was longer than 50 cm, while only 6% of the flowers from the annual harvest had stems longer than 50 cm. The biennial harvest reached picking maturity predominantly in February (Southern Hemisphere), 2 months earlier than the annual harvests (Gerber *et al*., 1995; Hettasch *et al*., 1997). The advantage of biennial flowering over annual flowering in terms of time, size and quality of harvest is due solely to the fact that flower initiation does not take place in the first year of the biennial cycle (Gerber *et al.*, 1995). Mature bushes harvested and pruned for the annual cycle can be induced into the biennial cycle of bearing by specific pruning techniques. Hettasch *et al.* (1997) proposed a model for pruning new plantings into the biennial cycle of production. For this strategy to be an economically viable option, the total yield must be increased, and management practices must therefore focus on increasing the shoot complexity of the plant and optimising fertilisation and irrigation (Gerber, 1998).

Protea species can be assigned to groups according to similar times of flower initiation and harvest. Insight into the time of flower initiation relative to vegetative growth could be used in making management decisions as well as forming a basis for manipulation of the flowering process (Gerber *et al*., 2001b). Knowledge of a specific flower initiation window and the time of pruning provide the opportunity of having flowers any time of the year or year-round (Gerber, 1998; G. Jacobs, pers. comm., 2002). Higher yields and better quality blooms can be obtained in both existing and new establishments (Hettasch *et al*., 1997).

Carbohydrate allocation in the plant affects the development of bearing branches. Hettasch *et al*. (2001) found that accumulation of carbohydrates (total sugars and starch combined) in the leaves of both 'Sylvia' and 'Cardinal' was significantly higher than in the stems in all the flushes. The lack of stored carbohydrates in stems and the dependence of new growth on carbohydrates produced from leaves highlights the need for retaining enough (3–4) healthy actively photosynthesising leaves on the bearers left after pruning or harvesting.

Weed control

The first weed control is undertaken during the preparation of the soil for planting. It is important at this stage to chemically kill all invasive grasses. Hand-weeding can be carried out within the plant rows once the field is established, or herbicides may be used with caution (Anon., 2000c).

Proteas are very easily damaged by herbicides registered for use against broad-leaf weeds, and if these are used, great care must be taken. Herbicides registered specifically against grasses generally do not affect pro-

teas, but care must be taken not to wet the proteas extensively with such herbicides. Broad-spectrum herbicides, such as those containing glyphosate, can be used only if the plants are screened from inadvertant spray drift, and only at long intervals. Proteas are very susceptible to damage from these herbicides, and the effects are not limited to short-term leaf burn, but include damage that results in atypical growth patterns, leaf yellowing and tip burn, making the plants unthrifty and prone to stress-related diseases. Weeds growing between the plant rows and any cover crop that is established between the plant rows should be kept mown with a bush cutter (Anon., 2000c).

Inorganic and organic mulches are widely used. The choice of the type of mulch depends on the soil type, soil temperatures, and cost of the mulch. Low-growing cover crops that have a low cutting frequency are recommended between rows to assist in weed control (Coetzee & Littlejohn, 2001).

Flowering pot plants

Attractive and unique pot plants can be produced from some of the South African *Proteaceae* (Ackerman *et al.*, 1995). The concept of protea flowering pot plants originated in 1975 when it was noticed that many selections, when rooted from terminal cuttings with an already induced floral bud, continued to develop and flower in the pot (Brits *et al*., 1992). Potted plants are sold on a small scale in South Africa, with Australia being the leading producer of potted *Proteaceae*, and developments taking place in Israel (Ben-Jaacov *et al*., 1989), Japan and the USA (Littlejohn & Robyn, 2000).

In Europe, particularly the Dutch markets (G.M. Littlejohn, pers. comm., 2002) and other countries, the market for flowering pot plants is expanding at the expense of traditional cut-flowers. The flowering pot-plant industry constantly demands new types of products. A special potential exists for pot plants of woody species such as *Protea* spp, because of their novelty value, hardiness and showiness (Brits, 1995). Quality must be produced at a reasonable price. In order to stay competitive, it is important to produce flowering protea pot plants in a year or less (Ackerman *et al*., 1992; Ben-Jaacov *et al*., 1986; Brits, 1989a, 1995; Brits *et al*., 1992).

Woody species such as proteas are difficult to develop as pot plants, and are therefore rather uncommon on the market. They are slow to root, do not flower readily at a young stage, and require high light intensities to flower (Ben-Jaacov *et al*., 1986; Brits, 1989b). *Leucospermum* spp., with their relative ease of rooting and attractive floral displays, have the greatest potential as pot plants (Sacks & Resendiz, 1996).

Proteaceae flowering pot plants (14–20-mo-old) can be produced conventionally from single-stem cuttings (Brits, 1995), or multi-branched cuttings. They are pruned and transplanted to 12 cm pots following rooting, grown for a single growth cycle, and are then naturally or artificially induced to flower. However, leaf drop on 12–14-mo-old wood results in unsightly plant bases (Brits *et al*., 1992).

A 'rapid production' method was developed (Ben-Jaacov *et al*., 1986) prompting autumn-rooted branched cuttings to flower in the following spring (Ben-Jaacov *et al*., 1986; Brits, 1989b). Flowering male plants of *Lcd. discolor* were produced in 3–5 months by rooting large, branched cuttings with initiated flowers (Ben-Jaacov *et al*., 1986). Plant vegetative structure development is completed on the mother plant, and only reproductive development occurs following rooting. Cuttings harvested during flower initiation resume flower development after rooting, producing flowering cuttings after 6–8 months (Brits *et al*., 1992). The date on which the cuttings are taken determines whether the apices will develop into flowerheads or not. Reverted or aborted buds were produced if the cutting was taken too early, before all flowers had formed on the receptacle. Taking cuttings too late in the season might not allow sufficient time for rooting, therefore a delicate balance between too early or too late for propagation is critical (Ben-Jaacov *et al*., 1986).

In South Africa, the growth and flowering of *Leucospermum* pot plant cultivars and selections were evaluated during production and for their post-harvest performance. Various production systems were evaluated based on different types of cuttings. Non-induced, unbranched hardwood cuttings rooted in spring. These cuttings were branched in pots during production, and flowered after 12 months. Non-induced, softwood cuttings, branched on the mother plant, rooted in late summer and flowered after 7–8 months. Shoots were induced to branch by spraying them with ethephon at 960 mg/L. Induced, branched semi-hardwood cuttings rooted in late autumn and flowered after 6 months. Large, single-flower cultivars of *Leucospermum* produced acceptable flowering pot plants, while small multi-headed species only produced flowering pot plants after 17 months of cultivation (Ackerman *et al*., 1995).

Ethephon spraying produced a well-branched *Leucospermum* cutting (primary shoot) on the mother plant in which axillary (secondary) shoots have both a good wide emergence angle of growth, and the desired thickness to produce a flower (Brits *et al.*, 1992; Brits, 1995). However, ethephon depressed growth in some selections. Ethephon was also effective in increasing the emergence angle of secondary shoots over manual pinching, as was found for *Leucospermum* 'Ballerina'. An emergence angle greater than what can be achieved with manual pinching is aesthetically desirable in *Leucospermum* (Brits *et al*., 1992).

It is difficult to control elongation growth of vigorous mother plants. In genera flowering from axillary buds, shoots headed back before or during flower initiation can initiate flower buds from undifferentiated axillary buds (Jacobs, 1983). It is therefore possible to head

back undesirable long shoots in branched cuttings to the desired length, as was done in *Leucospermum* 'Ballerina' (Brits *et al.*, 1992). Growth retardants can also be used to obtain a compact growth form. It could result in shorter shoots, increased leaf number per unit length of shoot, and increased shoot diameter (Brits *et al*., 1992). Paclobutrazol has shown good promise as a chemical shoot growth retardant without unwanted side effects (J. Ben-Jaacov *et al*., unpublished). Secondary shoot length was significantly reduced by paclobutrazol in some selections. Where ethephon caused shoot length reduction, the effect was additive to that of paclobutrazol. The number of secondary shoots also increased significantly in some cases, but generally little effect was found on shoot diameter (Brits, 1995).

Selections responding well to shaping with growth regulators on cutting mother plants are candidates for the 8-month rapid production system. However, some of the selections are clearly more suited to an 18-month conventional nursery production schedule. The rapid production technique requires material specially selected for propensity to flower following rooting (Ackerman & Brits, 1991). The selection must be able to develop a secondary or primary flower bud successfully into a normal flower (Brits *et al*., 1992). A series of protea clones has been identified which yield a high percentage of superior flowers when rapidly produced (Brits, 1995). Products must be selected and bred that can tolerate the various manipulations required in flowering pot plant production. Interspecies hybrids of proteas have exceptional potential as flowering pot plants, since the high vigour of these hybrids makes them suitable for the 'rapid production' technique (Brits *et al*., 1992).

Furthermore, the pot-plant rapid production technology was economised by rooting of cuttings using ambient seasonal temperature without bottom heating and by rooting cuttings early (mid-summer) to utilise the new root system for flower induction (G.J. Brits & A. Ackerman, unpublished; Brits *et al*., 1992). Normal inflorescence development in protea pot plants is strongly dependent on high light levels (Brits *et al*., 1992; Tal *et al*., 1992a) and promising results were found in preliminary tests in which cuttings were rooted in full sunlight during autumn. Early rooting, during the late summer season, achieved the combined purposes of providing a longer rooting period as well as utilising the relatively high ambient temperature of this season (Brits, 1989b), without the need for bottom heating. Early rooting will also utilise the high light levels of summer (Brits *et al*., 1992).

Problems are still being experienced with structure development on the mother plant as well as fertilisation, irrigation and disease control in the rooting and flower development stage (Malan, 1995a). Little research has been done on the planting media and fertilisation for pot plants. The medium must be light-weight and have sufficient water and cation retention capacity, and must also comply with international phytosanitary requirements used for the shipping of pot plants (Brits *et al.*, 1992). Ackerman *et al.* (1995) used a medium consisting of peat, pine bark and river sand (1:4:5).

HARVESTING

All proteas must be harvested at the right stage and when flowers are dry. If flowers are cut when wet due to rain or dew, they must be thoroughly dried before being cooled and packed, otherwise *Protea* species can develop leaf blackening, while *Rhizopus* can develop on *Leucospermum* flowers (Jacobs, 1982b).

Leucadendron

The female plants produce inflorescences in which the fruits are borne. The inflorescence becomes a woody cone after flowering (Rebelo, 2001), from which the mature fruits are released. The male inflorescence does not form woody cones (Littlejohn & Robyn, 2000). *Leucadendron* male clones are generally discarded and useless after flowering, due to poor shelf life and an extremely short marketing period (Littlejohn, 1997). *Leucadendron discolor* male stems are marketed during full-bloom stage before the pollen is released (Eigenhuis, 1999b). The female clones have an extended harvesting period. After flowering, the involucral leaves either become a tight bud around the cone or open up to expose a mature cone (Littlejohn, 1997). The stems used in the fresh cut-flower trade may be the green or red foliage of the plant prior to flowering, the flowering stems of the plant, or the stem bearing maturing cones (Littlejohn & Robyn, 2000). Foliage is harvested when the colour is favourable for the market (Eigenhuis, 1999b). The cone-bearing stems are most desired for the Christmas period. Harvesting of stems is limited to 9 months of the year (autumn, winter and spring), as the new shoots grow during the summer months and the young leaf tissue blackens and dries out easily (Littlejohn & Robyn, 2000).

Leucospermum

Littlejohn and Blomerus (1997) showed that yield was not correlated with the duration of flowering, and the stem yield of young plants is not a good indication of mature plant flower yield. Flowering periods varied from 2–14 weeks for *Lsp. cordifolium*, 1–10 weeks for *Lsp. cordifolium* hybrids, 3–15 weeks for *Lsp. lineare*, and 11 weeks for *Lsp. reflexum*.

Flowers can be harvested at 33–50% styles reflexed, resulting in effective style opening and optimum travel ability and shelf life. A flower with 100% styles reflexed, however, results in having a considerably poorer travel ability and shorter shelf life (Malan, 1995a; Eigenhuis, 1999b). The current trend is to market flowers with as little as 5% of styles reflexed (W. Verhoogt, Bergflora Protea exporter, South Africa, pers. comm., 2002).

Protea

Protea cultivars are generally able to bear marketable flowering stems 2–4 years after planting, depending on the parentage of the cultivar. Flowering stems are harvested at any stage between soft-bud or anthesis of the outer ring of florets (Coetzee & Littlejohn, 2001). The majority of proteas are harvested at the soft-tip stage, except for *P. aurea*, *P. lacticolor* and *P. mundii*, which are harvested at the emergence of the styles (Malan, 1995a). If flowers are picked too early, the flowers will not open (Eigenhuis, 1999b).

The stems must be placed in water immediately after harvest. Thereafter, the cool chain should be maintained until the stems are sold to the florist or consumer. In exporting countries, the cold chain is by necessity broken during air transport (Coetzee & Littlejohn, 2001).

POST-HARVEST

Leaf blackening

Leaf blackening is a common symptom of stress in *Protea* leaves and is one of the most important post-harvest problems in the protea industry (Ferreira, 1983). The vase life of *Protea* is generally 3–4 weeks, but post-harvest leaf blackening can reduce the vase life to 3–7 days after harvest (McConchie *et al*., 1991; Bieleski *et al*., 1992; Crick & McConchie, 1999). It is particularly important in *Protea* species such as *P. compacta, P. eximia*, *P. magnifica*, *P. neriifolia* and *P. repens* (Jacobs, 1982b; Ferreira, 1983; Van Doorn, 2001). Several hybrids derived from these species, such as 'Pink Ice' also have the problem (Crick & McConchie, 1999). Other species in the *Proteaceae* show the disorder to various degrees. Leaf blackening has been reported in *Leucadendron*, while *Leucospermum* shows little or no leaf blackening (Van Doorn, 2001).

Research on protea leaf blackening has been reviewed in detail by Jones *et al*. (1995). The extent of leaf blackening varies widely between species (McConchie & Lang, 1993a), cultivars within species (Paull *et al*., 1980; Paull & Dai, 1990), the time of year or season (Paull & Dai, 1990; Coetzee & Littlejohn, 2001), and from year to year (De Swardt *et al.*, 1987). It is clear that almost all proteas are to some extent susceptible to leaf blackening (Newman *et al*., 1990). The symptoms of this leaf disorder have been described many times (Paull *et al.*, 1980; Brink & De Swardt, 1986; De Swardt *et al*., 1987). Leaf blackening has been divided into four types, based upon the first appearance of the discoloration (De Swardt *et al.*, 1987).

PHYSIOLOGICAL CAUSES OF LEAF BLACKENING

Water stress has previously been reported as a cause of this disorder (Mulder, 1977; Paull *et al.*, 1980; Ferreira, 1983; De Swardt *et al.*, 1987; Paull & Dai, 1990). Water stress can be induced by transpiration from the flowerhead (Ferreira, 1982) or by occlusions, such as tannins, in the base of the stem (Paull & Dai, 1990). Water stress at the cellular level leads to an increase in membrane permeability (Ferreira, 1983), which plays an important role in tissue browning. The disorder can be eliminated by removal of the flower head (Paull *et al.*, 1980), a response that was suggested to be largely due to the reduced water demand of the headless branches. In many flowers, however, water loss from the flowers or inflorescences makes only a minor contribution to overall water loss (Halevy & Mayak, 1979). Newman *et al*. (1990) and Reid *et al*. (1989), furthermore, found strong evidence for the absence of a role of water stress in the development of early leaf blackening during vase life (Van Doorn, 2001). In conclusion, leaf water stress could be a contributing factor, but is not a primary cause of leaf blackening in cut *Protea* inflorescences (Jones *et al*., 1995).

The majority of studies conclude that carbohydrate depletion or stress of developing leaves through respiration/translocation to the flower head after harvesting is the main cause of leaf blackening (Brink & De Swardt, 1986; Reid *et al.*, 1989; Newman *et al*., 1990; McConchie *et al*., 1991, 1994; Bieleski *et al*., 1992; McConchie & Lang, 1993b; Dai & Paull, 1995; Van Doorn, 2001). The results of Newman *et al*. (1990) furthermore indicated that competition for carbohydrate, rather than water stress, is a major cause of leaf blackening in cut stems of *Protea* species. The ability of source leaves to sustain the flower sink demand, and hence flower longevity, was suggested to be determined by the number of leaves on the flower stem (Dai & Paull, 1995).

Flowers have high rates of respiration and growth, and therefore, they are major sinks for carbohydrates (Reid *et al.*, 1989). The leaves apparently serve as a source of carbohydrates for the flower head (Van Doorn, 2001). The concentration of sucrose and starch in the leaves drops rapidly after harvest (McConchie *et al*., 1991; Bieleski *et al*., 1992; McConchie & Lang, 1993b). These observations follow from the experiments on girdling, that blocks the pathway of sugars from the leaves to the flower head, and from experiments using darkness or additional light (Reid *et al*., 1989). In the light, leaf blackening is inhibited by the production of carbohydrates as a result of photosynthesis, or by the maintenance of an environment that prevents the final phenol oxidation (Jones & Clayton-Greene, 1992). Dai & Paull (1995) concluded that leaf blackening in protea is the result of depletion of carbohydrates by the expansion and respiration of the inflorescence, but most importantly due to the sugar demand for nectar production.

Flower head demand on carbohydrates appears to be linked with respiration and nectar production. The rate of respiration differs greatly from one part of the protea inflorescence to another. There is also a marked difference between the respiration rates of the florets in different positions on the protea inflorescence as well as of florets from inflorescences cut at different stages of de-

velopment. Respiration rates were reported to be higher in immature florets than mature florets of *P. neriifolia,* which therefore have a higher carbohydrate demand (Ferreira, 1986). The process of depletion of the substrates available for respiration begins soon after harvest of the cut-flower, and eventually leads to a decrease in the rate of respiration (Ferreira & De Swardt, 1980). This results in the hydrolysis of structural cell components (Halevy & Mayak, 1979). In senescing tissue, this is accompanied by a decrease in membrane permeability, followed by a loss of protoplasmic compartmentation (Sacher, 1973), the mixing of cellular contents (Lewis & Martin, 1969), and eventually leaf blackening. The maintenance of normal respiration does, however, not only depend on the available substrates, but also on an adequate water status in the cells to ensure the normal functioning of physiological processes in the plant (Rogers, 1973).

Furthermore, nectar production was reported to increase as flowers reached optimal picking stage (Dai & Paull, 1995). Species with a strong tendency to blackening, such as *P. eximia*, *P. compacta* and *P. neriifolia*, produce high volumes of nectar. Nectar production is a reason for the high carbohydrate demand by the flower heads (Dai & Paull, 1995). However, *P. cynaroides* also produces high volumes of nectar but leaf blackening is seldom observed (G.M. Littlejohn, pers. comm., 2002). Furthermore, nectar production poses a sink on carbohydrates prior to harvest, but upon harvest, this sink may become small compared to that of ongoing flower respiration (Van Doorn, 2001). McConchie & Lang (1993a) concluded that leaf blackening might be related more to inflorescence sink demand after harvest and oxidative substrate availability than preharvest reserve carbohydrate concentrations in various *Protea* species.

Transport of potted protea plants, particularly those transported in darkness and at high temperature, also resulted in leaf blackening. The leaves that blacken are those just below the vigorously growing top leaves. The uppermost growing leaves are likely a strong sink for carbohydrates and the subtending, full-grown leaves, a main source of these compounds (Van Doorn, 2001).

A long period of drought may result in leaf blackening in intact plants in the field. This may also relate to carbohydrate stress, as water stress may result in stomatal closure, and this in turn can reduce the rate of carbon dioxide fixation and the carbohydrate levels in the leaves (Van Doorn, 2001). *Protea* 'Sylvia' is particularly prone to leaf blackening on the plant during the period September–October (Southern Hemisphere). This is, however, not due to water stress (I.A. Stephens, Dept. of Horticulture, Univ. of Stellenbosch, South Africa, pers. comm., 2002).

Increasing temperatures enhance the rate at which leaf blackening occurs (Jacobs & Minnaar, 1977; Ferreira, 1983, 1986). The respiration rate of plant material increases with increasing temperatures (Ferreira, 1983, 1986). It is known that high temperatures increase membrane permeability and at extreme temperatures, membrane disruption occurs (Jacobs, 1982b).

Reid *et al*. (1989) observed that free water on the leaves, due to condensation, increased the incidence of blackening. Dew and rainfall may also relate to leaf blackening, but no data as yet can prove these claims, or explain the mechanism of leaf blackening as a result of free water on leaves (Van Doorn, 2001).

Evidence suggests that ethylene is unlikely to be involved. Ethylene released by the leaves was not related to blackening in a number of species (McConchie & Lang, 1993b). A short pulse with silver thiosulphate, an inhibitor of ethylene action, prior to vase life, had no effect on the time to leaf blackening in *P. eximia* (Reid *et al*., 1989; Bieleski *et al*., 1992).

Since relatively low light levels from incandescent lamps reduce leaf blackening, these reports require independent testing (Van Doorn, 2001).

Leaf blackening developed sooner in flowers harvested at the closed bud stage, with the slowest rate occurring in flowers harvested when the flower bracts had just started to unfold or at a later stage (Paull & Dai, 1990). Closed buds have a higher carbohydrate requirement than inflorescences harvested at a later stage, as they continue to expand and develop after harvest (Jones *et al*., 1995). Leaves are slower to blacken if harvested in the afternoon than in the early morning (Paull & Dai, 1990). This may also be due to an improved carbohydrate status of the inflorescence after a period of photosynthetic activity (Jones *et al*., 1995).

THE BIOCHEMICAL MECHANISMS OF LEAF BLACKENING

Although it is now clear that carbohydrate starvation is the main reason for blackening, the connection between this starvation and the blackening response is far from being elucidated (Van Doorn, 2001).

It is at present unclear to what degree leaf blackening is due to enzyme activity, and it is also not clear which enzymes, if any, are involved. Many researchers believe that the direct cause of the leaf blackening in *Protea*, induced by these stresses, is oxidation of the hydroxyphenols and tannins (Paull *et al*., 1980; Ferreira, 1983). This could be due to enzymatic activity of polyphenol oxidase (Jones & Clayton-Greene, 1992). *Protea* leaves contain many phenolic and flavonoid compounds, which are colourless in a reduced state, but rapidly turn brown or black when oxidation and polymerisation take place (De Swardt, 1979). According to Mulder (1977), blackening of *P. neriifolia* leaves is caused by oxidation of leucoanthocyanins. Although enzyme oxidation of phenolic compounds contributes to leaf blackening, Whitehead (1979) and Whitehead & De Swardt (1982) found that air oxidation of flavonoid compounds is the major cause of leaf blackening in *P. neriifolia*.

Carbohydrates in leaves of *Protea* species susceptible to leaf blackening have been reported to be bound

with phenolics in the form of an O-glycoside ester (Perold *et al*., 1973, 1979; Perold & Carlton, 1989; Perold, 1993). It has been hypothesised that liberation of the sugar component for use by the flower head results in a highly reactive free phenol which is oxidised non-enzymatically (Perold, 1993). This oxidation process appears to take place relatively slowly, and may explain the delay in appearance of leaf blackening. Phenolic concentration determined on removal from storage was significantly higher than pre-storage concentration, possibly as a result of synthesis (Stephens *et al*., 2001).

Blackening reactions in plants are often due to the activities of the peroxidase or polyphenol oxidase (PPO). *Protea* leaves had a higher protein and total phenolic content than *Leucospermum* leaves. Leaf PPO activity, measured *in vitro*, was high in *P. neriifolia* and absent in *Leucospermum*, which does not show leaf blackening. The latter was found to contain an inhibitor of *Protea* PPO activity (Dai & Paull, 1997). McConchie *et al*. (1994) found no association between PPO and leaf blackening, and no evidence of membrane degradation preceding the appearance of leaf blackening in *Protea* leaves. Therefore they concluded that osmotic stress may lead to a release of free phenol and non-enzymatic oxidation. Although PPO can thus not be excluded in the process of blackening, its role is still unclear. *In vitro* assays may not be of much relevance to the processes in the cell (Van Doorn, 2001).

The same criticism applies to the *in vitro* measurements of β-D-glucosidase activity, which seemed rather well correlated with blackening. This enzyme is inhibited by ethanol, and dip treatments of flowers in 30–50% ethanol considerably delayed leaf blackening (Jones & Cass, 1996). Obviously, this result is no proof that β-D-glucosidase is involved in blackening, as numerous other enzymes are inhibited by ethanol (Van Doorn, 2001).

The lack of convincing evidence linking enzymatic oxidation of phenol and leaf blackening has resulted in the search for alternative enzymes that may be involved (Jones *et al*., 1995).

According to the current ideas on blackening, the substrate for the reaction resides in the vacuole, while some of the enzymes that may be involved are present in the cytoplasm or in organelles such as the peroxisome (peroxidase) or the chloroplast (PPO). The mixing of the substrate and enzyme would require the breakdown of some of the membranes, or at least that of the vacuole (Van Doorn, 2001). McConchie & Lang (1993b) concluded that membrane degradation prior to blackening is unlikely as no degradation products were observed. It could not yet be excluded that blackening starts in the cell walls, but the role of the cell wall in leaf blackening has thus far not been investigated. Cellular localisation of blackening is therefore not yet resolved (Van Doorn, 2001).

CONTROL OF LEAF BLACKENING

Pulsing stems with sugars (20% sucrose), shortly after harvest, is effective in delaying blackening during transport (Jones, 1991; McConchie & Lang, 1993a, b). The treatment should be given within the first day after harvest, as leaves on *Protea* flowers tend to lose more than half of their starch reserves and considerable amounts of sucrose during the first 24 h (McConchie & Lang, 1993a, b). Although the sugar treatments reduce blackening during long-term dry storage, it is not clear how much benefit these treatments have for the blackening occurring during vase life. High sugar concentrations of 20% (Jones, 1991; McConchie & Lang, 1993a, b) are necessary to be effective for pulsing of *Protea* flowers. The inclusion of an antimicrobial compound is imperative as bacteria grow rapidly in sugar solutions. These bacteria will result in a blockage of the stems, thereby inhibiting water uptake and the uptake of sugars (Van Doorn, 2001).

Different carbohydrate sources were tested as pulsing and vase solutions. Earlier reports indicated that sucrose is the most effective, readily assimilable preservative in the vase solution, reducing the rate of leaf blackening (Akamine *et al*., 1979; Brink & De Swardt, 1986). The continued supply of sucrose to cut-flowers is essential for the maintenance of membrane integrity (Coorts, 1973). Adding 2.5–5% of sucrose to the vase solution was tested (Dai, 1993). At moderate concentrations of 0.5% sucrose (plus hypochlorite), addition to the vase solution substantially delayed the onset of leaf blackening, but at concentrations of 0.5–1% and higher, black spots developed on leaves of *P. eximia* (Newman *et al.*, 1990). Pulsing of *P. neriifolia* flowers with greater than 7.5% sucrose was harmful and accelerated leaf browning (Paull & Dai, 1990). With longer periods (18 h) of sucrose exposure, the flower head was the preferred sink (Brink & De Swardt, 1986). Leaves did receive significant amounts of sucrose if the pulses were less than 12 h. A 12-h pulse is therefore recommended (Brink, 1987). Dai & Paull (1995) found that a 5% sucrose solution stimulated nectar production and decreased leaf blackening of *Protea*. They found that sucrose at 0.5% used by McConchie *et al*. (1991) might not have been sufficient to promote inflorescence expansion. Paull & Dai (1990) found that the effect of flower carbohydrate supply on leaf blackening was modified by flower stem water uptake ability.

Stephens (2001) found a 2.5% glucose solution most effective in reducing leaf blackening in *Protea* 'Sylvia'. The proteas showed minimal blackening even after 20 days held in the solution. Genotypic differences between cultivars in their reaction to glucose as a vase solution were detected, indicating that no single vase solution is currently widely applicable. Each cultivar may require a unique vase solution for optimal effect. In some cultivars, sucrose was more effective. The combination of vacuum cooling of harvested stems and the use of a glucose vase solution for *Protea* 'Sylvia' was

very effective in reducing the incidence of leaf blackening, thus improving the transport ability of the product dramatically.

Rapid cooling of the produce as soon as it is packed and keeping the produce cold (approx. 1°C) is also an effective way to reduce blackening (Van Doorn, 2001). Quality of most cut-flowers is maintained effectively at storage temperatures of between 0 and 1°C (Reid, 1992). Vase-life of several *Protea* species stored at 2°C for 42 days was reported to be satisfactory (Haasbroek *et al*., 1973; Meynhardt, 1976). Suppression of leaf blackening was reported for *P. eximia* cut-flowers stored at 2°C (Paull *et al*., 1980). Stephens *et al*. (2001) reported that respiration and leaf blackening of 'Sylvia' correlated well with temperature and decreased significantly with decreasing temperature. Appearance of leaf blackening was delayed by 4 days in stems held at 0°C. Respiration rates decreased significantly with lower storage temperature, while starch, reducing sugar and total carbohydrate content decreased significantly with increasing temperature. The low carbohydrate content concurs with reports (Bieleski *et al*., 1992; McConchie & Lang, 1993b) that carbohydrate content of *P. eximia* and *P. neriifolia* declined rapidly after harvest. Phenolic concentration was significantly greater in subtending flush leaves than in leaves from other flushes. Flower-bearing stems held at 0°C had a significantly longer vase life than those held at 4.5°C. Leaf blackening increased significantly with length of storage (Stephens *et al*., 2001). The immediate removal of field heat from flowers was only worthwhile if low temperatures were maintained *en route* to the wholesaler or retailer. Flowers harvested in the hotter and drier part of the year are more prone to leaf blackening (Paull & Dai, 1990).

Vase life correlated well with girdling. Girdled flower-bearing stems had a significantly longer vase life than intact flower-bearing stems, due to removal of the flower head sink and retention of carbohydrates in the leaves (Brink & De Swardt, 1986; Dai & Paull, 1995). Removal of a ring of bark and phloem (5 mm) just below the flower head effectively delayed leaf blackening (Reid *et al*., 1989; Newman *et al*., 1990; Paull & Dai, 1990; Dai, 1993). Maintenance of low-temperature storage (0°C) during transit and girdling are recommended to prolong vase life of 'Sylvia' cut-flowers (Stephens *et al*., 2001).

Placing the floral stems in bright light (Reid *et al*., 1989; Paull & Dai, 1990; McConchie *et al*., 1991) delays or even prevents leaf blackening. The symptoms are more rapidly expressed when the flowers are held in the dark at room temperature (Newman *et al*., 1990). The starch and sucrose concentrations in leaves decline in stems held in the dark rather than in the light (McConchie *et al*., 1991; Bieleski *et al*., 1992).

Storage in an atmosphere containing a low oxygen concentration and a high carbon dioxide level, called controlled atmosphere (CA), also delayed blackening, at least during the period of storage (Jones & Clayton-Green, 1992). Crick & McConchie (1999) also reported a reduction of leaf blackening in the presence of elevated CO_2 levels. They suggested that the high CO_2 concentrations slowed down the respiration rate in the stems and consequently, the use of carbohydrates.

The inclusion of 1% ethanol in the vase solution also reduced blackening. A short pulse with 30–50% ethanol, shortly after harvest, was also effective (Jones & Cass, 1996). Ethanol vapour in the package had a similar effect (Crick & McConchie, 1999). It is difficult to draw parallels between these processes (Crick & McConchie, 1999). The effect of this treatment on the vase life remains to be determined (Van Doorn, 2001).

Finally, choosing the right species and cultivars may help to reduce the blackening problem. Breeding for reduced blackening will reduce the problem, since ample germplasm in the genus *Protea* shows little or no blackening (Van Doorn, 2001). However, G.M. Littlejohn (pers. comm., 2002) stated that only *P. cynaroides* shows no or limited leaf blackening, and this species is not used for breeding. Short-term solutions are, therefore, required (Paull & Dai, 1990). No study has yet compared the various methods in one species. One type of treatment may be more advantageous than another, also depending on the species and cultivar, and on the conditions during the distribution chain (Van Doorn, 2001).

Water relations

Vase life of cut-flowers depends on cell turgor or plant cell water relations. Vase life of mature stems of *Leucadendron* 'Silvan Red' fell from 6 to 4.5 weeks after exposure to a transient water stress (Street & Sedgley, 1990). Localised desiccation of the leaves started from the tips, extending towards the base of the leaf. Stressed leaves showed loss of dry weight, and a decrease in transpiration. These observations may be similar in kind and origin to leaf blackening, but they are far milder in intensity. Dessication may be due to a number of factors, including differences in the relative sizes of assimilate sinks and sources in the flower head, leaves and bracts, respectively, as well as in the abundance of specific compounds such as phenolics in the leaves. When bacterial plugging of xylem vessels is not a serious factor limiting the water supply, vase life of 'Silvan Red' tends to be correlated with a decline in leaf and bract turgor, caused by reduced osmotic pressure of the cell sap. A severe transient water stress may still override, and shorten, any slower, longer-term decline, and as a result, reduce the vase life of the stressed stems. Leaf turgor in 'Silvan Red' can be expected to respond to various treatments such as pulsing solutions to improve food supply, but it is not known whether such treatments will alleviate the effects of severe transient stresses on vase life (Street & Sedgley, 1990).

PHYTOSANITARY REGULATIONS AND EXPORT CONSIDERATIONS

Phytosanitary and export requirements vary according to the countries involved in the import and export of proteas. Export standards vary with respect to the percentage of leaves that must be retained on the stem, stem length, stem straightness, stage of development of the flower head, flower head size and shape, the presence of blemishes and insects, carton type and marking, etc. Standards may also differ between genus and even sometimes between species (Coetzee & Eigenhuis, 1999; Anon., 2000c; Coetzee & Littlejohn, 2001). Phytosanitary and quality control inspections are carried out in both the countries of export and import. At the ports where proteas are exported from South Africa, up to 1% of all shipments are rejected (S.A. Perishable Products Evaluation Control Board, 1998–1999). Of those shipments that reach the importing country, further quarantine inspections cause more rejections. These are even more critical as shipping costs and destruction/removal costs must be covered.

Diseases and insect pests

Pathogens which are exclusive to countries where *Proteaceae* breeding programmes are carried out may be introduced when the new cultivars are imported, or re-enter the original country of origin. Thus, in recent years research efforts have sought to address some of these issues, and a scientific and systematic survey of the pathogens in the various countries where proteas are cultivated is in progress. The increased knowledge of the biodiversity of the fungi and insects on *Proteaceae* will support the development of science-based plant quarantine regulations and decisions (Taylor, 2001). Pests and diseases that have evolved together with African *Proteaceae* also have their origins in South Africa, with potential to become serious pests in other regions where proteas are cultivated, especially in areas with great climatic similarity (Wright & Saunderson, 1995). Pathogens permissible are controlled by the phytosanitary regulations of the importing country, but it is obvious that the presence of disease lesions that detract from the flower's appearance will negatively influence the price.

The presence of insects in cut-flowers is one of the most serious limiting factors influencing the South African protea industry. A wide range of species occurs normally at high population levels (Visser, 1992) in the flowers (Coetzee & Giliomee, 1985). Insect damage causes flowers to become unacceptable for aesthetic reasons, while the presence of insects in flower heads makes them unacceptable for phytosanitary reasons (Plate 12). Flower visitors, feeding on nectar and pollen, cause phytosanitary problems, as well as predators of other insects present in the blooms. Sap suckers, such as mealy bugs, scale insects, aphids, as well as stink bugs and borers, may be present in the flower head or on the stem (Coetzee & Wright, 1999). If blooms are picked very late, pollen has already been released and large numbers of insects may be present (Wright, 1998).

The phytosanitary regulations of the American market are particularly strict. The presence of any insect, dead or alive, in a consignment of flowers, will result in rejection of the consignment. Flowers packed for export must therefore be free of any insect infestation. USA regulations state that *Proteaceae* must be cultivated (Wright, 1998).

It is evident that the protea cut-flower industry has considerable potential, but diseases which blemish foliage and blooms are one of the problems which make this a high-risk crop (Greenhalgh, 1981). Cultivated proteas are subject to fungal diseases of the stems and roots. Post-harvest diseases of the flowers are mainly caused by widespread fungi with little host specificity, whereas diseases of the leaves are caused by protea-specific pathogens, many of which originated in South Africa. The former group of diseases mainly causes a loss in productivity for the producer, whereas the latter group is more important in affecting the aesthetics of the flowers, lowering their value, and causing rejection at quarantine inspection points.

Various pre-harvest and post-harvest practices ensure that insect-free products are packed. Before flowers are picked for export, a pyrethroid insecticide application (e.g. deltamethrin) should be made. Pyrethroids have wide-spectrum insecticidal effects, and will kill most insects present on the plants, thereby reducing the number of insects that infest flowers at a later stage. Flowers must be picked in the 'soft-bud' stage, when the bracts of the flower are beginning to open. Almost all proteas will continue to open after being picked at soft-bud. At this stage, no insects are attracted to the flower since no pollen or nectar has been released. This practice will reduce insect numbers inside the flowers dramatically. Stems should be inspected at harvest for borers, mealy bugs and scale insects. Any flowers infested with borers should be discarded, and any with mealy bugs or scale insects should be cleaned (Wright, 1998).

After picking, stems should be placed into water as soon as possible, and a 1% solution of dichlorvos EC can be injected into each flower at this stage (1 mL per flower) (Wright & Coetzee, 1992). This practice will reduce insect numbers by about 85%, and should be adequate for European markets, but not for the USA markets. Flowers exported to the USA should be fumigated. The most effective way to fumigate proteas is to apply forced-ventilation fumigation. This requires a fumigation chamber that is air-tight with suitable air circulation equipment. The most suitable fumigant is dichlorvos AE 9.3%, registered in South Africa on proteas (Nel *et al*., 1999). This aerosol formulation provides small particles that are able to penetrate the flowers and kill insects inside. Optimal results were obtained with fumigating at 12°C for 12–14 h. At tem-

peratures greater than 20°C, leaves will blacken rapidly and flowers will also be damaged. Flowers must stand in water to ensure that they remain well hydrated (Wright, 1992, 1998).

Once flowers have been fumigated, they should be shaken to remove any dead insects. Un-fumigated flowers should be removed out of the vicinity of fumigated flowers. Each flower should be carefully inspected prior to final packing, and any dead insect should be removed. If the flowers were picked at the soft-bud stage, flowers should be insect-free at this stage (Wright & Saunderson, 1995; Wright, 1998).

Countries without indigenous *Proteaceae* (e.g. the USA) seem not to have any of the most injurious guilds of insect pests noted in countries with indigenous *Proteaceae*. This is definitely the result of strict phytosanitary control applied by countries importing plant material (Wright & Saunderson, 1995).

Global trade and the role of phyto-sanitary regulations

The introduction of the Sanitary and Phytosanitary Agreement (SPS) by the WTO (World Trade Organisation, 1994), has significantly transformed phytosanitary legislation. The WTO was established in 1994, to replace GATT (General Agreement on Tariffs and Trade). The roles of both GATT and the WTO are to promote free trade and reduce barriers or tariffs (Anon., 1999). However, as it has become more difficult to use tariffs and other barriers as trade protection measures, some countries could potentially turn to using health protection measures to restrict trade. Any move to liberalise trade must try to stop this from happening, while still allowing legitimate steps to protect health. Therefore, as part of a bid to tackle agricultural trade issues, the Sanitary and Phytosanitary Agreement (SPS) was introduced (World Trade Organisation, 1994). This agreement is about how to apply sanitary (human and animal health) and phytosanitary (plant health) measures. It establishes principles that countries are committed to uphold when they work to protect health while trading in plants, animals and their products (World Trade Organisation, 1994).

The basis for the phytosanitary part of the SPS agreement is the International Plant Protection Convention. The IPPC plays a vital role in trade as it is recognised by the WTO in the SPS Agreement as the source for International Standards for Phytosanitary Measures (ISPMs) affecting trade (Anon., 2000b). The IPPC is an international treaty in plant protection to which 110 countries currently adhere (Anon., 2000b). The IPPC's main objectives are to take specific actions to prevent the introduction and spread of plant pests, and to promote measures for pest control. To summarise, the SPS Agreement makes provisions for plant protection in a trade agreement, whereas the IPPC makes provisions for trade in a plant protection agreement (Taylor, 2001).

The international standards for the phytosanitary measures or ISPM's provide guidelines for the assessment of potential pathogens and the risks they pose. There are currently 10 ISPMs already approved by the IPPC, with a further two under consultation. Signatories to the WTO must abide by these guidelines on phytosanitary issues, and quarantine requirements must be based on scientific principles and a thorough pest risk analysis (Anon., 2000b). Prior to the WTO/SPS Agreement, phytosanitary outcomes were covered by Article XX(b) of the General Agreement which permitted countries to take measures 'necessary to protect human, animal or plant life or health' as long as these were not applied in an arbitrary or unjustified manner (Anon., 2000a). The current SPS agreement applies to all measures developed to protect human, animal and plant life and health, which may directly or indirectly impact on international trade (Article 2), provided that they are based on *scientific principles* (World Trade Organisation, 1994).

Phytosanitary measures and protea production

Article 5 of the SPS Agreement deals with the 'Assessment of Risk and Determination of the Appropriate Level of Sanitary or Phytosanitary Protection', and ISPM 2 - Guidelines for Pest Risk Analysis (Anon., 2000b), provides information on the standards and procedures (World Trade Organisation, 1994). A pest risk analysis (PRA) is carried out on a chosen commodity, and involves a science-based process. The aims of a PRA are to identify hazards (pests and diseases) that might be recorded on the commodity when examined and to assess the likelihood of the hazard becoming established on the plant populations of the importing country and the potential impact of such an establishment (Anon., 2000a). Risk management options are then developed for the commodity, which may range from no restriction on trade, through various treatments or testing procedures, to a complete prohibition on trade. The most suitable management option is then selected so that an adequate level of protection can be ensured for the importing country. Finally quarantine procedures are developed and implemented (Anon., 2000a). The regulators of phytosanitary decisions in some countries where proteas are cultivated include APHIS (Animal and Plant Health Inspection Service) in the U.S.A.; AQIS (Australia Quarantine and Inspection Service) in Australia; and the National Department of Agriculture, Directorate Plant Health and Quality (South Africa).

No country has ever requested a PRA on proteas or even started one, as proteas have already been imported to many countries prior to the more formalised PRA process now in place. Also, the plants are mainly sent as cut-flowers, rather than propagation material, which

is treated differently. Therefore cut-flower products are still subject to standard inspection on entry.

During the inspection process quarantine officials of an importing country may refer to an actionable pathogen list for that country, which is based on the known pathogens of South African proteas and on previous interceptions. Shipments of proteas with lesions may be rejected if the lesions are caused by a quarantine significant organism. However, this was previously based on little or no prior knowledge of the status of that pathogen in the importing country. Thus there was a need for a more formalised approach in importing countries, although up until recently only South Africa had carried out a survey of protea pathogens, which were subsequently in public depositories (Herbaria). In Hawaii, a survey was carried out in local protea plantations (Anon., 1991, 1993), but the diseased specimens were never deposited and these records cannot be verified. Therefore, in order to begin to redress this balance and to provide data for *science-based* plant quarantine regulations and decisions, a survey of orchards and nurseries of some of the major protea-growing areas outside of South Africa (Australia and the USA) has been undertaken.

Symptomatic leaf and stem samples of cultivated *Proteaceae* hosts (*Banksia*, *Grevillea*, *Hakea*, *Leucospermum*, *Leucadendron, Protea* and *Telopea*) were collected and surveyed for the presence of fungal fruiting structures. Herbarium specimens are lodged at PREM and HKU(M). Where possible, isolates were cultured and reference cultures are maintained in the culture collection of the Centraalbureau voor Schimmelcultures (CBS) in Utrecht, the Netherlands. The results of this study have been published (Taylor *et al.*, 2000a, b; Crous *et al.*, 2001) and are summarised in Table 2. Only reports of fungal pathogens recorded from diseased *Proteaceae* hosts occurring in two or more countries are included and also only verified records are cited. More details can be accessed from the separate publications on Australia (Crous *et al.*, 2001, Crous *et al.,* unpubl. data), California (Taylor *et al.*, 2000b), and Hawaii (Taylor *et al.*, 2000a), or from records in South Africa (Crous *et al.*, 2000).

Table 2. Records of the most common diseases from hosts including *Banksia* (*B.*)*, Buckinghamia* (*Bu.*), *Dryandra* (*D.*), *Grevillea* (*G.*)*, Hakea* (*H.*), *Leucadendron* (*Lcd.*)*, Leucospermum* (*Lsp.*)*, Protea* (*P.*), and *Telopea* (*T.*), in the countries or regions surveyed (Taylor, 2001).

Fungal pathogen	Australia	California	Hawaii	South Africa
Batcheloromyces leucospermi	-	*P.*	-	*P.*
Botryosphaeria dothidea	*Bu.*	*Lcd., P.*	*G., Lcd., Lsp., P.*	*Lcd., Lsp., P.*
Botryosphaeria proteae (≡ *Saccharata proteae*, see Leaf Diseases)	*P.*	*P.*	*Lsp., P.*	*Lsp., P.*
Botryosphaeria ribis	*B.*	-	*G*	-
Botrytis cinerea	*Lsp.*	*P.*	*Lsp., P.*	*Lsp., P.*
Cercostigmina protearum var. *hakeae* (≡ *Pseudocercospora protearum*, see Leaf Diseases)	*H.*	-	-	*H.*
Cladosporium spp.	*H., Lsp.*	*Lsp., P.*	*P.*	-
Coleroa senniana	*P.*	*Lsp., P.*	*P.*	*Lsp., P.*
Colletotrichum gloeosporioides	*G., Lsp., P.*	*P.*	*B., Lcd., Lsp., P., T.*	*Lsp., P.*
Coniothyrium leucospermi	-	*P.*	-	*Lsp., P.*
Didymosphaeria futilis	-	*P.*	-	*P.*
Drechslera dematoidea	*Lsp.*	-	*Lsp.*	*Lsp.*
Elsinoë banksiae	*B.*	-	*B.*	-
Elsinoë leucospermi	-	*Lsp.*	*Lcd., Lsp.*	*Lsp.*
Fusarium sp.	-	*P.*	-	*P*
Fusicoccum sp.	*P., T., Lsp.*	*P.*	*Lsp., P., T.*	*Lcd., Lsp., P.*
Leptosphaeria protearum	-	-	*P.*	*P.*
Lophiostoma fuckelii	-	*P.*	*Lsp., P.*	*Lsp., P.*
Mycosphaerella jonkershoekensis	*P*	-	-	*P*
Pestalotiopsis spp.	*B., Lcd., Lsp.*	*Lsp., P.*	*Lsp., P., T.*	*Lsp., P.*
Phyllosticta telopeae (see Leaf Diseases)	*Lcd., H., T.*	-	*B., G., Lcd., Lsp., P., T.*	-
Phytophthora cinnamomi	*B., G., H., Lcd., Lsp., P.*	*Lcd., Lsp., P.*	*B., Lcd., Lsp., P.*	*Lcd., Lsp., P.*
Phytophthora nicotianae	*B., D., G., H.*	-	*B., Lsp.*	-
Pyrenochaeta-like sp.	*G.*	*G.*	-	-
Pythium spp.	*B., G., Lcd., P.*	*Lcd., Lsp., P.*	*Lsp., P.*	*P.*
Sarcostroma grevillieae	*G.*	*G.*	-	-
Seimatosporium sp.	*H., T.*	*P.*	-	-
Vizella interrupta	*B., G.*	-	-	*Lcd., Lsp., P.*

SECTION 3

INFECTIOUS DISEASES

CHAPTER 1

FOLIAR DISEASES

Batcheloromyces leaf spot

Species of *Batcheloromyces* are known only from *Proteaceae*, and the three known species also appear to be host-specific at the host genus level. *Batcheloromyces leucadendri* causes lesions on *Leucadendron* spp., *B. leucospermi* on *Leucospermum* and *Protea* spp., and *B. proteae* on *Protea* spp. (Van Wyk *et al*., 1975a; Knox-Davies *et al*., 1987; Sutton & Pascoe, 1989; Crous & Braun, 1996; Taylor *et al*., 1999). *Batcheloromyces proteae* is only a problem of commercial importance on *Protea cynaroides* (Von Broembsen, 1989), as the presence of the leaf spots lowers the value of the bloom. Although *Batcheloromyces leucandendri* is often more inconspicuous, it is frequently intercepted at quarantine inspection points (Taylor, 2001). Results from a recent molecular study have shown *Batcheloromyces* to be an anamorph of *Mycosphaerella* (Taylor *et al*., 2003).

Batcheloromyces leucadendri P.S. Van Wyk, Marasas & Knox-Dav., *South African Journal of Botany* **51**: 344 (1985). Figs 4, 7–12, Plate 1.

Symptoms: *Leaf spots* containing numerous flattened, brown sporodochial plates, amphigenous, circular to irregular, radiating, initially discrete, becoming confluent and ultimately covering large areas of the leaf, non-necrotic, brown to black, sometimes causing discoloration of the leaf, 1–5(–8) mm diam.

Causal organism: *Conidiomata* sporodochial, composed of a single layer of radiating, septate, brown hyphae which are formed from a stroma within the sub-stomatal cavity, composed of thick-walled, dark brown mycelial plugs and brown hyphae in the stomatal cells, mycelial plugs erumpent through stomata forming black, pulvinate sporodochial plates, 25–110 µm diam; hyphae radiating from sporodochial plates, adhering closely to the host surface, following the contours of the cell borders, 2.5–4 µm wide. *Conidiophores* erect or ascending lateral branches on the superficial hyphae, simple, brown, effuse but occurring mainly towards the centre of the sporodochial plate, above the stomata, sometimes proliferating to form hyphae. *Conidiogenous cells* doliiform to calyciform, holoblastic with percurrent proliferation resulting in up to four irregular, ragged annellations, (2.5–)3.5–4(–5) µm high, (2.5–)3.5–4(–5) µm wide, narrowing to (2–)2.5–3 µm at the base. *Conidia* produced singly from blown out ends of conidiogenous cells, solitary, separating rhexolytically from the conidiogenous cell, one- or multi-celled, with the latter ultimately separating into single-celled entities, but often remaining attached to form fragile chains of up to 3 cells, oblong or bacilliform, with thick, verrucose walls, marginal frill at one or often both ends remaining after conidial secession; apically produced conidia with a rounded apex and truncate base with a marginal frill, apex and base of subapical truncate and marginal frills at each end; aseptate conidia, (4–)5.5–6.5(–8) × (3–)4–5(–6) µm, two-celled structures, (6.5–)7.5–9(–11.5) × (3.5–)4.5–5.5(–7) µm, three-celled structures, 11–13 × 6–7 µm.

Cultural characteristics: *Colonies* iron grey (25’’’’k), reverse olivaceous black (27’’’’k), smooth with irregular margins; aerial mycelium sparse and grey or lacking; moderately slow growing, up to 40 mm in diam after 60 days,.

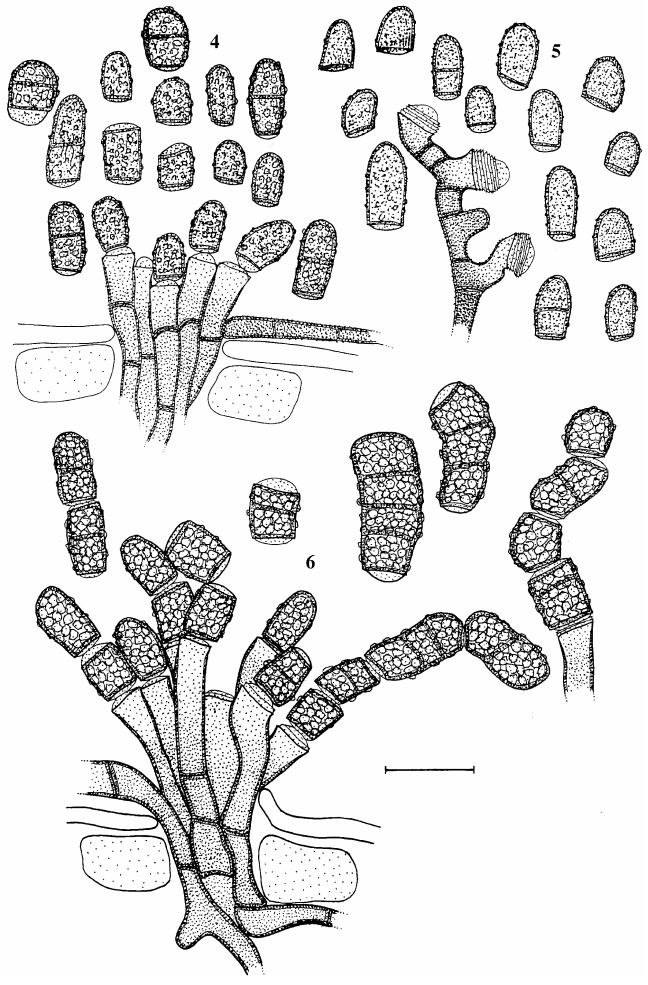
Host range and geographic distribution: *Lcd. argenteum, Lcd. coniferum, Lcd. discolor, Lcd. elimense*, *Lcd. gandogeri, Lcd. laureolum, Lcd.* ‘Pisa’, *Lcd. salicifolium, Lcd. salignum, Lcd. uliginosum, Lcd. xanthoconus, Leucadendron* spp. (**South Africa**) (Van Wyk *et al.*, 1985b; Taylor *et al*., 1999).

Batcheloromyces leucospermi Joanne E. Taylor & Crous, *Mycological Research* **103**: 1479 (1999). Figs 5, 13–19.

Symptoms: *Leaf spots* containing sparsely scattered, effuse, flattened, brown sporodochial plates, 2–5 mm diam, mainly epiphyllous, circular to irregular, radiating, initially discrete, becoming confluent, covering large areas of the leaf, non-necrotic, pale to dark brown and darker in the centre, causing discoloration of the leaf.

Causal organism: *Conidiomata* sporodochial, effuse, spreading, composed of a single layer of radiating, branched, septate, brown hyphae which are formed from stromata within the substomatal cavities composed of thick-walled, dark brown mycelial plugs, 55–106 µm; hyphae radiate from the sporodochial plates

and adhere closely to the host surface, 2.5–4 µm wide. *Conidiophores* erect or ascending lateral branches on the superficial hyphae, simple or branched, brown, effuse but occurring mainly towards the centre of the sporodochial plates, above the stomata, sometimes proliferating to form hyphae. *Conidiogenous cells* doliiform to cylindrical, proliferating percurrently resulting in up to three irregular, ragged annellations, (4–)5.5–6.5(–10) × (2.5–)3–3.5(–4) µm. *Conidia* arising singly from blown out ends of the conidiogenous cells, one-celled, often remaining as multicellular structures in fragile chains of up to 8 conidia; ellipsoidal or oblong, with thick, verrucose walls, ornamentation appearing to occur in irregular rough plates. Marginal frills at one or often both ends of the conidia remaining after conidial secession; single or apically produced conidia with a rounded apex and truncate base with a marginal frill, subapical conidia truncate ends, both with a marginal frill; aseptate conidia, (6–)7–9(–14) × (4–)5.5–6.5(–8) µm, two-celled structures, (5.5–)8–9.5(–12) × (4–)5–5.5(–7) µm, three-celled structures, 9–15 × 5–7 µm, four-celled structures, 12–18 × 4–7 µm.



Figs 4–6. Conidia and conidiophores of *Batcheloromyces* spp. Fig. 4. *B. leucadendri.* Fig. 5. *B. leucospermi*. Fig. 6. *B. proteae.* Scale bar = 10 µm. (From Taylor *et al*., 1999).

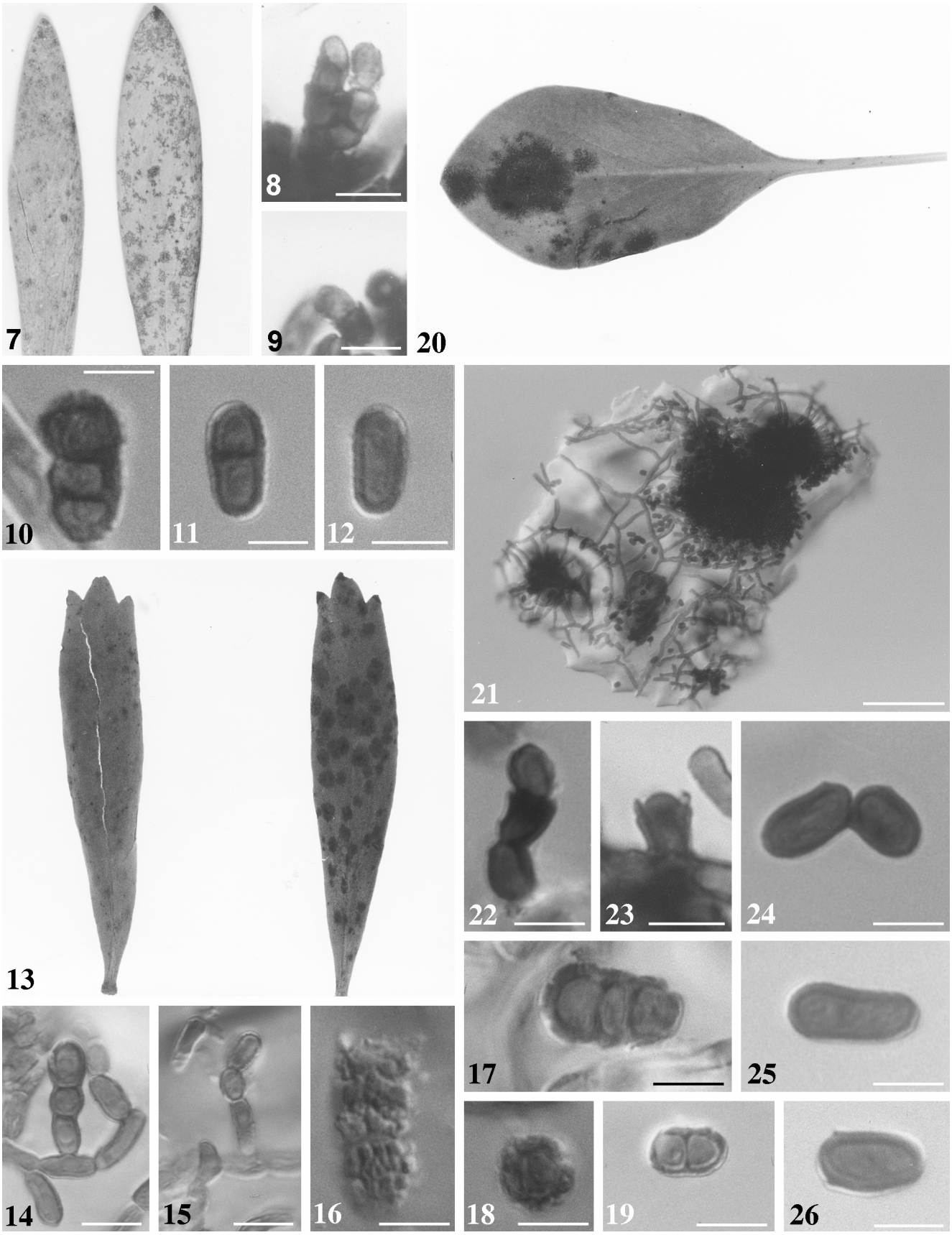
Cultural characteristics: *Colonies* iron grey (25”””k), olivaceous black (27”””k) in reverse, smooth with irregular margins; aerial mycelium sparse and grey or lacking; moderately slow growing, up to 40 mm diam after 60 days.

Host range and geographic distribution: *Leucospermum* spp. (**South Africa**) (Taylor *et al*., 1999); *Protea* sp. (**USA, California**) (Taylor *et al*., 2001b).

Batcheloromyces proteae Marasas, P.S. Van Wyk, & Knox-Dav., *Journal of South African Botany* **41**: 43 (1975). Figs 6, 20–26, Plate 1.
≡ *Stigmina proteae* (Marasas, P.S. Van Wyk, & Knox-Dav.) B. Sutton & Pascoe, *Mycological Research* **92**: 214 (1989).

Symptoms: *Leaf spots* containing numerous black sporodochial plates, amphigenous, circular, radiating, discrete but sometimes becoming confluent and covering large areas of the leaf surface, non-necrotic, highly variable, sometimes restricted and consisting only of groups of sporodochia causing little discoloration and disruption of the host tissue; or becoming somewhat erumpent; or more commonly causing red-brown discoloration which may be visible on corresponding areas of the opposite leaf surface, 1–25 mm. Lesions sometimes spreading to petioles but not encountered on stems.

Causal organism: *Conidiomata* sporodochial, composed of a single layer of radiating, septate, branched, pale to dark brown, thick-walled hyphae which are formed from a stroma within the substomatal cavity, composed of thick-walled, dark brown mycelial plugs, plugs erumpent through the stomata and forming black, pulvinate plates, 100–120 µm diam; hyphae radiating from sporodochial plate and adhering closely to the host surface or growing into the stomata, 2.5–4 µm wide. *Conidiophores* erect or ascending, short lateral branches on the superficial hyphae, simple, brown, effuse, but occurring mainly towards the centre of the sporodochial plates, above the sto-mata, terminating to produce conidiogenous cells or sometimes proliferating to form hyphae. *Conidiogenous cells* mainly calyciform, but occasionally doliiform, proliferating percurrently resulting in up to three irregular, ragged annellides, (3–)3.5–4(–5) µm high × (3–)3.5–4(–5) µm diam, narrowing to (2–)2.5–3(–3.5) µm at the base (or 3.5–4 × 2.5–3.5 µm when doliiform). *Conidia* arising singly from blown out ends of the conidiogenous cells, solitary, unicellular, but often remaining in fragile chains of 2 conidia or appearing as multicellular structures, ellipsoidal, oblong or bacilliform, with thick, smooth to verrucose walls, a marginal frill at one or often both ends resulting from conidial secession, (4–)6.5–7(–9) × (3–)3.5–4(–5) µm.



Figs 7–26. Species of *Batcheloromyces*. Figs 7–12. *B. leucadendri*. Fig. 7. Leaf spot on *Leucadendron* sp. Figs 8–9. Conidiogenous cells. Figs 10–12. Aseptate and multiseptate conidia. Figs 13–19. *B. leucospermi*. Fig. 13. Leaf spot on *Leucospermum* sp. Figs 14–15. Conidiogenous cells giving rise to conidia. Figs 16–19. Aseptate and septate conidia, showing ornamentation. Figs 20–26. *B. proteae.* Fig. 20. Leaf spot on *Protea cynaroides*. Fig. 21. Sporodochia developing from substomatal stromata. Figs 22–23. Conidiogenous cells giving rise to conidia. Figs 24–26. Aseptate conidia occurring singly, or in chains. Scale bars: 8–12, 16–19, 22–26 = 5 µm, 14–15 = 10 µm, 21 = 50 µm. (From Taylor *et al*., 1999).

Cultural characteristics: *Colonies* olivaceous (19”k), the same in reverse, smooth with irregular margins, sectored; aerial mycelium lacking; slow-growing, approximately 10–18 mm diam after 60 days.

Host range and geographic distribution: *P. cynaroides* (**Madeira**) (Moura & Rodrigues, 2001); *P. cynaroides*, *P. grandiceps, P. magnifica*, *P. neriifolia*, *P. punctata*, *P. repens* (**South Africa**) (Marasas *et al.*, 1975; Smit *et al*., 1983; Van Wyk *et al.*, 1985b; Knox-Davies *et al*., 1986); *P. magnifica* (**Zimbabwe**) (Masuka *et al*., 1998).

PATHOLOGY OF BATCHELOROMYCES *SPP.*

Although studies have thus far only been carried out on symptom development of *B. proteae*, the pathogenic habit of all three species is similar. *Batcheloromyces* species are biotrophic, the stromata are restricted to the stomata, and leaf spots are due mainly to the presence of the fruiting bodies. Sometimes the leaf tissue is discoloured but no chlorosis or necrosis occurs. On *P. cynaroides,* which is an extremely variable species, *B. proteae* causes a wide variety of symptoms (Smit *et al.*, 1983). Smit *et al*. (1983) also noted that some variants were particularly susceptible to *B. proteae*, whereas others showed no symptoms. It was suggested that variation is due to physiological differences in host genotype as different individuals of the same host showed similar symptoms. Von Broembsen (1989) stated that resistant plants had been observed, but not selected for use in resistant cultivars.

Disease cycle and epidemiology: The life cycle is unknown, but Smit *et al*. (1983) noted that the lesions have a dry, sooty covering of spores in moist weather. It is likely that new infections are initiated by windborne conidia. Von Broembsen (1989) suggested that *B. proteae* might be seedborne since it had appeared in areas where the sole form of propagation material was seed. However, its absence from nearly every area surveyed outside of South Africa would suggest that it is probably not seedborne. No species of *Batcheloromyces* has ever been recorded as an endophyte from symptomless material or from treated or untreated seeds.

Control: There are no fungicides registered for use against *Batcheloromyces* spp. Von Broembsen (1989) noted that control has been poor even when spray programmes with general fungicides have been carried out in the field.

KEY TO BATCHELOROMYCES *SPECIES*

See key to *Mycosphaerella* anamorphs.

Botryosphaeria leaf blight and stem canker

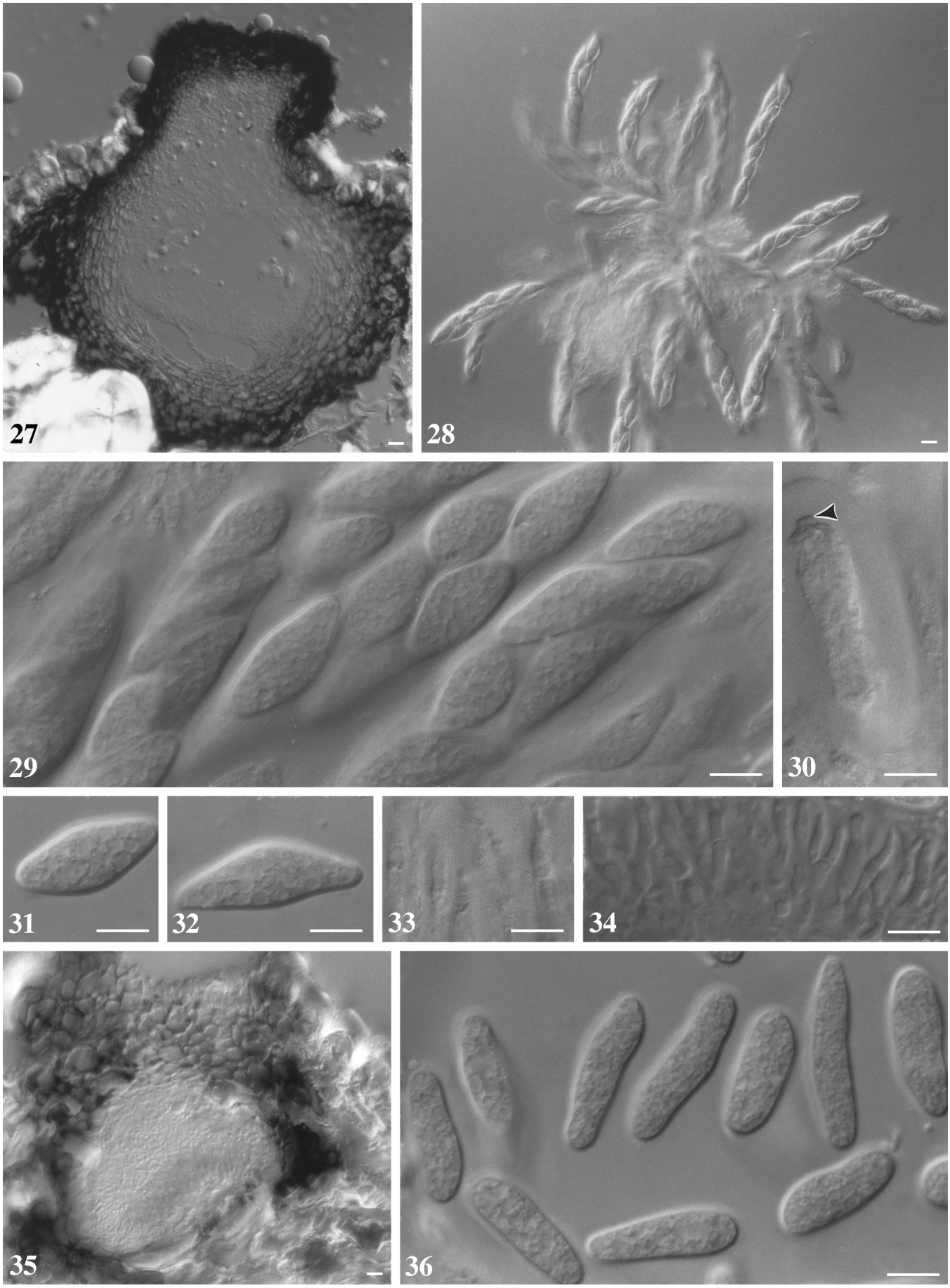
Botryosphaeria protearum Denman & Crous, *Mycologia* **95**: 301 (2003). Figs 27–39, Plates 14, 15.

Anamorph: ***Fusicoccum protearum*** Denman & Crous, *Mycologia* **95**: 301 (2003).

Symptoms: *Leaf spots* pale to medium brown, usually associated with blight along the veins or margins, lesions extending down the petiole into the stem from where they mainly spread downwards causing stem cankers. Stem cankers sunken, dark brown to black, usually killing the whole branch but sometimes also leading to death of the whole plant.

Causal organism: *Ascomata* pseudothecial, embedded in host tissue, up to 600 µm diam, becoming erumpent, solitary, stromatic, dark brown to black, with central, black ostioles; pseudothecial wall 6–15 cell layers thick, composed of brown *textura angularis*. *Asci* clavate to subcylindrical, 110–200 × 15–21 µm, 8-spored, bitunicate, with a well-developed apical chamber, which becomes inconspicuous at maturity, interspersed amongst filiform, branched, septate, 3–5 µm wide pseudoparaphyses. *Ascospores* irregularly biseriate, hyaline, nonseptate, granular, becoming pale brown with age, (25–)26–33(–37) × (9–)10–12(–13) µm, sometimes inequilateral, fusiform, widest in the middle with obtuse ends.

Conidiomata pycnidial, embedded in host tissue, solitary or botryose, stromatic, globose, up to 500 µm diam, pycnidial wall, 4–8 cell layers thick, composed of brown *textura angularis*, becoming hyaline towards the inner region. *Conidiophores* 0–1-septate, hyaline, subcylindrical, rarely branched, 7–20(–30) × 3–5 µm. *Conidiogenous cells* holoblastic, hyaline, subcylindrical, 7–12 × 3–5 µm, rarely proliferating percurrently with 1–2 proliferations, predominantly proliferating at the same level with minute periclinal thickening, which becomes more prominent in older conidiogenous cells. *Conidia* hyaline, granular, ovoid to clavate when young, becoming irregularly fusoid when mature, widest in the middle with an obtuse apex and bluntly rounded or minutely flattened base (inconspicuous in older, permanent mounts), (20–)25–30(–40) × 7–8(–10) µm *in vivo*. *Spermatial state* occurring in conidiomata with the *Fusicoccum* anamorph, or in separate spermogonia. *Spermatiophores* hyaline, smooth, branched, cylindrical, 0–2-septate, straight, unbranched or branched above, 12–17 × 2–3 µm. *Spermatiogenous cells* discrete or integrated, hyaline, smooth, cylindrical, proliferating via determinate phialides with periclinal thickening, 5–12 ×



Figs 27–36. *Botryosphaeria protearum* and its anamorph *Fusicoccum protearum*. Fig. 27. Vertical section through a pseudothecium. Figs 28–29. Asci and ascospores. Fig. 30. Ascus with apical chamber (arrowed). Figs 31–32. Ascospores. Fig. 33. Pseudoparaphyses. Fig. 34. Conidiophores. Fig. 35. Vertical section through a pycnidium. Fig. 36. Conidia. Scale bars = 10 µm. (From Denman *et al*., 2003).

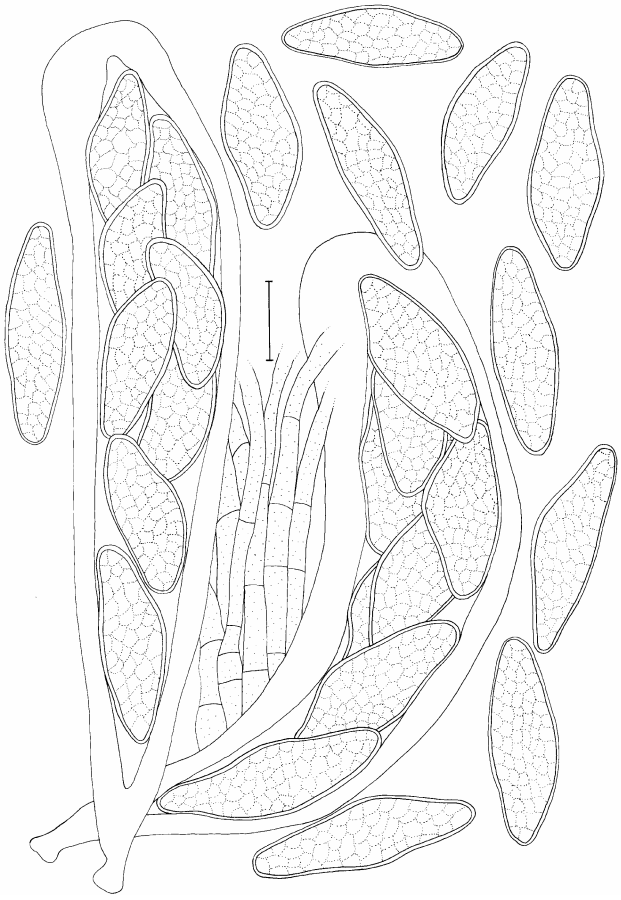


Fig. 37. Asci, ascospores and pseudoparaphyses of *Botryosphaeria protearum.* Scale bar = 10 µm. (From Denman *et al*., 2003).

1.5–2.5 µm. *Spermatia* hyaline, smooth, aseptate, rod-shaped with rounded ends, 3–6 × 1–1.5 µm.

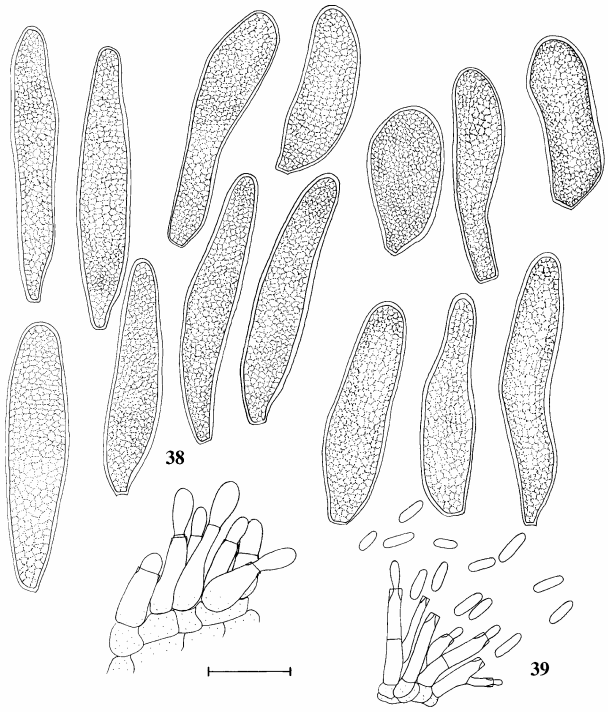
Cultural characteristics: *Colonies* initially translucent to white, gradually darkening from the centre, olive-green to grey (4–7 days), ultimately becoming charcoal-black (14–21 days). Initially with a moderate amount of aerial mycelium, but this collapses as hyphae reach the edge of Petri dishes, resulting in flat colonies with rims of loose aerial mycelium at the edge of the dish. Black conidiomatal initials sometimes formed, beginning in the centre of the colonies and spreading over the entire colony surface. Sporulation not occurring *in vitro*. Colonies olivaceous-grey (23$^{''''''}$i) to iron-grey (25$^{''''''}$k), reverse greenish-black (33$^{''''''}$k); aerial mycelium around the edge of the dish ranging from smoke-grey (21$^{''''}$f) to pale olivaceous-grey (21$^{''''''}$f); 25.5 mm/d at 25°C. Cardinal temperatures for growth, minimum below 5°C, optimum 25°C, maximum above 35°C. No growth was recorded at 40°C.

Host range and geographic distribution: *Leucadendron* sp., *P. magnifica* (**Australia**); *Lcd. tinctum, P.* 'Pink Ice' (**Madeira**); *Leucadendron* sp. (**Portugal**); *Lcd.* 'Silvan Red', *Leucadendron* spp., *P. cynaroides, P. eximia*, *P. magnifica, P. neriifolia*, *P. repens* (**South Africa, WC**); *P. cynaroides* (**USA, Hawaii**).

Disease cycle and epidemiology: *Botryosphaeria protearum* exists as an endophyte in leaves. Symptoms of disease first become evident in spring and usually do not occur in the top flush but rather in the previous year's growth. Leaf necrosis is thus initiated in spring and summer, and stem cankers are evident towards the end of summer and in autumn. This is also when the fungus usually forms fruiting structures. Von Broembsen & Van der Merwe (1990) reported that spore dispersal took place in spring and summer, especially after rain events in the latter season.

Frequently, necrosis begins on only one or two leaves from where the fungus spreads into the branch, killing other leaves in its passage. The disease can be recognised at a distance by branches bearing healthy-looking upper flushes followed by a completely necrotic flush supported by a healthy-looking flush beneath the dead part.

Control: Integrated control using a variety of strategies is advocated to reduce the impact of *Botryosphaeria* infection on *Protea* spp. (Von Broembsen & Van der Merwe, 1990; Forsberg, 1993; Denman, 2002). Cultivation sites that have a dry microclimate and avoid stress through over- or under-irrigating plants should be selected. Sanitation pruning alone can reduce disease occurrence by at least 30% and this should be



Figs 38–39. *Fusicoccum protearum*. Fig. 38. Conidia and conidiophores. Fig. 39. Spermatia and spermatiophores. Scale bar = 10 µm. (From Denman *et al*., 2003).

practiced fortnightly from spring to late autumn. All prunings must immediately be removed and burnt.

Fungicides applied prophylactically give a high degree of disease control but curative treatments are less successful. If disease is visible, diseased material must be removed and a regular fungicide application programme implemented, taking special care to keep the leaves protected after rain or high humidity events. The standard practice of alternating mancozeb fortnightly with prochloraz-manganese chloride gives excellent protection if adhered to strictly. After each rainfall, a systemic fungicide or a combination of contact and systemic fungicides must be applied. In field trials, Denman *et al*. (2004) obtained good results with the contact fungicide iprodione and the systemic fungicides bitertanol, fenarimol and tebuconazole.

KEY TO BOTRYOSPHAERIA *SPECIES ON* PROTEACEAE

See Section 3, Chapter 2, *Botryosphaeria* stem pathogens of *Proteaceae* and a key to *Botryosphaeria* species.

Calonectria leaf spot

Cylindrocladium colhounii, the anamorph of *Calonectria colhounii* and the most frequently encountered form of the fungus, has a wide geographic distribution and host range. It causes diseases ranging from leaf spot to fruit rot and stem canker. Generally it is not host-specific, and requires high humidity and rainfall conditions for disease development, hence it is more important in the tropics (Crous, 2002).

Other *Cylindrocladium* species that have been isolated from *Proteaceae* include *C. parasiticum* (Hawaii), and *C. pauciramosum* (South Africa). The latter two species have been associated with root rot and are sometimes isolated from nursery cuttings, but their relative importance as a pathogen of proteas remains to be determined.

Calonectria colhounii Peerally, *Transactions of the British Mycological Society* **61**: 92 (1973).

Figs 40–43.

Anamorph: ***Cylindrocladium colhounii*** Peerally, *Transactions of the British Mycological Society* **61**: 92 (1973).

Symptoms: Leaf spots circular, varying from tan to brown in colour.

Causal organism: *Ascomata* perithecial, solitary or in groups, bright yellow; in cross-section subglobose to ovoid, 350–500 µm high, 320–400 µm diam, base turning dark red in KOH; perithecial apex consisting of flattened, thick-walled hyphal elements with rounded tips forming a palisade, discontinuous with warty outer layer, gradually becoming thinner towards the ostiolar canal, and merging with outer periphyses; perithecial walls rough, consisting of two thick-walled layers: outer layer of *textura globulosa*, 35–80 µm thick, becoming more compressed towards inner layer of *textura angularis*, 10–15 µm thick, becoming thin-walled and hyaline towards the centre, outer cells 20–40 × 12–38 µm, inner cells 8–25 × 2–6 µm; perithecial base up to 170 µm wide, consisting of dark red, angular cells, merging with an erumpent stroma, cells of the outer wall layer merging with the pseudoparenchymatous cells of the erumpent stroma. *Asci* (1–)4-spored, clavate, 80–180 × 15–30 µm, tapering into a long thin stalk. *Ascospores* aggregated in the upper third of the ascus, hyaline, guttulate, fusoid with rounded ends, straight to slightly curved, (1–)3-septate, granular, not or slightly constricted at the septa, (30–)50–65(–75) × (4–)5–6(–8) µm (mean = 55 × 6 µm).

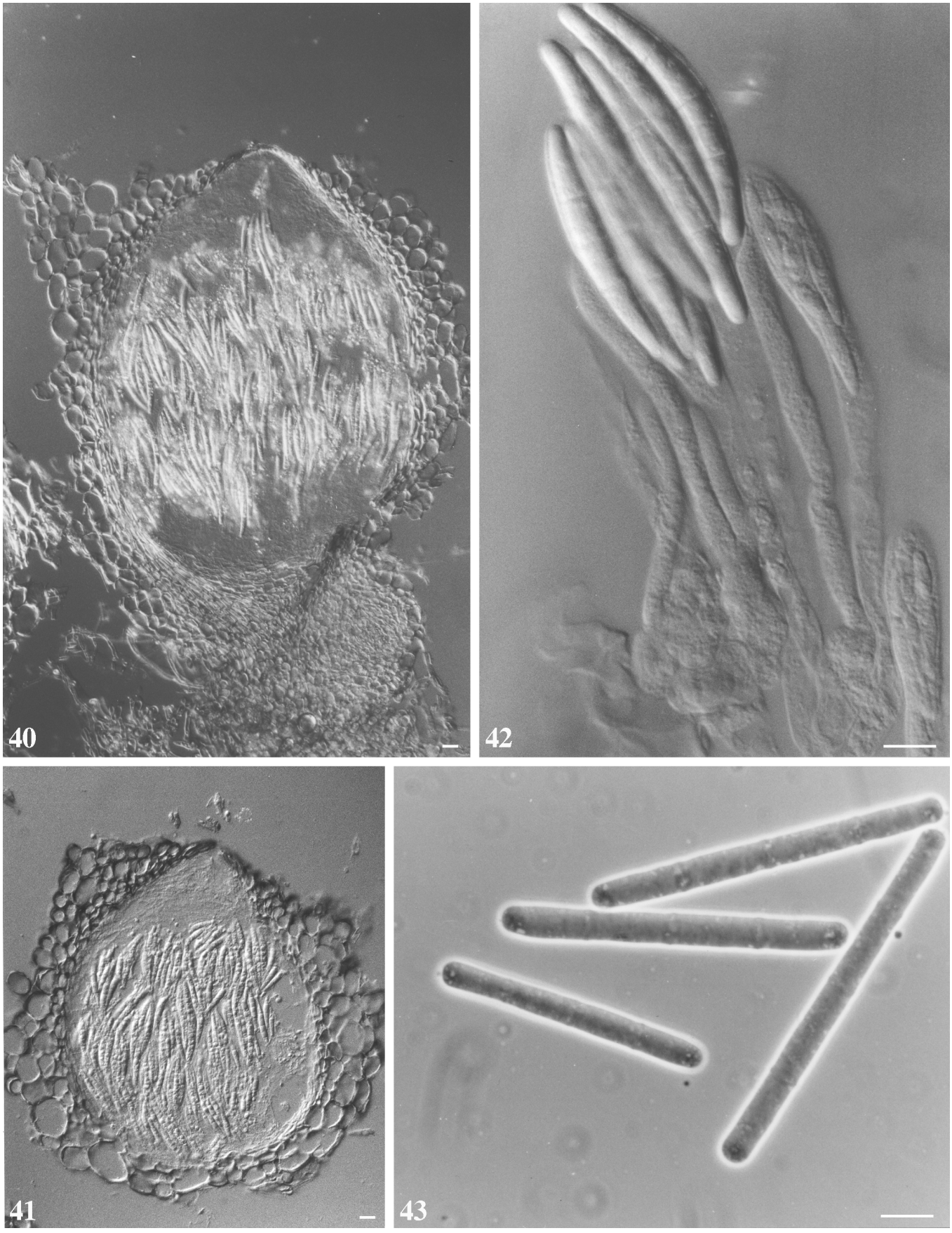
Conidiomata consisting of a stipe, a penicillate arrangement of fertile branches, a stipe extension, and a terminal vesicle; stipe septate, pale brown at base, becoming hyaline, smooth, 80–190 × 6–7 µm; stipe extension septate, straight to flexuous, 160–280 µm long, 3–4 µm wide at apical septum, terminating in a clavate vesicle, 3–4 µm diam. *Conidiophores* 50–80 µm long, 40–80(–140) µm wide; primary branches aseptate or 1-septate, 12–30 × 3–7 µm; secondary branches aseptate, 12–20 × 3–5 µm, tertiary and additional branches (up to 5) aseptate, 7–13 × 3–5 µm, each terminal branch with 2–6 phialides; *conidiogenous cells* phialidic, elongate doliiform to reniform, hyaline, aseptate, 7–16 × 3–4 µm, apex with minute periclinal thickening and inconspicuous collarette. *Conidia* cylindrical, rounded at both ends, straight, (45–)60–70(–80) × (4–)5(–6) µm (mean = 65 × 5 µm), (1–)3-septate, lacking a visible abscission scar, held in cylindrical clusters by colourless slime.

Cultural characteristics: *Colonies* tawny (13'i) (reverse); chlamydospores abundant, in coarse chains throughout the medium, eventually forming microsclerotia. Cardinal temperatures for growth, minimum above 5°C, maximum below 35°C, optimum 25°C.

Host range and geographic distribution: *Lsp. conocarpodendron, Lsp. cordifolium*, *Leucospermum* spp. (**USA, Hawaii**) (Uchida *et al*., 1996; Uchida & Aragaki, 1997). The fungus occurs in South Africa and Australia but has not been reported on *Proteaceae* (Crous, 2002).

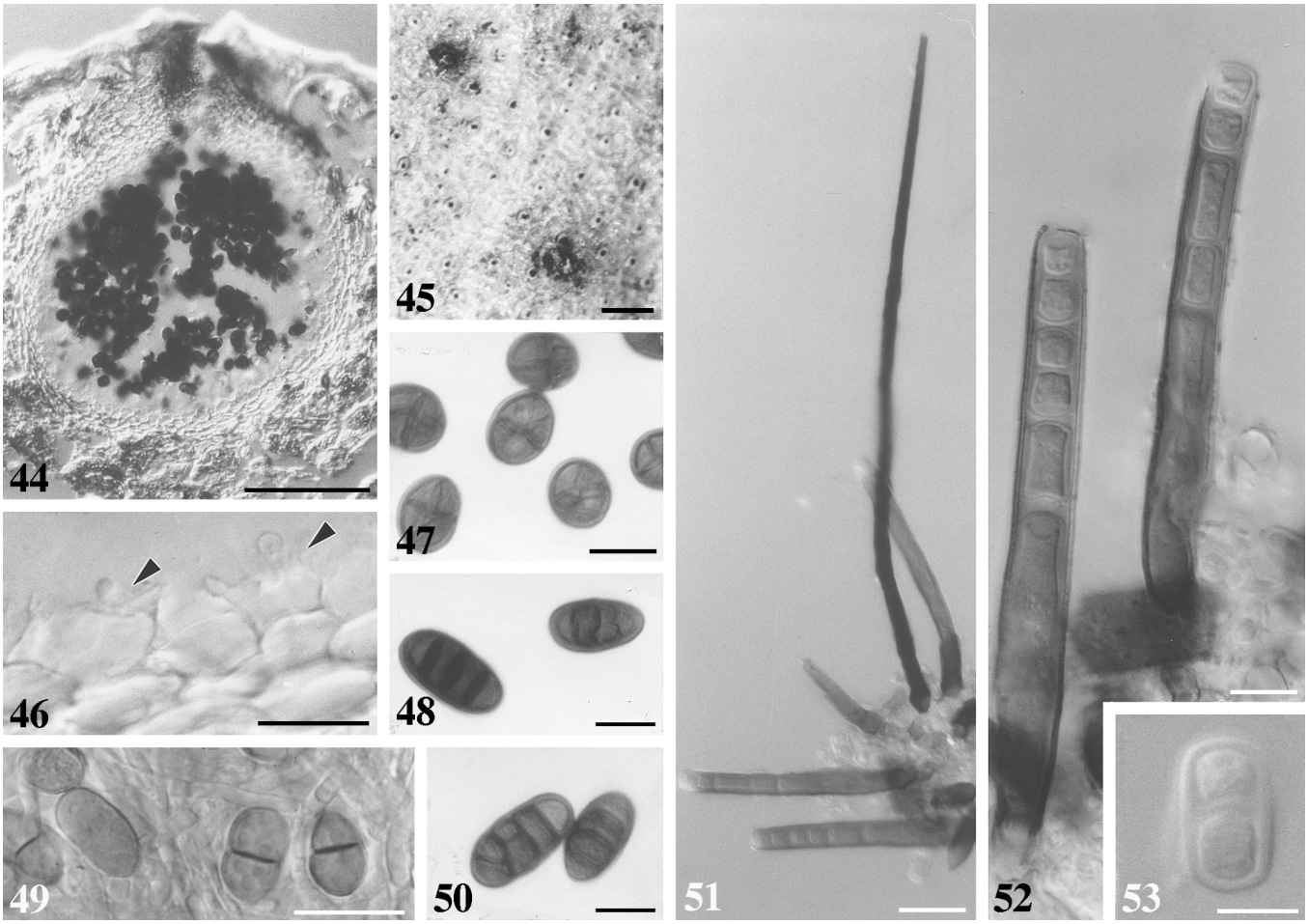
Disease cycle and epidemiology: Ascospores are wind-dispersed and conidia splash-dispersed. The fungus forms microsclerotia in debris and soil, which act as survival propagules and can remain viable for several years in soil. Microsclerotia can also initiate new infections.

Control: Leaf spot diseases caused by species of *Cylindrocladium* can be effectively controlled by a combination of good nursery hygiene and the application



Figs 40–43. *Calonectria colhounii* and its anamorph *Cylindrocladium colhounii*. Figs 40–41. Vertical sections through perithecia. Fig. 42. Asci containing ascospores. Fig. 43. Conidia. Scale bars = 10 µm.

of systemic fungicides. Diseased plants should be removed immediately, potting medium should be disinfested and allow good drainage, plants should be watered minimally, and overhead- or mist-irrigation should be avoided. Nair & Jayasree (1986) found foliar applications of Bordeaux mixture, carbendazim



Figs 44–53. *Camarosporium* sp. and *Chaetochalara proteae*. Figs 44–50. *Camarosporium* sp. Fig. 44. Vertical section through a conidioma. Fig. 45. Conidiomata on host substrate. Fig. 46. Conidiogenous cells showing wide phialides (arrowed). Figs 47–48, 50. Conidia *in vivo*. Fig. 49. Conidia *in vitro*. Figs 51–53. *Chaetochalara proteae*. Fig. 51. Setae and conidogenous cells. Figs 52. Conidiogenous cells. Fig. 53. Conidium. Scale bars: 44–45 = 100 µm, 46–50, 52 = 10 µm, 51 = 20 µm, 53 = 5 µm. (From Taylor *et al*., 2001a).

and chlorothalonil to be effective against *C. colhounii*. Keeping plants well spaced to facilitate air circulation and reduce humidity is fundamental to suppressing disease caused by *Cylindrocladium* in nursery environments.

Camarosporium leaf spot

Camarosporium is a widespread genus with approximately 100 species which are anamorphs of *Cucurbitaria*, *Leptosphaeria* and *Pleospora* (*Cucurbitariaceae*, *Dothideales*) (Kirk *et al*., 2001). The *Camarosporium* sp. occurring on *Proteaceae* has not been described to species level as the genus is in need of revision. It is possible, however, that the collections from *Proteaceae* represent the anamorph of one of the *Leptosphaeria* or *Pleospora* spp. which have been collected from *Proteaceae* (Taylor *et al*., 2001b) but for which connections in culture could not be confirmed.

Camarosporium sp. Figs 44–50.

Symptoms: *Leaf spots* circular to irregular, necrotic, sunken, grey-brown with a raised dark brown margin, up to 25 mm diam.

Causal organism: *Conidiomata* amphigenous, pycnidial, substomatal, subepidermal, not erumpent but raising the host surface slightly, dark brown, 90–170 µm diam; globose in cross section, with a central, non-papillate ostiole; reduced stroma consisting of brown fungal hyphae in host cells surrounding the ostiole; approximately 270–300 µm high, 250–300 µm diam. *Peridium* composed of an outer stratum of thick-walled, small-celled, pale brown *textura angularis* becoming thin-walled in the inner layer; with the innermost cells thin-walled and hyaline, 20–25(–45) µm diam. *Condiogenous cells* lining conidiomal cavity, subglobose, hyaline, determinate to indeterminate, 6–9 × 5–8 µm; conidiogenesis holoblastic, infrequently proliferating percurrently resulting in up to three, wide annellations. *Conidia* red-brown, muriform, with transverse, longitudinal and oblique eusepta, oblong, ellipsoidal to globose, base truncate, apex obtuse,

thick-walled, verruculose, (9–)12–14(–17) × (6–)7.5–8(–10) µm. *In vitro conidiogenous cells* pyriform to globose, hyaline, 7–12 × 7–9 µm. *Conidia* initially hyaline, becoming medium red-brown at maturity, 0–1(–3)-septate, thinner-walled, smooth, 7–12 × 7–9 µm.

Cultural characteristics: *Colonies* circular with undulate margins at 25°C in the dark, buff (19"d), often with darker patches, reverse brown-vinaceous (84"'m), with broad concentric bands of buff (19"d) and brown-vinaceous (84"'m); moderately dense, felty to woolly aerial mycelium; 60 mm diam after 20 days.

Host range and geographic distribution: *Lsp. cordifolium, P. cynaroides* (**USA, California**) (Taylor *et al*., 2001b).

Chaetochalara leaf spot

Chaetochalara is similar to *Chalara*, differing from it only in the production of setae (Nag Raj & Kendrick, 1975; Kirk & Spooner, 1983). *Chaetochalara aspera and C. proteae* have been recorded on *Proteaceae*, but *C. aspera* is not a significant pathogen and is known only on *Knightia excelsa* in New Zealand. The two species are similar in that the conidiogenous cells of both have asperate or roughened walls, but differ in that *C. aspera* produces longer, narrower, thin-walled conidia (10–27 × 2.5–4 µm) and longer conidiophores (48–140 µm).

Chaetochalara proteae Joanne E. Taylor, Crous & M.E. Palm, *Mycotaxon* **78**: 451 (2001). Figs 51–53.

Symptoms: *Leaf spots* circular, necrotic, sunken with raised borders, pale yellow-brown.

Causal organism: *Colonies* amphigenous, diffuse, sparse, dark brown to black. *Fertile hyphae* superficial, branched, arising above or from stomata, hyaline or brown, smooth-walled, bearing phialides and setae. *Setae* simple, erect, up to 8-septate, uniformly dark brown to red-brown, smooth-walled, up to 250 µm long, slightly bulbous at the base, approx. 7.5 µm diam, tapering gradually towards the apex, 2–3 µm diam, *Conidiogenous cells* phialidic, mainly borne directly on the fertile hyphae, or singly on short conidiophores, subcylindrical, pale to medium brown, verruculose, regeneration of the conidiogenous cell can occur at the 7–10 µm wide base, narrowing slightly to 6–8 µm at the apex, and 70–90 µm long. *Conidia* in chains, cylindrical, rounded at the apex and truncate at the base, with an inconspicuous frill, 1-septate, hyaline, with a smooth and relatively thick (0.5–1 µm wide) wall, not constricted at the septum, (9–)10.5–12(–14) × (5–)6.5–7 µm.

Host range and geographic distribution: *P.* 'Pink Ice' (**USA, Hawaii**) (Taylor *et al*., 2001a).

Coleroa leaf spot

Coleroa senniana causes lesions on leaves of species of *Protea* and *Banksia* and occasionally on *Leucadendron* and *Leucospermum*. Although infected plants rarely die, economically significant losses occur through reduction in aesthetic value of blooms and rejections due to phytosanitary concerns. *Coleroa* mainly causes lesions on older material. Furthermore, there have been reports of secondary invasion of the *Coleroa* lesions by *Fusicoccum* "*aesculi*", which invades the inner host tissues when the ascomata of *C. senniana* erupt through the cuticle. On *P. magnifica* this relationship results in necrotic lesions, whereas on *Leucospermum* spp. it is associated with symptoms of "copper-leaf" (Serfontein & Knox-Davies, 1990a).

Coleroa senniana is the most frequently encountered organism on South African proteas at quarantine inspection points (Taylor, 2001). However, it is no longer considered of quarantine significance by the United States due to recent reports of its occurrence in Australia (Crous *et al*., 2000b), California (Taylor *et al*., 2001b), Hawaii (Taylor *et al*., 2001a), and Madeira (Moura & Rodrigues, 2001).

Coleroa senniana (Sacc.) Arx, *Die Gattungen der Didymosporen Pyrenomyceten. Beiträge zur Kryptogamenflora der Schweiz* **11 (2)**: 418 (1962). Figs 54–61, Plates 2, 3.

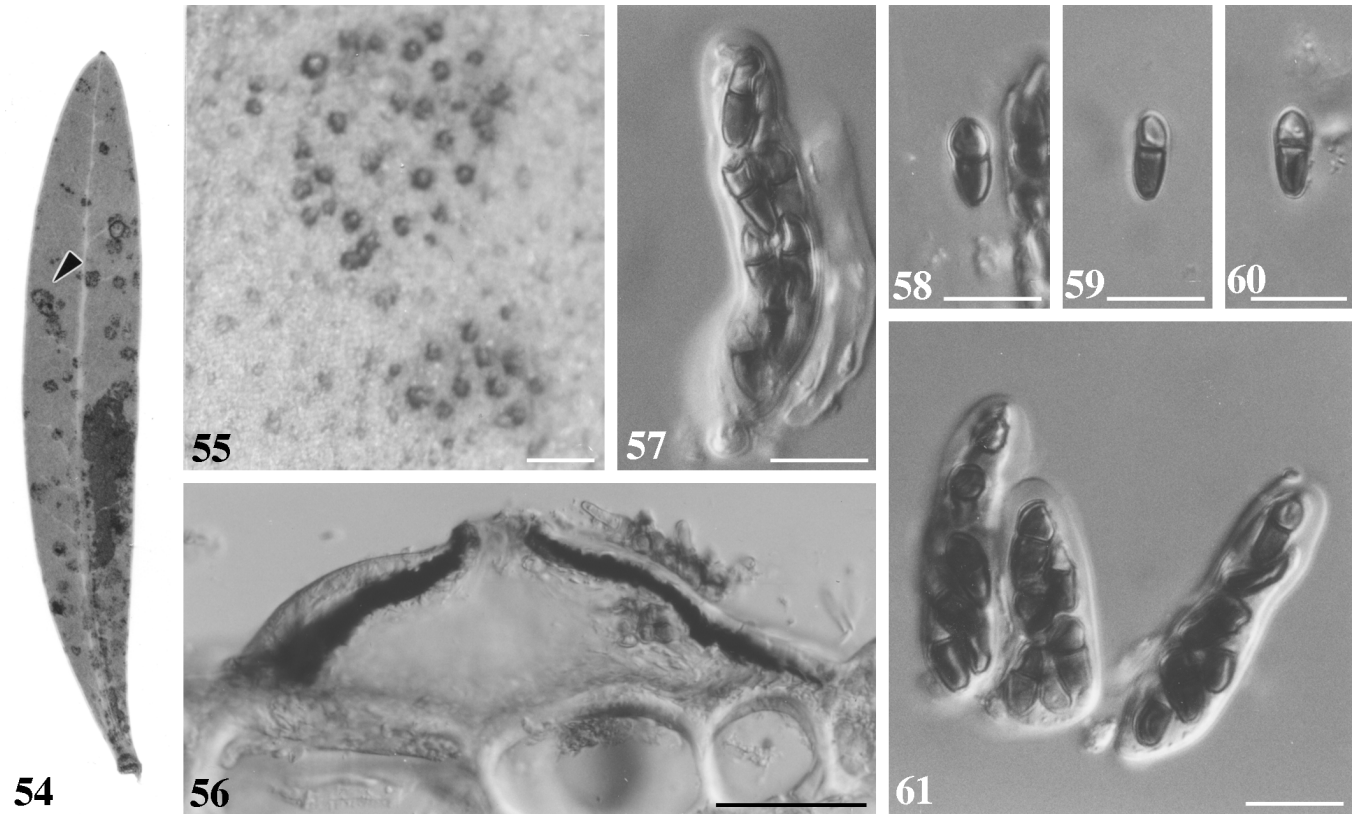
≡ *Phaeosphaerella senniana* Sacc., *Annales Mycologici* **8**: 337 (1910).

≡ *Aphysa senniana* (Sacc.) Doidge, *Bothalia* **4**: 213 (1941).

= *Thyriopsis proteae* Van der Byl, *South African Journal of Science* **26**: 318 (1929).

Symptoms: *Leaf spots* irregular, seldom circular in outline, discrete or confluent, variable according to host, from raised, yellow-brown zones to apparently healthy leaf tissue in which few to many ascomata are embedded, often forming distinct spots or large irregular patches, <10 mm diam.

Causal organism: *Ascomata* amphigenous, grouped and widely separated, scattered or gregarious, black, angular to irregular or circular in outline, cuticular or subcuticular, (79–)125–188(–210) µm diam; in cross section shallow-conical with a flat base, asci radiating from the margin of the ascoma with the apices pointing towards the centre, often with a central column of sterile material, ostiolar pore central or excentric, more or less circular and 25 µm diam , (48–)51.5–61(–70) µm high, (158–)167–205(–237) µm diam. *Peridium* at the base comprised of thin-walled, hyaline, compressed cells, 5–10 µm diam, upper wall comprised of



Figs 54–61. *Coleroa senniana*. Fig. 54. Leaf spot on a *Protea* sp. Fig. 55. Leaf spot with subcuticular ascomata. Fig. 56. Vertical section through an ascoma. Figs 57, 61. Asci. Figs 58–60. Ascospores. Scale bars: 55 = 2 mm, 56 = 50 µm, 57–61 = 10 µm. (From Taylor & Crous, 1998).

1–2 layers of dark brown, thick-walled, melanised, compressed cells, 5–8 µm diam., cells 3-5 um diam near pore and nearly opaque, thinner-walled at the margin, uniting at an acute angle with basal layer or extending beyond the edges of the basal layer. *Paraphyses* deliquescent. *Asci* clavate to obclavate, short-pedicellate, bitunicate, with a wide rounded apex and an indistinct ocular chamber, 8-spored, (30–)38–41.5(–48) × (9–)11–12(–16.5) µm. *Ascospores* mainly biseriate, broadly ellipsoidal, obtuse at ends, with one supra-median septum, upper cell shorter and broader, pale yellow-brown, smooth to finely verrucose, mainly straight or asymmetrical, slightly constricted at the septum, (10.3–)12–12.5(–14) × (3.5–)5–5.5(–6.5) µm (Taylor & Crous, 1998a).

Cultural characteristics: *Colonies* grey, raised, felty and convoluted; slow growing, 2–3 mm diam in 7 d on PDA, ceasing growth and remaining sterile after attaining colony diameter of 5−7 mm.

Host range and geographic distribution: *Banksia* spp., *P. lepidocarpodendron, P. magnifica, P.* 'Pink Ice', *P. pudens*, *P. susannae*, *P. lorifolia*, *Protea* sp. (**Australia, NSW, Tasmania**) (Crous *et al*., 2000b), *P. gaguedi* (**Eritrea**) (Saccardo, 1910); *P.* 'Pink Mink', *Protea* spp. (**Madeira**) (Moura & Rodrigues, 2001); *Protea* spp. (**New Zealand**) (Von Broembsen, 1989); *Lcd. argenteum*, *Lsp. cordifolium, P. acaulos, P. amplexicaulis, P. burchellii, P. caffra, P. compacta, P. grandiceps, P. lepidocarpodendron, P. longifolia*, *P. magnifica, P. nitida, P. punctata, P. repens, P. scabra*, *Protea* spp. (**South Africa, EC, NC, WC**), *P. simplex*, *Protea* spp. (**South Africa, KN**), *P. caffra, P. gaguedi* (**South Africa, GT**) (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); *Lsp. cordifoilum, P.* 'Pink Ice', *P. laurifolia*, *P. laurifolia* × *neriifolia, P. magnifica, P.* 'May Day', *P. neriifolia* × *magnifica, P.* 'White Owl', *P. obtusifolia, P. pudens, P.* 'Queen Mink', *Protea* sp. (**USA, California**) (Taylor *et al*., 2001b); *P.* 'Carnival', *P.* 'Lady Di', *P.* 'May Day', *P.* 'Mink', *P.* 'Pink Ice', *P.* 'Ivory Musk', *Protea* sp. (**USA, Hawaii**) (Taylor *et al*., 2001a); *Protea* spp., *P.* 'Brenda', *P.* 'Susara', *P.* 'Moonshine', *P.* 'Red Baron', *Protea* sp. (**Zimbabwe**) (Masuka *et al*., 1998; L. Swart, unpubl. data).

Notes: Symptoms due to *C. senniana* are variable. On *P. caffra* the lesions are small (< 2 mm), yellow-brown and with a few small, black ascocarps widely separated in the lesion or the ascomata can be clustered in groups of 3–5 mm diam as described by Doidge (1941), with no leaf discoloration. *Coleroa senniana* is associated with oily, yellow confluent spotting (so-called 'copper leaf') of *Lsp. cordifolium* (Serfontein & Knox-Davies, 1990a). On *P. magnifica* leaves, the fungus produces tiny, black leaf specks (actually the ascomata) to raised, yellow to brown discoloured

spots. The pseudothecia are found on whichever leaf surface is facing upwards (Serfontein & Knox-Davies, 1990a) and are 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 pale brown discolouration.' Fruiting bodies occur in groups, which are more or less sharply defined and often coalescent (Doidge, 1941). The underlying leaf tissue is apparently health, without any 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*. These were ascribed to secondary pathogens such as *Fusicoccum* "*aesculi*" invading the *Coleroa* lesions.

Coleroa senniana is largely confined to the cuticle but Serfontein & Knox-Davies (1990a) reported that the hyphae also can occur subcuticularly, causing the discoloration seen in the underlying tissue. The pseudothecia are also formed within the cuticle, which ruptures as they enlarge. The basal layer of the pseudothecium 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. 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).

Disease cycle and epidemiology: The disease cycle of this fungus is unknown. It has been suggested that *C. senniana* is seed-borne as it occurs everywhere outside South Africa where proteas have been cultivated and often occurs in plantations raised entirely from seeds. However, it has never been recorded from treated or untreated seeds or as an endophyte in symptomless material. It is biotrophic and does not cause chlorosis or necrosis of the leaf tissue. New growth is usually unaffected by *Coleroa* (Von Broembsen, 1989) but, if infection of young leaf tissue does occur, symptoms tend not to appear until leaves are over 3 months old (Serfontein & Knox-Davies, 1990a). Infection of new growth can occur on *P. magnifica* in the Western Cape after an unusually heavy rainfall during the hot summer months.

Control: In fungicide trials carried out in the field using a variety of systemic fungicides (benomyl, fenarimol, nuarimol, penconazole, triadimefon), all proved effective to varying degrees (Serfontein & Knox-Davies, 1990a). Von Broembsen (1989) stated that effective control of *Coleroa* is achieved with regular spray programmes, which include a benzimidazole fungicide. In view of the risk of building up resistance to the latter fungicides, growers are cautioned to restrict their use to twice a season, and are advised to combine the benzimidazoles with compatible contact fungicides such as mancozeb.

Colletotrichum anthracnose

See Section 3, Chapter 2 on flower, shoot and stem diseases.

Coniothyrium leaf spot

Species of *Coniothyrium* often are associated with necrotic spots at the tips or margins of leaves of *Leucadendron, Leucospermum* and *Protea* spp. These fungi are not a problem of commercial importance, although they can cause significant leaf tip dieback of new growth flushes if plants are severely stressed. *Coniothyrium* spp. are commonly intercepted during phytosanitary inspections. Many of these species have no known teleomorph but *Leptosphaeria protearum* has recently been found to have a *Coniothyrium* anamorph (Taylor & Crous, 1998c). The latter pathogen is treated under *Leptosphaeria*.

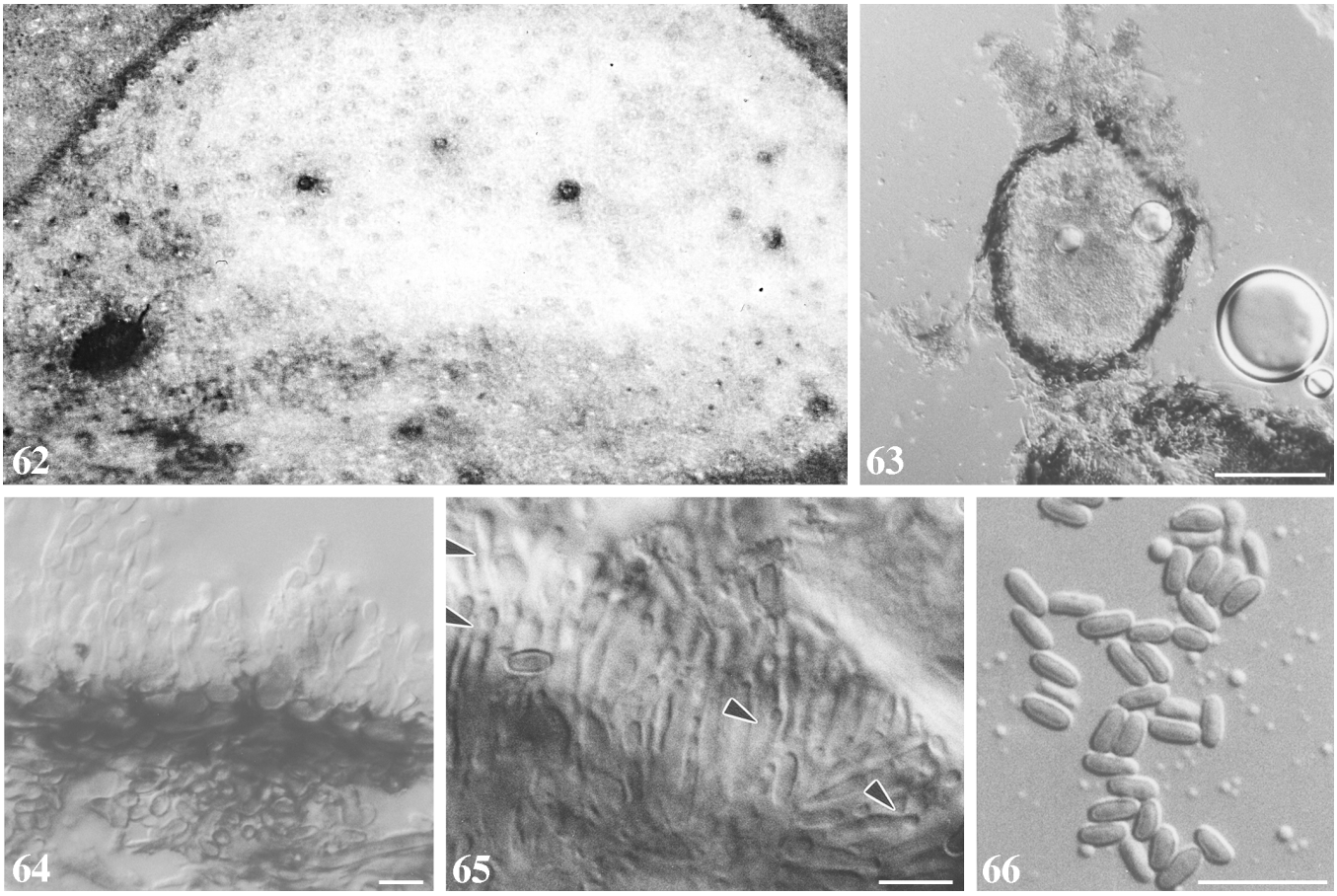
Coniothyrium grandicipis Joanne E. Taylor & Crous, **sp. nov.** Figs 62–66.

Conidiomata pycnidialia, subepidermalia, sub stomatibus, amphigena; in sectione (in cultura) globosa ad irregularia, unilocularia ad multilocularia, papilla carentia, (163–)205–259(–285) µm lata, (138–)165–237(–285) µm diam. *Peridium* ex 2 stratis cellularum brunnearum texturae angularis constans, introrsum pallescens et hyalinum factum, (22–)29–40(–50) µm. *Conidiogenae cellulae* discretae, laeves, pallide brunneae, cylindricae, semel vel ter enteroblastice et percurrenter proliferentes, (3–)5.5–6.5(–9) × (1–)1.5–2 µm. *Conidia* pallide ad medio-brunnea, parietibus tenuibus, laevia, aseptata, ellipsoidea ad oblonga, apicibus obtusis, (3.5–)4–4.5(–5) × (1.5–)2(–2.5) µm *in vivo*; (3–)4–4.5(–5) × (1.5–)2(–2.5) µm *in vitro,* hyalina vel pallide brunnea.

Etymology: In reference to the *Protea* host from which this fungus is described.

Holotype: South Africa, Western Cape Province, Elgin, leaves of *Protea grandiceps*, J.E. Taylor & S. Denman, 20 Jul. 1998, PREM 56616, cultures ex-type STE-U 1852–1853, CBS 111702.

Symptoms: *Leaf spots* amphigenous, irregular, grey to pale brown with a raised, dark brown border.



Figs 62–66. *Coniothyrium grandicipis*. Fig. 62. Leaf spot with immersed pycnidia. Fig. 63. Vertical section through a pycnidium. Figs 64–65. Conidiogenous cells, with percurrent proliferation (arrows). Fig. 66. Conidia. Scale bars: 62 = 100 µm, 63–66 = 10 µm.

Causal organism: *Conidiomata* pycnidial, subepidermal, substomatal, amphigenous, separate, visible as dark brown spots; in section (*in vitro*) globose, unilocular, apapillate, (163–)205–259(–285) high, (138-)165–237(–285) µm wide, often associated with a large complex stroma of dark red-brown, thin-walled fungal hyphae. *Peridium* of two strata of *textura angularis,* outer stratum of thick-walled brown cells which are unevenly thicker in places, becoming thinner-walled and hyaline in the inner stratum, (22–)29–40(–50) µm. *Conidiogenous cells* discrete, smooth, pale brown, cylindrical, indeterminate, (3–)5.5–6.5(–9) × (1–)1.5–2 µm; conidiogenesis holoblastic, proliferating 1–3 times percurrently. *Conidia* pale to medium-brown, smooth, aseptate, ellipsoidal to oblong, apex obtuse, (3.5–)4–4.5(–5) × (1.5–)2(–2.5) µm *in vivo*; *in vitro* (3–)4–4.5(–5) × (1.5–)2(–2.5) µm, hyaline to pale brown.

Cultural characteristics: *Colonies* woolly with dense aerial mycelium, margins slightly undulate, surface smoke-grey (19””d), reverse honey (19”d) to cinnamon (13”), 6 mm diam after 2 weeks in the dark at 25°C. Cardinal growth requirements, minimum 10°C, optimum 25°C, maximum above 25°C.

Host range and geographic distribution: *P. grandiceps* (**South Africa, WC**).

Notes: This *Coniothyrium* differs from previous species described or collected from *Proteaceae* hosts (Van Wyk, 1973b; Swart *et al*., 1998) and is therefore described as new. *Coniothyrium grandicipis* most closely resembles *C. proteae* in morphology, size and cultural characteristics. However, *C. proteae* has larger, ellipsoidal to globose conidia measuring (5–)5.5–7(–8) x 3.5–4 µm, and doliiform to ampulliform conidiogenous cells. The dimensions and morphology of the conidia of *Coniothyrium grandicipis* were nearly identical on the host and in culture, whereas many species produce conidia of a different and often smaller size in culture.

Coniothyrium leucospermi Crous & Denman, *South African Journal of Botany* **64**: 139 (1998), *emend.* Joanne E. Taylor & Crous, *Mycoscience* 42: 266 (2001). Figs 67–75, 87.

Symptoms: *Leaf spots* amphigenous, irregular, grey to pale brown with a raised, dark brown border, frequently associated with tip blight or leaf margins.

Causal organism: *Conidiomata* pycnidial, subepidermal, substomatal, amphigenous, separate, visible as dark brown spots; *in vivo* globose, apapillate, sometimes surrounded by a stroma composed of host cells and dark brown fungal hyphae, 55–185 µm high, 55–205 µm wide; *in vitro* globose, sometimes becoming cupulate at maturity, 125–295 high, 100–300 µm wide. *Peridium* consisting of two strata of slightly compressed *textura angularis* to *globulosa,* outer stratum of thick-walled, brown cells sometimes having almost occluded lumina, thinner-walled and hyaline in the inner stratum, 10–42 µm; *in vitro* consisting of two strata of *textura globulosa,* outer stratum of thick-walled, brown cells, thinner walled and hyaline in the inner layer, 8–50 µm. *Conidiophores* reduced to conidiogenous cells, often invested in mucus. *Conidiogenous cells* discrete, smooth or slightly verruculose, hyaline to pale brown, doliiform to ampulliform, proliferating 1–3 times percurrently, 5–12 µm high, 4–9 µm wide at the base, and 1.5–3 µm wide at the apex. *Conidia in vivo* varying in size, colour and ornamentation but broadly similar in shape, varying from hyaline to dark red-brown, thick-walled, faintly verruculose to echinulate, aseptate, ellipsoidal to globose, apex obtuse, base bluntly rounded to truncate, 6–12 × 3–8 µm; *in vitro* initially hyaline and thick-walled, becoming medium brown, smooth to verruculose, ellipsoidal to globose and often irregularly shaped, with an obtuse apex, and a bluntly rounded to truncate base, 6.5–15 × 3.5–8 µm. Conidia more variable in shape *in vitro*, but more consistent in size.

Cultural characteristics: *Colonies* circular, smooth to slimy, finely radiating with regular margins, surface fuscous-black (7””k), reverse olivaceous-black (27””m); aerial mycelium sparse, forming fine concentric zonations; 14–19(–31) mm diam after 2 weeks in the dark at 25°C. Cardinal temperatures for growth, minimum 5°C, optimum 20–25°C, maximum below 30°C.

Host range and geographic distribution: *Leucospermum* sp. (**Dominican Republic**) (Swart *et al*., 1998); *Lcd. sessile*, *Leucadendron* sp., *Lsp. conocarpodendron*, *P. nitida*, *P. obtusifolia*, *P. repens*, *P. magnifica*, *P. neriifolia*, *Protea* sp. (**South Africa, WC**) (Swart *et al*., 1998; Taylor & Crous, 2001); *P. neriifolia, P. obtusifolia, Protea* sp. (**USA, California**) (Taylor *et al*., 2001b).

Coniothyrium nitidae Crous & Denman, *South African Journal of Botany* **64**: 138 (1998). Figs 83–84, 86.

Symptoms: *Leaf spots* pale brown, amphigenous, variable in shape and size, frequently associated with tip dieback or situated along leaf margins.

Causal organism: *Mycelium* immersed, hyphae septate, medium brown, finely verruculose, 3–4 µm wide *in vivo*, 2–5 µm *in vitro*, finely verruculose, pale 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*. *Conidiogenous cells* discrete, smooth, hyaline to pale olivaceous, doliiform to ampulliform, proliferating 1–4 times percurrently, 5–8 × 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) × 3–4(–4.5) µm *in vivo*, (5.5–)6–8(–9) × 3–4 µm *in vitro*.

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

Host range and geographic distribution: *P. nitida* (**South Africa, WC**); *P.* ‘Carnival’ (**USA, Hawaii**) (Taylor *et al*., 2001a).

Coniothyrium proteae Crous & Denman, *South African Journal of Botany* **64**: 139 (1998). Figs 82, 85.

Symptoms: *Leaf spots* pale brown, amphigenous, variable in shape and size, frequently associated with tip dieback or situated along leaf margins.

Causal organism: *Mycelium* immersed, hyphae septate, branched, pale to medium brown, smooth to finely verruculose, 3–4 µm wide. *Conidiomata* pycnidial, subepidermal, globose, separate, brown, 60–120 µm diam. *Peridium* consisting of 2–3 layers of brown cells of *textura angularis*. *Conidiogenous cells* discrete, smooth, hyaline, doliiform to ampulliform, proliferating 1–2 times percurrently, 3–8 × 3–4 µm. *Conidia* pale to medium brown, thin-walled, smooth to finely verruculose, aseptate, ellipsoidal to globose, rarely pyriform, with an obtuse apex, and a bluntly rounded to truncate base, (5–)5.5–7(–8) × 3.5–4 µm *in vivo*, (3–)3.5–4 × 2–2.5 µm *in vitro*.

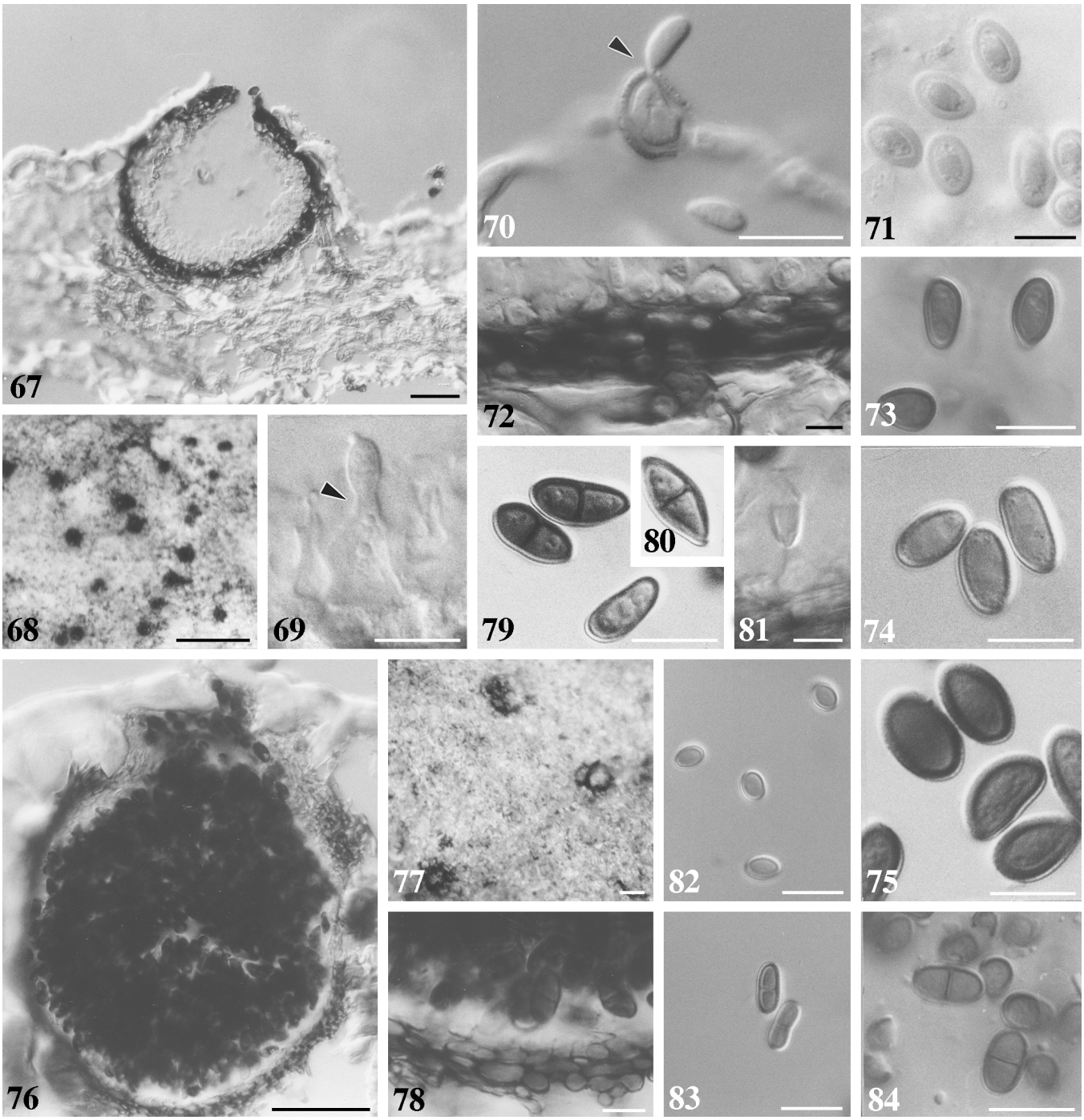
Cultural characteristics: *Colonies* with smooth, sinuate margins, surface smoke-grey (21””f), reverse olivaceous-grey (23’””i); aerial mycelium sparse to moderate; 4 mm diam after 2 weeks in the dark at 25°C.

Host range and geographic distribution: *P. nitida*, *P. repens* (= *P. mellifera*) (**South Africa, WC**) (Swart *et al*., 1998).

Coniothyrium septatum Joanne E. Taylor & Crous, *Mycotaxon* **78**: 79 (2001). Figs 76–81.

Symptoms: *Leaf spots* amphigenous, irregular, grey to pale brown with a raised, dark brown border, mainly situated along leaf margins, 3–17 mm.

Causal organism: *Conidiomata* pycnidial, subepidermal, substomatal, separate, visible as dark brown spots, 90–130 µm diam; in section globose to subglobose, with a central apapillate ostiole, 130–180(–230) µm high, (150–)130–160(–200) µm diam. *Peridium* consisting of two strata of *textura angularis,* outer stratum of thick-walled brown cells, becoming thinner-walled and hyaline in the inner layer, (12–)14–23(–32) µm thick, often thicker around the ostiole. *Conidiogenous cells* hyaline, discrete, smooth, pyriform to ampulliform, determinate to indeterminate, 4–6(–7) × 3–5(–6) µm; conidiogenesis holoblastic, proliferating 1–3 times percurrently. *Conidia* pale to dark



Figs 67–84. *Coniothyrium* spp. Figs 67–75. *C. leucospermi*. Fig. 67. Vertical section through a pycnidium. Fig. 68. Conidiomata on leaf surface. Fig. 69. Conidiogenous cell with percurrent proliferations (arrow). Fig. 70. Germinating conidium *in vitro*. Fig. 71. Conidia *in vitro*. Fig. 72. Pycnidium wall *in vivo*. Figs 73–75. Conidia *in vitro*. Figs 76–81. *C. septatum*. Fig. 76. Vertical section through a pycnidium. Fig. 77. Conidiomata on host surface. Fig. 78. Pycnidial wall *in vivo*. Fig. 79–80. Conidia. Fig. 81. Conidium attached to conidiogenous cell. Fig. 82. Conidia of *C. proteae*. Figs 83–84. Conidia of *C. nitidae*. Scale bars: 67 = 30 µm, 68 = 500 µm, 69–75, 78–80, 82–84 = 10 µm, 76 = 50 µm, 77 = 100 µm, 81 = 5 µm.

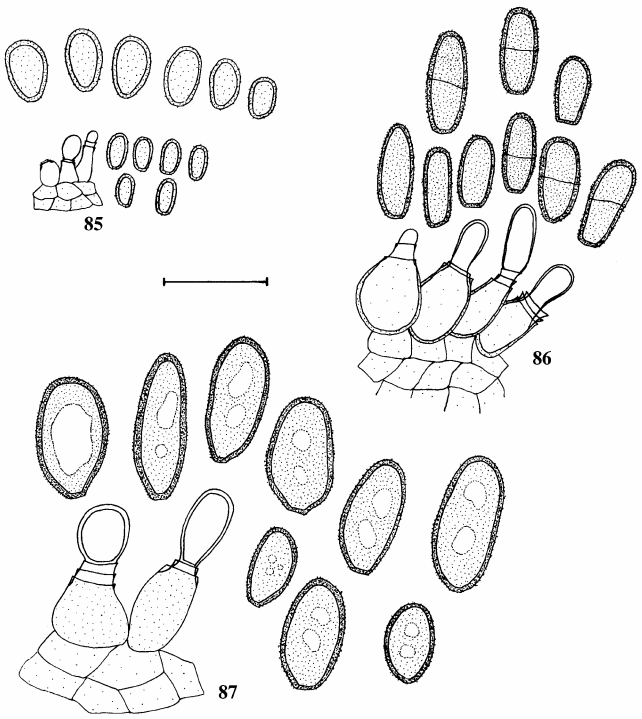
yellow-brown, thick-walled, smooth to finely verruculose, (0–)1-euseptate, fusiform, occasionally narrowing at the septum, with an obtuse apex, and a truncate base, (8–)10.5–11.5(–13) × (4.5–)5–5.5(–6) µm.

Cultural characteristics: *Colonies* circular with entire margins, white on both surfaces, becoming olivaceous (23'"b) in the centre at 25°C in the dark; aerial mycelium sparse, finely cottony; slow-growing; 20 mm diam after 28 days.

Host range and geographic distribution: *Lsp.* 'High Gold', *P.* 'Susara' (**Canary Islands, Tenerife**); *Lsp. cordifolium, P. laurifolia* (**USA, California**) (Taylor *et al*., 2001b).

***KEY TO* CONIOTHYRIUM *SPECIES ON* PROTEACEAE**

1 Conidia frequently 1-septate **2**
1 Conidia aseptate **3**
2 Conidia fusiform, pale to dark yellow-brown, smooth to finely verruculose, (8–)10.5–11.5(–13) × (4.5–)5–5.5(–6) µm ..... ***C. septatum***
2 Conidia ellipsoidal to subcylindrical, medium brown, verruculose, (6.5–)8–9(–11) × 3–4(–4.5) µm ***C. nitidae***
3 Conidia generally longer than 8 µm and wider than 4 µm **4**
3 Conidia shorter than 8 µm and narrower than 4 µm **5**
4 Conidiogenous cells smooth, hyaline, 4–6 × 3–4 µm; conidia pale to medium brown, 5–10 × 3–7 µm; colonies growing normally, not yeast-like ……..... ***C. protearum* (*Leptosphaeria protearum*)**
4 Conidiogenous cells smooth or slightly verruculose, hyaline to pale brown, 5–12 µm × 4–9 µm; conidia hyaline to dark red-brown, 6–12 × 3–8 µm; young colonies having a slimy, yeast-like growth ... ***C. leucospermi***
5 Conidia smooth to finely verruculose, ellipsoidal to globose, rarely pyriform, with an obtuse apex, and bluntly rounded to truncate base, (5–)5.5–7(–8) × 3.5–4 µm ..... ***C. proteae***
5 Conidia smooth, ellipsoidal to oblong with obtuse apices, (3.5–)4–4.5(–5) × (1.5–)2(–2.5) µm .... ***C. grandicipis***



Figs 85–87. Conidia and conidiogenous cells of *Coniothyrium* spp. *in vivo*. Fig. 85. *C. proteae*. Fig. 86. *C. nitidae*. Fig. 87. *C. leucospermi*. Scale bar = 10 µm. (From Swart *et al*., 1998).

Didymosphaeria leaf spot

Didymosphaeria futilis (Berk. & Broome) Rehm, *Hedwigia* **18**: 167 (1879). Figs 88–92.

Symptoms: *Leaf spots* circular to irregular, discrete to confluent, necrotic, sunken with a raised dark brown margin and with conspicuous black ascomata in the dead tissue, 4–20 mm diam associated with necrotic leaf spots and on stems.

Causal organism: *Ascomata* amphigenous, immersed to semi-immersed, non-erumpent, black, singular, scattered, 180–320 µm diam; in section substomatal, subepidermal, pyriform or globose with a papillate, periphysate ostiole, that appears slot-like in some sections, 190–225 µm high, 180–210 µm diam, with a clypeus surrounding the ostiole, consisting of host cells filled with fungal hyphae. *Peridium* of two strata of *textura intricata*, an outer stratum of medium brown, thick-walled cells, inner stratum becoming hyaline and thin-walled, 22–30 µm thick at the sides, 20–50 µm thick at the base. *Pseudoparaphyses* trabecular, numerous, anastomosing between the asci, 1–2 µm wide. *Asci* cylindrical, tapering gradually to a long pedicel, with a rounded apex with an indistinct ocular chamber, 8-spored, bitunicate, (58–)64–78(–90) × (5.5–)6–6.5(–7) µm. *Ascospores* uniseriate, slightly

overlapping, ellipsoidal, medium red-brown, 1-distoseptate, with fine spinulose ornamentation, (7.5–)8.5–10.5(–12) × (3.5–)4–4.5(–6) µm.

Host range and geographic distribution: *Leucadendron* sp., *P. susannae, Protea* sp. (**South Africa**, **WC**); *P. magnifica* (**USA, California**).

Notes: This fungus has been reported from stems, wood, and leaves of various plants (Aptroot, 1995a). It has been associated with *Phoma*-like and *Fusicladiella* synanamorphs (Aptroot, 1995a), though none were observed on the collections from *Proteaceae*. *Didymosphaeria futilis* appears to be commonly associated with lesions of *Proteaceae* but it is likely a saprobe or weak pathogen.

Drechslera blight

See Section 3, Chapter 2 on flower, shoot and stem diseases.

Elsinoë scab disease

See Section 3, Chapter 2 on flower, shoot and stem diseases.

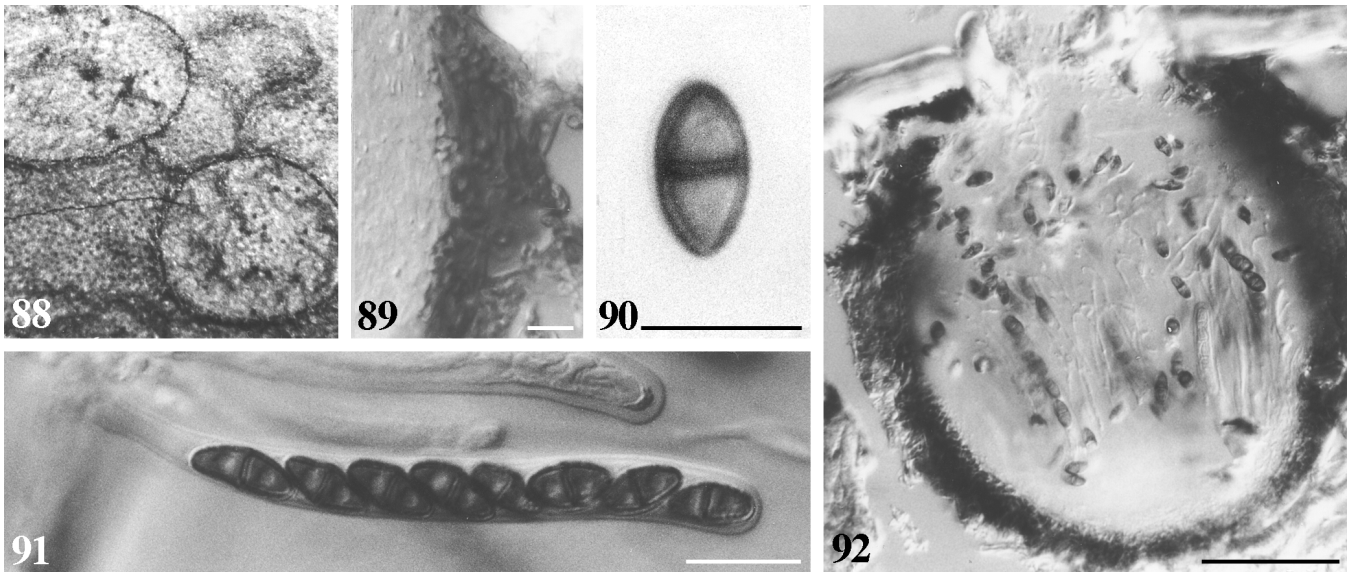
Guignardia leaf spot

Species of *Guignardia* have *Phyllosticta* anamorphs. Three species of *Phyllosticta* are known to occur on members of the *Proteaceae*, only one of which has a known sexual state. *Phyllosticta capitalensis,* teleomorph *Guignardia mangiferae,* is known from proteas as well as many other hosts worldwide and is considered an endophyte (Baayen *et al*., 2002). In South Africa, *P. owaniana* is associated with distinctive leaf spots of *Brabejum stellatifolium* (Swart *et al*., 1998). *P. telopeae* occurs on *Telopea* in Australia. An undescribed species is known from *Banksia.*

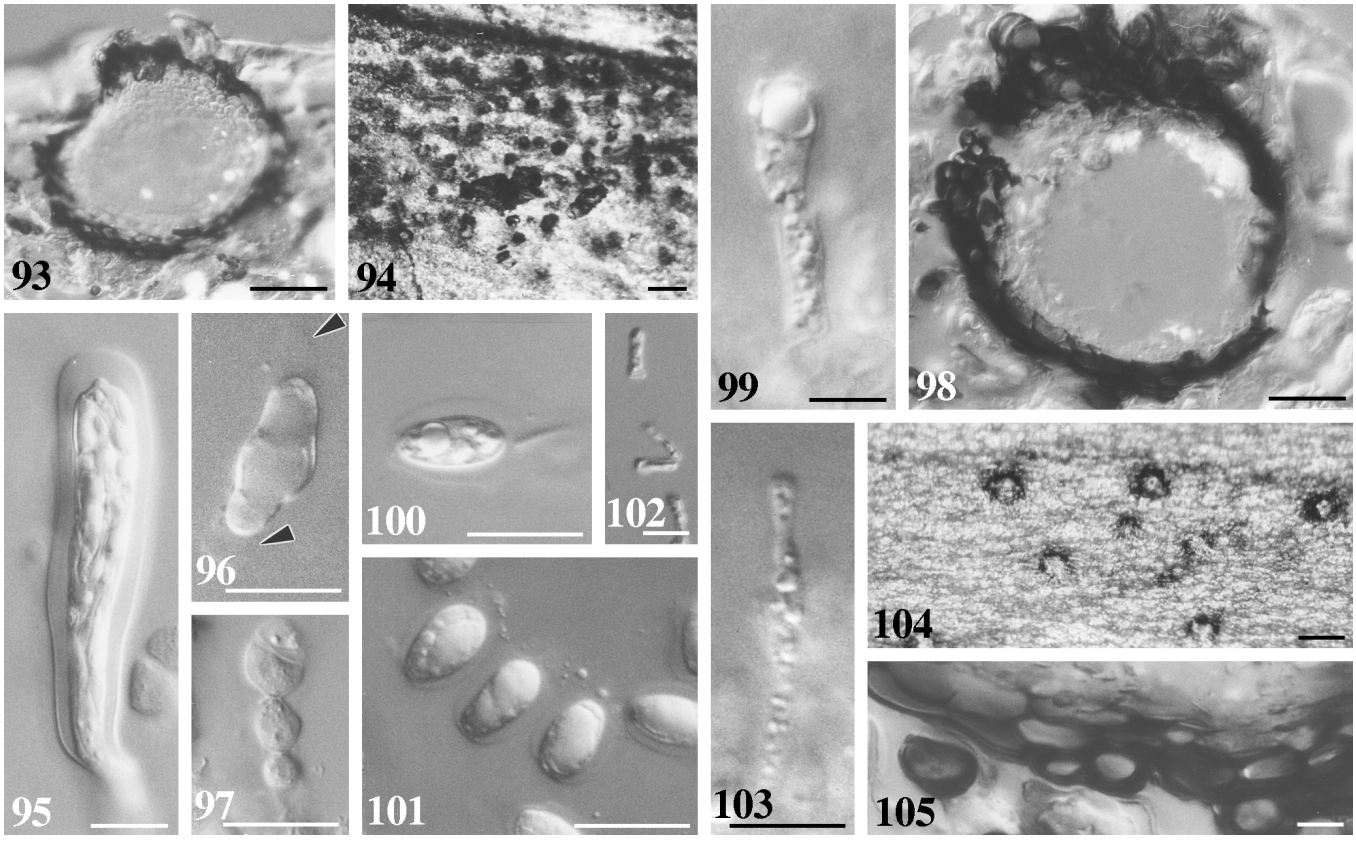
Crous *et al*. (2000b) reported that *Phyllosticta telopeae* occurred on *Telopea speciosissma* and *Telopea* spp. in Australia (NSW, Tasmania), as well as in the USA (Hawaii) (Taylor *et al*., 2001a). Swart *et al*. (1998) and Crous & Palm (1999) noted another *Phyllosticta* sp. associated with species of *Protea*, *Leucospermum* and *Leucadendron* and these collections were all treated as *P. telopeae* in a later study (Taylor *et al*. 2001a). It was noted that conidial sheaths were up to 2 µm wide and that both the teleomorph and the anamorph formed in culture. Baayen *et al*. (2002) included these isolates in a larger study and found that two species were involved. One was *P. telopea* which is a distinct species known from *Telopea* only. Many of the other isolates studied by Taylor *et al*. (2001a) were a widespread species, *P. capitalensis* (teleomorph *Guignardia mangiferae*), which can co-inhabit lesions of *Telopea* caused by *P. telopeae*. Phyllosticta capitalensis is considered a non-pathogenic, cosmopolitan endophyte on other *Proteaceae* as well as plants in many other families. Its pathogenicity on proteas is unclear but, because *P. capitalensis* is often associated with leaf lesions of proteas, maybe it is a latent pathogen.

Guignardia mangiferae A.J. Roy, *Indian Phytopathology* **20**: 340 (1968) Figs 93–108.
Anamorph: ***Phyllosticta capitalensis*** Henn., *Hedwigia* **48**: 13 (1908).

Symptoms: *Leaf spots* necrotic, yellow-brown, often with whole leaves affected, ascomata visible as raised, slightly darkened spots, mainly in discrete groups.



Figs 88–92. *Didymosphaeria futilis*. Fig. 88. Lesions on leaves of *Protea* sp. Fig. 89. Ppseudothecium wall layer. Fig. 90. Ascospore. Fig. 91. Ascus with ascospores. Fig. 92. Vertical section through a pseudothecium. Scale bars: 89–91 = 10 µm, 92 = 50 µm. (From Taylor *et al*., 2001a).



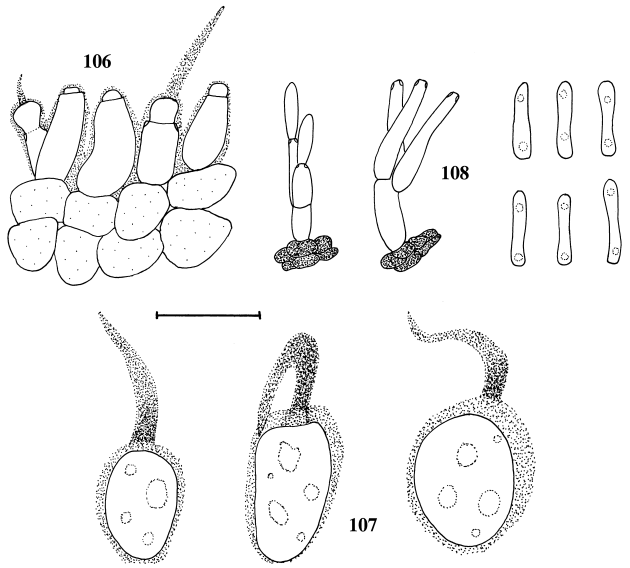
Figs 93–105. *Guignardia mangiferae* and its anamorph *Phyllosticta capitalensis*. Fig. 93. Vertical section through a pseudothecium. Fig. 94. Pseudothecia on host surface. Fig. 95. Ascus. Fig. 96. Ascospore. Fig. 97. Pseudoparaphyses. Fig. 98. Vertical section through a pycnidium. Fig. 99. Conidium on a conidiogenous cell. Figs 100–101. Conidia with mucous appendages. Fig. 102. Spermatia. Fig. 103. Spermatiophore. Fig. 104. Pycnidia on host surface. Fig. 105. Pycnidial wall layer. Scale bars: 93 = 50 µm, 94, 104 = 200 µm, 95–97, 99–101, 103, 105 = 10 µm, 98 = 20 µm, 102 = 5 µm. (From Taylor *et al*., 2001b).

Causal organism: *Ascomata* amphigenous, subepidermal, substomatal, immersed, becoming erumpent, visible as a slightly raised, darkened area, often with a pale centre, apapillate, 130–200 µm diam; in section globose to pyriform, often irregularly shaped, unilocular, central ostiole forming by dehiscence when mature, 143–252 µm high, 138–213 µm diam. *Peridium* comprising three strata, an outer stratum of thick-walled, small-lumened, brown *textura angularis*, becoming thin-walled with larger lumina in the middle layer, inner layer of thin-walled, hyaline *textura angularis*, altogether 14–45 µm thick. *Stroma* well-developed, composed of host cells and thick-walled, dark brown fungal hyphae surrounding the ascomata, sometimes reduced to the peridium surrounding the locule. *Pseudoparaphyses* pseudoparenchymous, hyaline. *Asci* attached to the basal peridium, clavate, with a wide, slightly squared apex, tapering gradually to a small pedicel, bitunicate, with a well-developed ocular chamber, 8-spored, 58–77 × 11–15 µm. *Ascospores* limoniform, sometimes slightly elongated, aseptate, hyaline, thick-walled, refractive, with a large central guttule and large mucilaginous polar appendages, overlapping biseriate, 15–17 × 5–6 µm, 3.5 µm wide at each end.

Conidiomata in the same leaf spots as the ascomata, visible as raised, slightly darkened spots, scattered or in discrete groups; in section substomatal, subepidermal, unilocular, globose, immersed in a sparse stroma comprising host cells and dark brown, smooth-walled fungal hyphae, 70–110 µm high, 75–110 µm diam. *Peridium* a single stratum of thick-walled, small lumened, brown *textura angularis*, with one layer of thin-walled, hyaline cells on the inner surface, 20–30 µm. *Conidiogenous cells* cylindrical, hyaline, holoblastic, proliferating percurrently resulting in 1–3 annellations, 6–18 × 2.5–4 µm. *Conidia* ovoid to obovoid, sometimes slightly constricted at the centre, apex rounded, base with an inconspicuous flattened scar, hyaline, guttulate, often with one large guttule and many smaller ones, (8–)9–11(–14) × (5–)5.5–6.5(–9) µm, surrounded by a distinct mucilaginous sheath, 2 µm wide, with a single, unbranched apical mucoid appendage which tapers to a bluntly rounded tip, 6–18 µm long. *Spermatia* forming in spermogonia, hyaline, oblong to bone-shaped, 4.5–6 × 1.5–2 µm.

Cultural characteristics: *Colonies* irregular with fimbriate margins, green-black, the same in reverse; dense aerial mycelium with a granulose appearance; 20 mm diam in 14 days, fertile, producing both anamorph and teleomorph.

Host range and geographic distribution: Plurivorous and widespread; *Grevillea*, *Leucadendron*,



Figs 106–108. *Guignardia mangiferae* and its anamorph *Phyllosticta capitalensis*. Fig. 106. Conidiogenous cells. Fig. 107. Conidia. Fig. 108. Spermatophores and spermatia. Scale bar = 10 µm. (From Swart *et al*., 1998).

Leucospermum, *Telopea* spp. (**Australia, NSW, Tasmania**); *Banksia dryandroides, Grevillea robusta*, *Lcd.* 'Safari Sunset', *Leucadendron* sp., *Lsp.* 'Coral', *Lsp.* 'High Gold', *Lsp.* 'Hybrid 24', *Leucospermum* sp., *P.* 'Pink Ice', *P. cynaroides*, *Telopea* sp. (**USA, Hawaii**) (Taylor *et al*., 2001a).

Notes: Phyllosticta telopeae differs from *P. capitalensis* in having a thin, mucous conidial sheath ca. 0.5 µm (Yip, 1989), an apical appendage that is often greater than 20 µm long, and conidia that measure 11.5–15.5 × 7–10 µm. Conidia of *P. capitalensis* have a thicker sheath up to 2 µm thick (Taylor *et al*., 2001a), measure (8–)9–11(–14) x (5–)5.5–6.5(–9) µm and have an apical appendage 6–18 µm long. Conidia of *Phyllosticta owaniana* are (10–)12–14(–15) × 7–8(–9) µm with a sheath up to 3 µm thick and an apical appendage 5–8(–14) µm long.

Disease cycle and epidemiology: The fungus sporulates readily on necrotic leaves and litter, forming ascomata with ascospores, which initiate new infections. However, pathogenicity needs to be proven.

Control: Unknown.

Harknessia leaf spot

Harknessia leucospermi Crous & Viljoen, *South African Journal of Botany* **64**: 140 (1998). Fig. 109.

Symptoms: Commonly found on litter of *Proteaceae*, not associated with disease symptoms, probably saprobic.

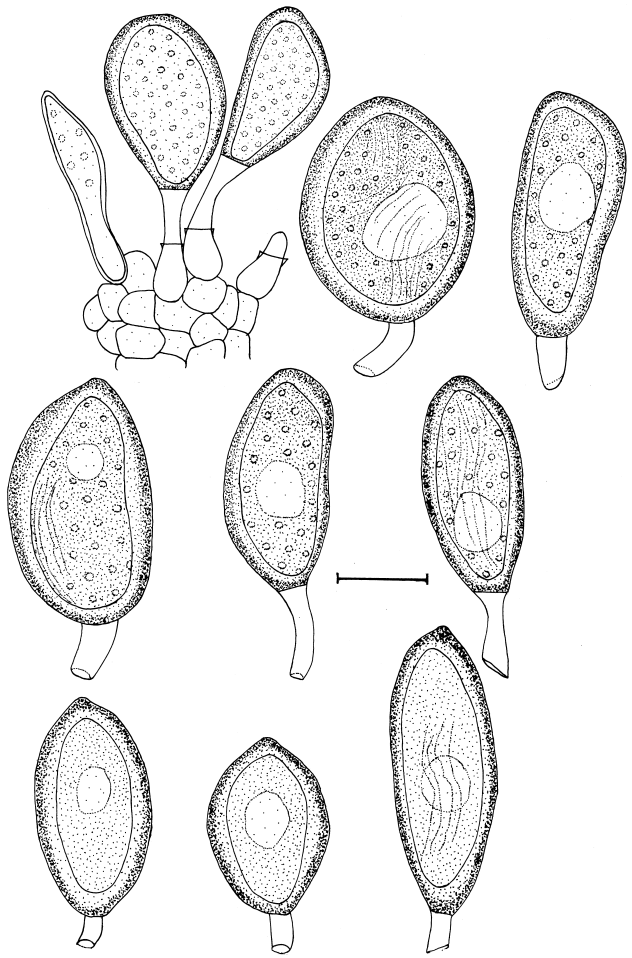


Fig. 109. Conidia and conidiogenous cells of *Harknessia leucospermi*. Scale bar = 10 µm. (From Swart *et al*., 1998).

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

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

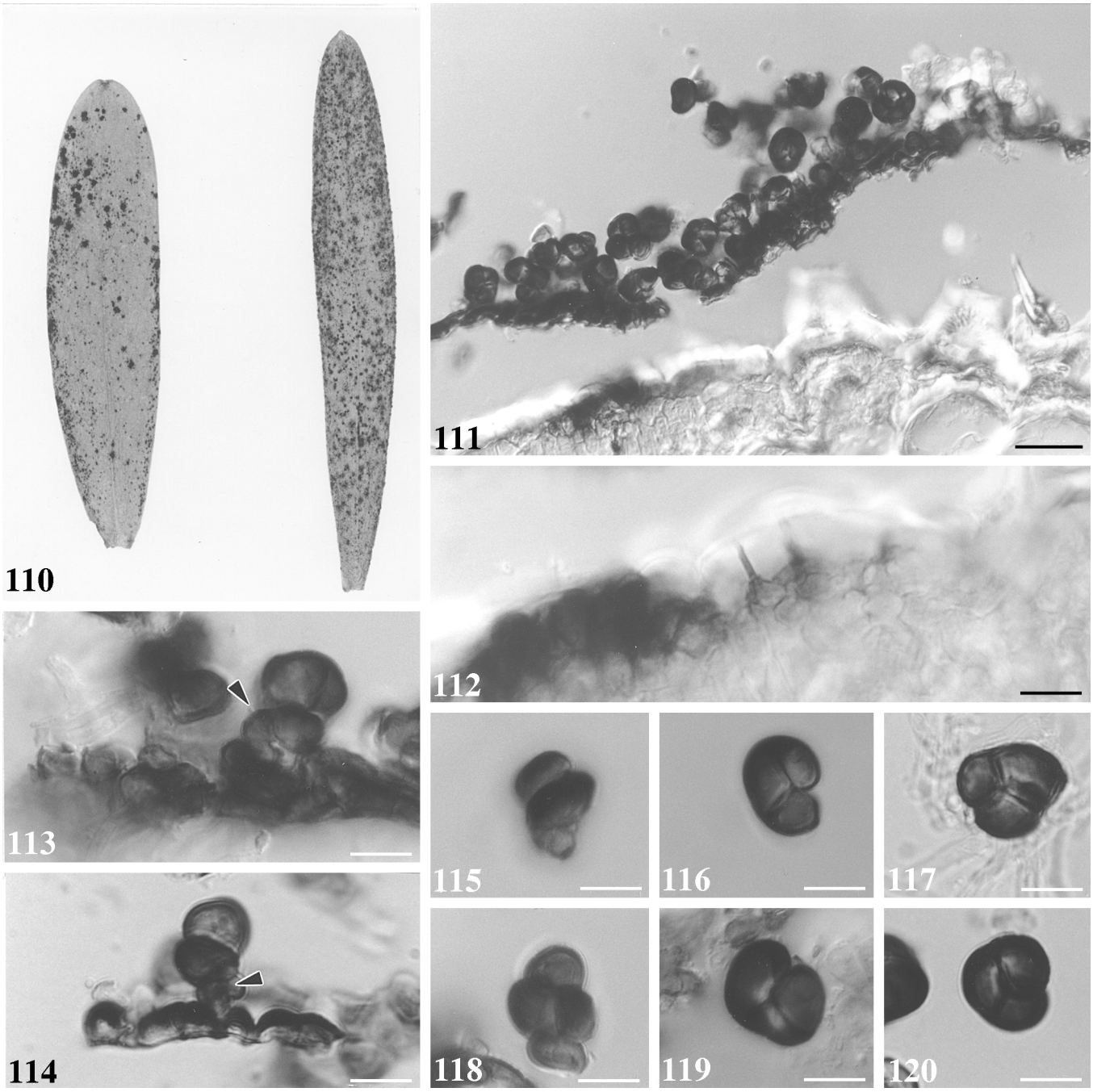
Host range and geographic distribution: *Leucadendron, Leucospermum* and *Protea* spp. (**South Africa, WC**).

Helicosingula leaf spot

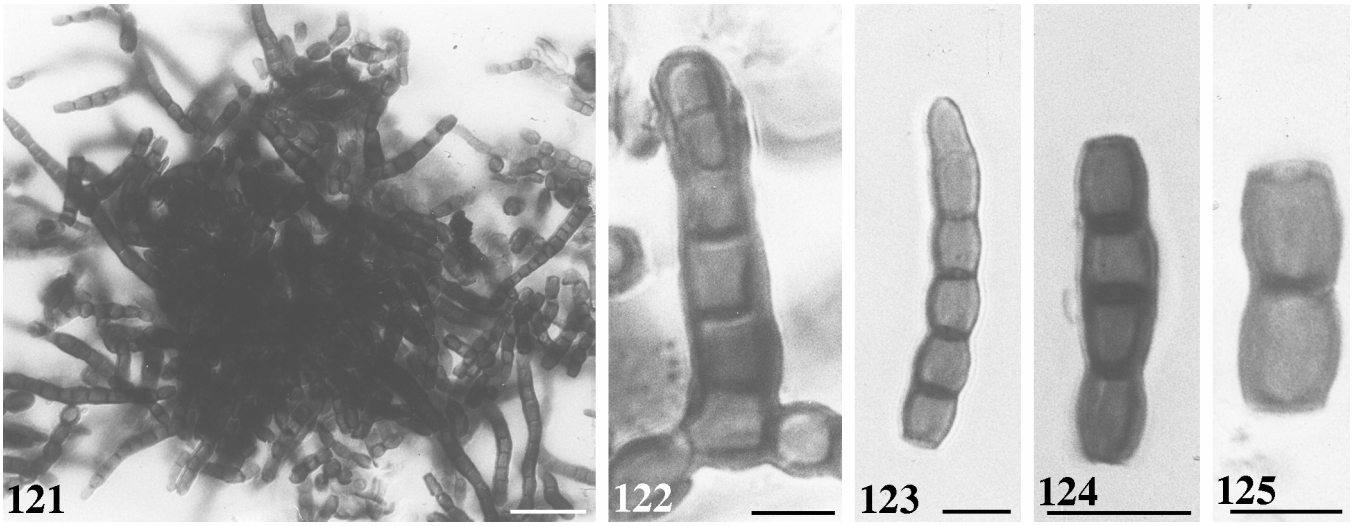
Helicosingula leucadendri is restricted to *Leucadendron* spp. and, although recorded on cultivated hosts and often on new growth, it does not appear to be a serious pathogen. *Helicosingula leucadendri* is biotrophic and leaf spots are due to the presence of hyphae and conidia of the fungus. Colonies are not associated with necrosis or chlorosis. Attempts to isolate this pathogen in culture have been unsuccessful.

Helicosingula leucadendri P.S. Van Wyk, Marasas & Knox-Dav., *Transactions of the British Mycological Society* **85**: 183 (1985). Figs 110–120, Plate 11.

Symptoms: *Leaf spots* amphigenous, circular, radiating, discrete or confluent, sometimes covering large areas of the leaf surface, non-necrotic, dry, black, with a densely sooty appearance, up to 3 mm diam.
Causal organism: *Mycelium* forming a dense crust-like network on the host surface, consisting of branched, anastomosed and intertwined dark brown, smooth hyphae, 3–6 µm wide; subcuticular hyphae stroma-like, hyaline, becoming thick-walled and dark brown, connecting to superficial mycelium via thin, brown hyphae which penetrate the cuticle. *Conidiophores* indistinguishable from vegetative hyphae. *Conidiogenous cells* holoblastic, globose, occurring laterally on the vegetative superficial mycelium, (3.8–)5.5–7(–7.5) µm diam, basal portion of the conidiogenous



Figs 110–120. *Helicosingula leucadendri*. Fig. 110. Leaf spots on a *Leucadendron* sp. Fig. 111. Superficial network of mycelium on host surface. Fig. 112. Subcuticular stromatic tissue. Figs 113–114. Conidiogenous cells occurring on superficial hyphae (arrowed). Figs 115–120. Conidia. Scale bars: 111 = 30 µm, 112–120 = 10 µm. (From Taylor & Crous, 1998).



Figs 121–125. *Heteroconium neriifoliae*. Fig. 121. Superficial mycelium and conidiophores. Fig. 122. Conidiophore. Figs 123–125. Conidia. Scale bars: 121 = 20 µm, 122–125 = 5 µm. (From Taylor *et al*., 2001b).

cell remaining as a cup-shaped cell following rhexolytic secession of conidium. *Conidia* helicoid, tightly coiled in three dimensions, often resembling a dictyospore, solitary, dry, dark brown, multiseptate, not constricted at the septa, (12.5–)14.5–18(–22.5) µm high, (12.5–)15.5–17(–19) µm diam.

Host range and geographic distribution: *Lcd. tinctum*, *Leucadendron* spp. (**South Africa, WC**) (Van Wyk *et al*., 1985a; Taylor & Crous, 1998b).

Disease cycle and epidemiology: Propagules are probably splash-dispersed.

Control: Unknown.

Heteroconium leaf spot

The genus *Heteroconium* presently consists of approximately ten species (Castañeda Ruiz *et al*., 1999), which occur mainly in warmer areas of the world. Compared to the other species described in *Heteroconium*, *H. neriifoliae* most closely resembles *H. citharexyli* in conidium shape, septation and dimensions. However, the conidia and conidiogenous cells of that latter species are described as smooth, as are those of all other previously described species of *Heteroconium*, whereas those of *H. neriifoliae* are distinctly ornamented.

Heteroconium neriifoliae Joanne E. Taylor, Crous & M.E. Palm, *Mycotaxon* **78**: 453 (2001). Figs 121–125.

Symptoms: *Leaf spots* necrotic, pale brown, covering almost the whole leaf, colonies diffuse, abundant, dark brown to black.

Causal organism: *Mycelium* superficial, hyphae pale to medium brown, smooth-walled to verrucose, branched, reticulate, forming a network over the leaf surface, 2–5 µm wide. *Conidiophores* macronematous, mononematous, unbranched, arising as lateral branches on the superficial hyphae, erect, straight, cylindrical, red-brown, multiseptate with septal pores faintly visible, verrucose with longitudinal cracks or creases, 8–64 × 4–6 µm. *Conidiogenous cells* holoblastic, determinate or percurrent, integrated terminally on the conidiophore, 4–8 × 4–6.5 µm. *Conidia* subcylindrical, tapering to one or both ends, catenate, dry, simple, verrucose with longitudinal striations or creases, either obtuse at the apex and truncate at the base or truncate at both ends, with up to 14 septa, (6–)18–25(–45) × (3.5–)4–4.5(–6) µm, becoming slightly constricted at the septa.

Host range and geographic distribution: *P. neriifolia*, *P.* 'Pink Ice' (**USA, Hawaii**) (Taylor *et al*., 2001a).

Kabatiella leaf spot

The genus *Kabatiella* comprises approximately 15 widespread, and mostly north-temperate species (Kirk *et al*., 2001). Although similar to *Aureobasidium*, species of *Kabatiella* are pathogenic, causing leaf spots, while *Aureobasidium* species are generally regarded as saprobes.

Kabatiella is an important but rare pathogen of *Proteaceae* and has been reported from South African material intercepted by the USDA Animal and Plant Health Inspection Service (APHIS) in the USA (Taylor, 2001). *Kabatiella proteae* has recently been recorded from *Protea* in Australia (Crous *et al*., 2000b). Therefore, members of this genus are of phytosanitary importance and of relevance to plant pathologists and protea producers alike.

Kabatiella proteae Joanne E. Taylor & Crous, *Mycological Research* **104**: 619 (2000). Figs 126–133.

Symptoms: *Leaf spots* irregular, occurring on the petiole and base of lamina, extending up the leaf, necrotic, sunken, pale to medium brown with a raised, dark brown margin; areas where sporodochia occur often darkened.

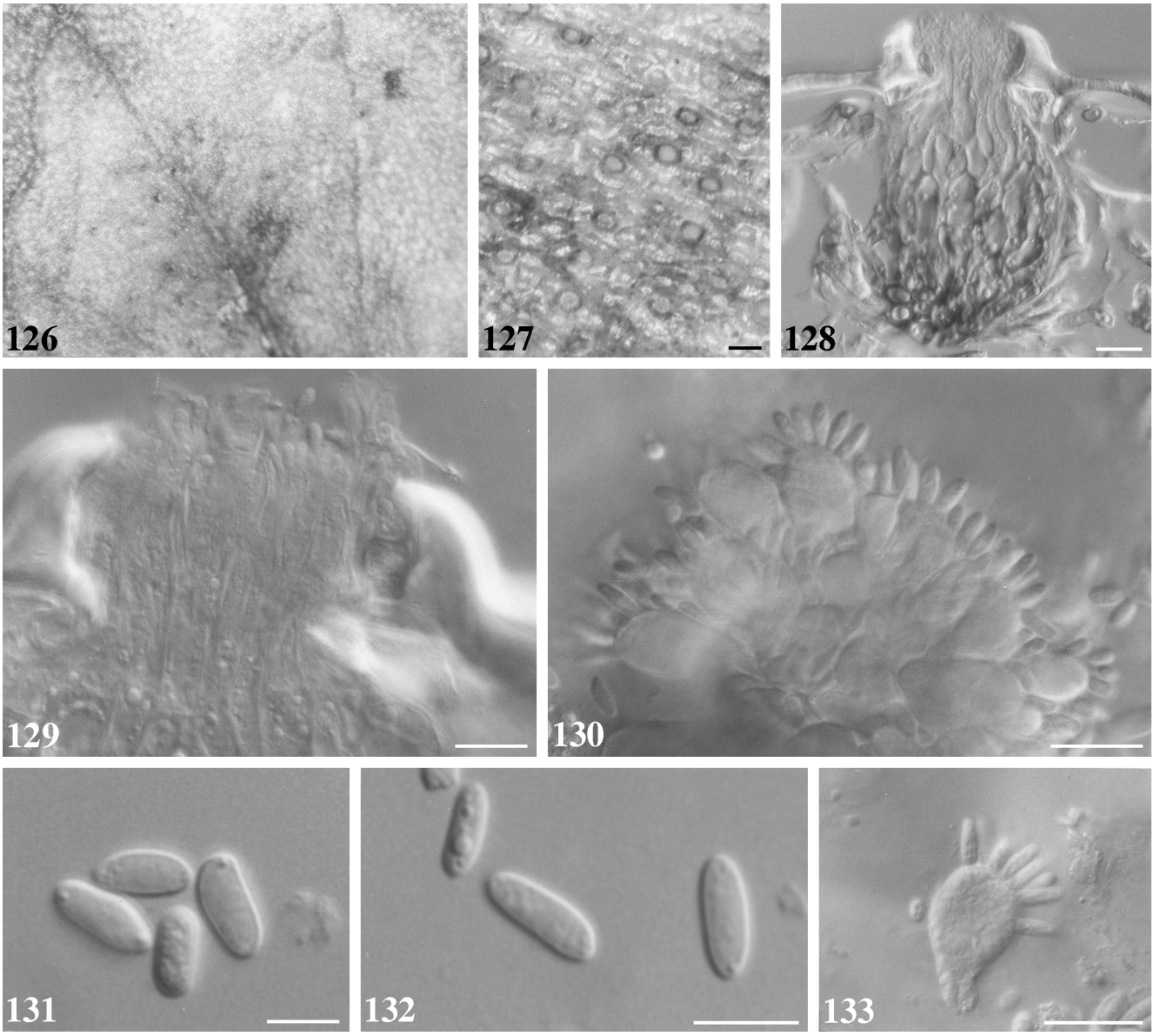
Causal organism: *Mycelium* immersed. *Conidiomata* acervular, amphigenous, substomatal, subepidermal, pulvinate, dry or crystaline in appearance, pale brown, discrete, 60–100 µm diam. *Stroma* visible in substomatal cavity, dark brown, mainly consisting of elongated pseudo-parenchymatous cells with large lumina, becoming hyaline, thinner-walled at the apex, (80–)101–121(–125) × (50–)72–92(–100) µm. *Conidiogenous cells* cylindrical, clavate or globose, integrated, terminal, conidial ontogeny holoblastic, with numerous synchronously produced conidia, (13–)16–20(–27) × (4–)6–8(–11) µm. *Conidia in vivo* solitary, aseptate, ellipsoidal to spherical, occasionally with a slightly truncate base, hyaline, thin-walled, smooth, often with small guttules, (5–)6.5–7.5(–10) × (2–)2.5–3(–3.5) µm; conidia *in vitro* similar, (4–)6–7(–9) × (2–)2.5–3(–3.5) µm.

Cultural characteristics: *Colonies* with moderate to sparse aerial mycelium, surface olivaceous-grey (23''''i), reverse olivaceous-grey to iron-grey (23''''i–23''''k), radial striations appearing in the agar and visible from underneath; 76 mm diam after 14 days at 25°C.

Host range and geographic distribution: *P. cynaroides* (**South Africa, WC**), *P.* 'Pink Ice' (**Australia, NSW**) (Crous *et al*., 2000b; Taylor & Crous, 2000).

Disease cycle and epidemiology: The fungus sporulates profusely once symptomatic material is placed in moist chambers. Conidia are probably splash-dispersed.

Control: Unknown.



Figs 126–133. *Kabatiella proteae*. Lesion on *Protea cynaroides*. Fig. 127. Conidiomata on host substrate. Fig. 128. Substomatal stroma. Figs 129–130, 133. Conidiogenous cells. Figs 131–132. Conidia. Scale bars: 127 = 100 µm, 128 = 20 µm, 129–130, 133 = 10 µm, 131–132 = 5 µm. (From Taylor & Crous, 2000).

Lembosia "Clasterosporium" leaf spot

Alternative common name: 'Greasy spot'

Lembosia proteae and its anamorph, *Clasterosporium proteae*, have thus far only been recorded on *Protea* spp. in South Africa. The fungus is biotrophic, causing no damage to the leaves, and the lesions are encountered infrequently. It is not a problem of commercial importance and is rarely observed during phytosanitary inspections.

Lembosia proteae Joanne E. Taylor & Crous, *Mycological Research* **104**: 621 (2000).
Figs 134–143, Plate 2.
Anamorph: ***Clasterosporium proteae*** M.B. Ellis, in Ellis, *More Dematiaceous Hyphomycetes*: 100 (1976).

Symptoms: *Leaf spots* circular with an uneven margin, effuse, discrete or occasionally confluent, non-necrotic, dark brown to black, 3–13 mm diam.

Causal organism: *Mycelium* superficial, consisting of branched, septate, medium brown hyphae, roughened, growing over surface of the upper peridium of the ascomata (3.5–)4.5–6(–7) µm wide, forming lateral, simple, smooth, dark brown, rounded hyphopodia, with a small pore, (4–)4.5–6.5(–8) µm diam. *Ascomata* commonly epiphyllous, initially shield-shaped, becoming hysterothecioid, with a central longitudinal slit-like ostiole; isodiametric peridium cells radiating out from the ostiolar slit; margin of ascomata uneven with rounded ends, black, ascomata single and separate or sometimes coalescing, (250–)342–459(–510) µm long, (120–)126–151(–180) µm diam; in section shallowly conical, base applanate, superficial with a central ostiolar cleft, forming haustoria consisting of thick-walled brown fungal hyphae in epidermal cells; (120–)165–220(–250) µm diam, (50–)60–75(–85) µm high. *Peridium* on the upper side of one layer of black melanised cells, (14–)15–23(–30) µm thick, at the ostiolar cleft and at the base becoming thin-walled, pale brown *textura angularis*, and becoming hyaline in the inner layer, (8–)9–16(–25) µm diam. *Pseudoparaphyses* embedded in a gelatinous matrix, simple or branched, hyaline, septate, thick-walled, hypha-like, longer than the asci, with swollen tips, 2–3 µm wide. *Asci* mainly saccate, some clavate to globose, bitunicate, sessile, parallel on the basal layer of the peridium, with a broad rounded apex, ocular chamber lacking, 4–8-spored, (30–)34–39(–48) × (12–)13–15(–17) µm. *Ascospores* fusiform, two-celled, prominently constricted at the septum, upper cell broader, and widest at the base, hyaline and smooth, becoming pale to medium brown and finely verrucose at maturity, overlapping pluriseriate, apices obtuse, (13–)13.5–14(–16) × (4.5–)5–6(–6.5) µm, coated in a thin mucilaginous sheath.

Conidiophores developing laterally on the hyphae, erect, cylindrical, mainly straight, dark brown, 1–2-septate, smooth or roughened, (7–)11–13.5(–16) × (4–)4.5–5(–6) µm. *Conidiogenous cells* monoblastic, integrated, terminal, cylindrical, dark brown, conidia detach by schizolytic secession, (3–)9–11(–18) µm. *Conidia* obclavate, base truncate, apex rounded, narrow, mid to dark brown, verrucose with vertical cracks or wrinkles, 1–9-distoseptate, darkened with a distinct pore visible in each septum, solitary, dry, slightly constricted at the septa, (16–)40–46(–58) × (7–)8–9 µm diam in the broadest part.

Cultural characteristics: *Colonies* circular, convex with entire margins, top and reverse fuscous black (7""k); with dense, felty aerial mycelium; 5 mm diam after 150 days. No anamorph produced in culture.

Host range and geographic distribution: *P. acaulos* (teleomorph), *P. amplexicaulis*, *P. eximia, P. magnifica* (anamorph only) (**South Africa**) (Taylor & Crous, 2000; Knox-Davies *et al.,* 1987).

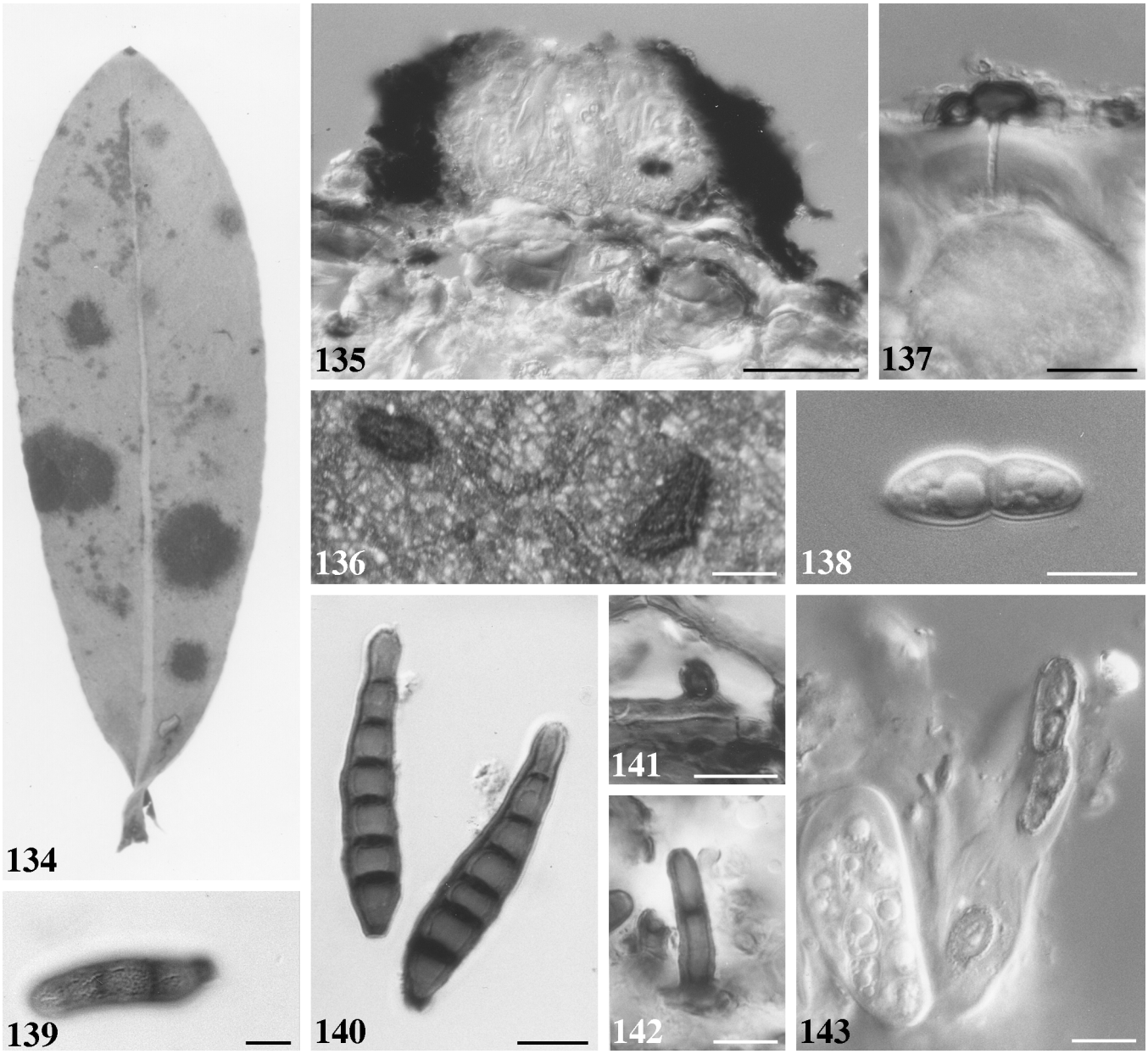
Disease cycle and epidemiology: The disease cycle is unknown but the fungus appears to prefer humid conditions. It was first described from *P. acaulos* that has a low, sprawling habit; the fungus is commonly recorded on the lower, inner leaves of *Protea* spp. with erect or upright habits.

Control: Unknown.

Leptosphaeria leaf blight

Species of *Leptosphaeria* are widespread and commonly cause diseases of dicotyledonous hosts. Two species have been recorded on proteas, namely *Leptosphaeria leucadendri,* which is rarely encountered and recorded on a *Leucadendron* sp., and *Leptosphaeria protearum* which causes severe leaf blight on *Protea* spp. Crous *et al*. (2000b) described *Leptosphaeria telopeae*, which occurs on *Telopea speciosissima* in Australia, but that species is not treated here.

On proteas, *Leptosphaeria protearum* is a problem of commercial importance as it produces necrotic lesions which can develop into severe leaf blight, on both young and old growth. It can even kill young plants or branches of mature plants (Von Broembsen, 1989). In addition, *L. protearum* is an important phytosanitary organism and is commonly intercepted on *Protea* spp. at quarantine inspection points (Taylor, 2001), resulting in rejection of the shipment.



Figs 134–143. *Lembosia proteae* and its anamorph *Clasterosporium proteae*. Fig. 134. Leaf spots on *Protea magnifica*. Fig. 135. Vertical section through an ascoma. Fig. 136. Ascomata on leaf surface. Fig. 137. Superficial hypha with infection peg extending through epidermis. Fig. 138. Ascospore. Fig. 139–140. Conidia. Fig. 141. Hyphopodia. Fig. 142. Conidiogenous cell. Fig. 143. Asci. Scale bars: 135 = 50 µm, 136 = 200 µm, 137–143 = 10 µm. (From Taylor & Crous, 2000).

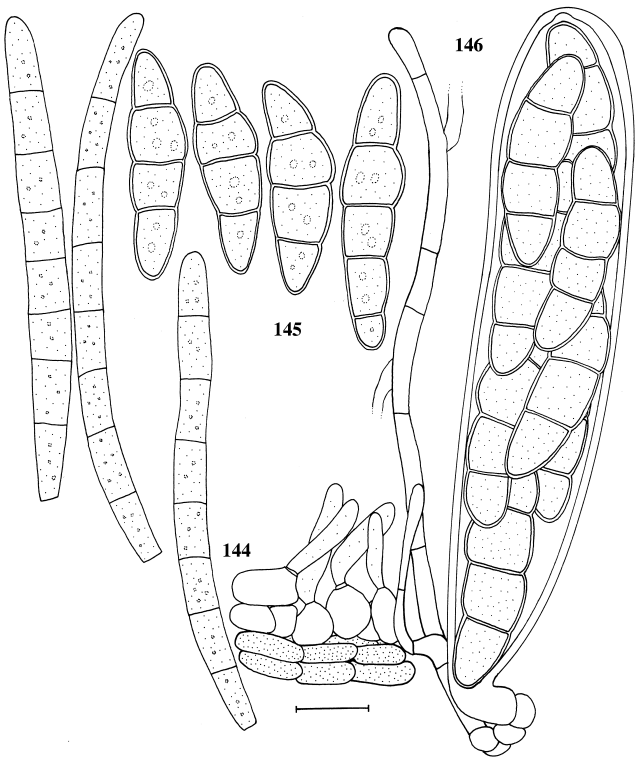
Leptosphaeria leucadendri Crous & M.E. Palm, *Mycological Research* **103**: 1299 (1999).

Figs 144–145.

Anamorph: ***Sclerostagonospora leucadendri*** Crous & M.E. Palm, *Mycological Research* **103**: 1299 (1999).

Symptoms: *Leaf spots* amphigenous, up to 5 mm diam, subcircular, situated along leaf margins, grey, with a raised, dark brown border, surrounded by a diffuse red-purple margin.

Causal organism: *Mycelium* immersed, hyphae pale brown, smooth, branched, septate, 2–3.5 µm wide. *Ascomata* sparse, scattered, globose to subglobose, becoming erumpent, up to 250 µm diam, 200 µm high, with a central papillate ostiole, up to 60 µm diam, wall up to 30 µm thick, consisting of 8–12 layers of *textura angularis*, outer region of 5–8 layers of brown scleroplectenchymatous cells, inner region of 3–4 layers of hyaline, more elongated cells. *Pseudoparaphyses* numerous, initially attached to the top and bottom of the cavity, 1–3 µm wide, septate, branched. *Asci* 60–90 × 12–16 µm, numerous, sessile, cylindric-clavate, thin-walled, short-stalked, apex rounded, with eight 2–3 seriate ascospores. *Ascospores* (20–)23–25(–28) × (5–)6–7 µm, fusoid to ellipsoidal, straight to slightly curved with obtuse ends, 3(–4)-septate, primary septum median, subapical cell becoming slightly swollen, constricted at septa, yellow-brown, smooth, guttulate. *Conidiomata* pycnidial, separate, up to 150 µm diam, 130 µm high, immersed, subcuticular, brown, thin-walled, wall up to 10 µm thick, consisting of 2–3 layers of medium brown *textura angularis*,



Figs 144–146. *Leptosphaeria leucadendri* and its anamorph *Sclerostagonospora leucadendri*. Fig. 144. Conidia and conidiogenous cells. Fig. 145. Ascospores. Fig. 146. Ascus and pseudoparaphyses. Scale bar = 10 µm. (From Crous & Palm, 1999).

ostiole single, central. *Conidiogenous cells* lining the cavity, holoblastic, discrete, proliferating 1(–2)-times percurrently, hyaline, smooth, ampulliform to doliiform, formed from the inner layer of the pycnidial wall, 4–6 × 3.5–5 µm. *Conidia* pale brown, thin-walled, cylindrical, smooth, guttulate, straight to variously curved, apex obtuse, base truncate or tapering from basal septum to an obconically truncate base, (6–)7–9(–11)-septate, not constricted at the septa, (45–)50–60(–70) × (3–)3.5–4(–5) µm.

Host range and geographic distribution: *Leucadendron* sp. (**Australia, NSW**) (Crous & Palm, 1999).

Leptosphaeria protearum Syd. & P. Syd., *Annals of Mycology* **10**: 441 (1912). Figs 146–158, Plate 4.
Anamorph: ***Coniothyrium protearum*** Joanne E. Taylor & Crous, IMI Descriptions of Fungi and Bacteria No. **1343** (1998).

Symptoms: *Leaf spots* circular to irregular, discrete to confluent, variable in size, under conditions favourable to disease symptoms more similar to a blight than a leaf spot, necrotic, sunken with a raised dark brown margin and with conspicuous black ascomata in the dead tissue, 4–30 mm diam.

Causal organism: *Ascomata* amphigenous, immersed to semi-immersed, not erumpent, black, single, gregarious, 180–320 µm diam; in section, substomatal, subepidermal, pyriform or globose with a papillate, periphysate ostiole, immersed in a stroma consisting of deteriorated host mesophyll cells filled with fungal hyphae, (210–)230–264(–288) µm high, (180–)200–255(–300) µm diam. *Peridium* consisting of three strata of slightly compressed *textura angularis*, an outer stratum of dark brown, thick-walled cells, becoming paler in the central stratum, and hyaline, thin-walled in the inner stratum, altogether (20–)24.5–37.5(–50) µm thick. *Pseudoparaphyses* numerous, 3–5 µm wide, deliquescing to form a gelatinous mass surrounding the asci. *Asci* clavate to cylindro–clavate, often curved, tapering to a pedicel, narrowing slightly to a rounded apex with an indistinct ocular chamber, 8-spored, bitunicate with fissitunicate dehiscense, (70–)80–87.5(–105) × (13.5–)14.5–16(–21.5) µm. *Ascospores* biseriate, fusiform, broader at the apical end, initially hyaline and 1-septate, becoming yellow-brown and 3-septate at maturity, slightly constricted at median to supra-median septum, (21.5–)27.5–29.5(–37.5) × (6.3–)7.5–8(–10) µm in water mounts, (21–)25.5–27.5(–31) × (5.5–)6–7(–8) µm in lactophenol.

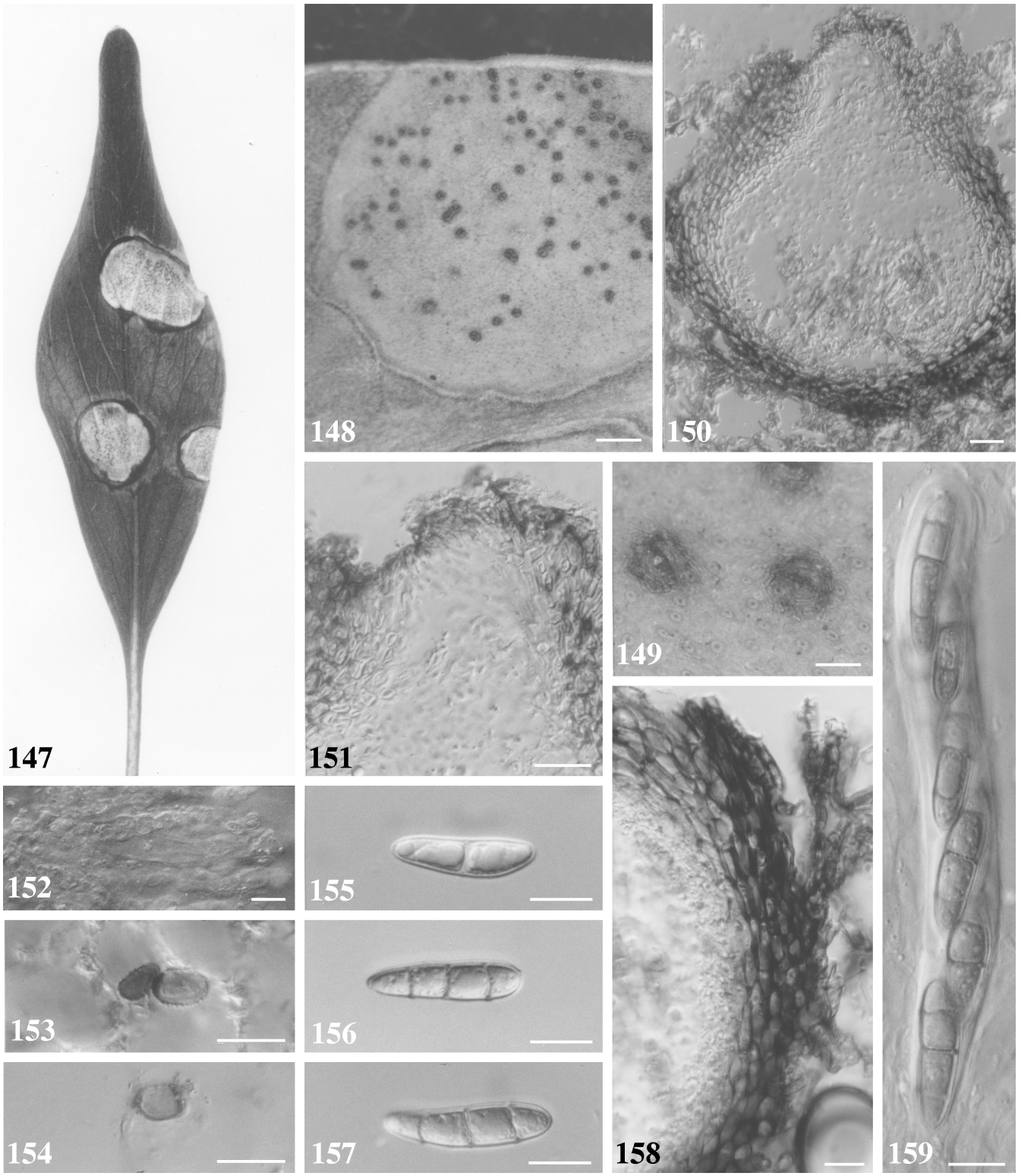
Conidiomata barely visible and interspersed between ascomata, pycnidial, subepidermal, substomatal, separate, globose to pyriform, occasionally with well-developed papilla, dark brown, < 200 µm diam. *Conidiophores* reduced to conidiogenous cells. *Conidogenous cells* discrete, smooth, hyaline, doliiform to ampulliform, holoblastic, proliferating 1–2 times percurrently, 4–6 × 3–4 µm. *Conidia* pale brown to medium brown, thick-walled on maturity, smooth to finely verruculose, eguttulate, ellipsoidal to globose, often truncate at one end, 5–10 × 3–7 µm.

Host range and geographic distribution: *P. cynaroides*, *P.* 'Susara' (**Madeira**) (Moura & Rodrigues, 2001); *P. caffra*, *P. compacta, P. cynaroides*, *P. gaguedi, P. grandiceps, P. lacticolor, P. lepidocarpodendron, P. lorifolia*, *P. magnifica, P. punctata, P. repens, P.* 'Sheila', *Protea* spp. (**South Africa**) (Sydow & Sydow, 1912; Doidge, 1927, 1950; Nel, 1942; Van Wyk, 1973a; Van Wyk *et al*., 1975a; Knox-Davies *et al*., 1986, 1987); *P. cynaroides*, *P. laurifolia*, *P. neriifolia*, *P.* 'Ivory Musk', *P.* 'Mink', *P.* 'Pink Ice', *P.* 'Rose Mink', *P. susannae, Protea* sp. (**USA, Hawaii**) (Taylor *et al*., 2001a); *P. cynaroides*, *P. gaguedi, P. neriifolia, Protea* sp. (**Zimbabwe, Inyanga**) (Doidge, 1950; Masuka *et al*., 1998).

Disease cycle and epidemiology: *Protea magnifica* and cultivars of this species are particularly susceptible to Leptosphaeria leaf spot (Knox-Davies *et al.*, 1986). This is particularly true when they are cultivated outside their natural habitats, which for *P. magnifica* is montane (1200–2700 m, rarely 600 m). At lower altitudes or in warmer or summer rainfall habitats, the susceptible hosts become blighted. *L. protea-*

rum causes severe losses in plantations of South African *Protea* spp. in Hawaii. This condition is never seen on hosts growing in the wild. *L. protearum* has been recorded in many countries where South African proteas are cultivated. It has never been recorded as an endophyte from symptomless material or from treated or untreated seeds. The life cycle is unknown but ascospores and conidia likely are wind- and splash-dispersed (Taylor & Crous, 1998c).

Braun & Crous (1992) reported that *Dactylaria leptosphaeriicola* frequently colonised pseudothecia of *L. protearum* when leaves were incubated in moist chambers. This hyperparasite has been observed sporadically, always associated with pseudothecia of *L. protearum*.



Figs 147–159. *Leptosphaeria protearum* and its anamorph *Coniothyrium protearum*. Fig. 147. Leaf spots on *Protea cynaroides*. Fig. 148. Leaf spot with pseudothecia. Fig. 149. Pseudothecia on host surface. Fig. 150. Vertical section through a pseudothecium. Fig. 151. Ostiolar area. Fig. 152. Hamathecium. Fig. 153–154. Conidia. Fig. 155–157. Ascospores. Fig. 158. Perithecium wall layer. Fig. 159. Ascus. Scale bars: 148 = 2 mm, 149 = 100 µm, 150 = 20 µm, 151–159 = 10 µm. (From Taylor & Crous, 1998).

Control: Specific control measures are unknown. Avoiding planting sites that have high humidity and utilizing humidity-reducing practices such as good weed control, an open canopy and spacing plants for maximum air movement, disease may be reduced. Overhead or mist irrigation is to be avoided. There is good potential for fungicide protection against this disease but research is needed to determine effective chemicals and optimal spray programmes.

Lophiostoma leaf spot

Lophiostoma fuckelii is a widespread fungus on numerous hosts (Chesters & Bell, 1970; Holm & Holm, 1988). It is probably a saprobe or weak, opportunistic pathogen of *Protea* and allied hosts (Taylor & Crous, 2000, Taylor *et al*., 2001a). Many species of *Massarina* have recently been transferred to *Lophiostoma* based on results of molecular studies (Hyde *et al.*, 2002; Liew *et al*., 2002).

Lophiostoma fuckelii Sacc., Michelia 1: 336 (1878). Figs 159–166.

Symptoms: *Leaf spots* circular to irregular, necrotic, sunken, grey-brown with a raised dark brown margin, often situated at the margin of the leaf, 3–13 mm diam; ascomata visible as blackened dots, scattered within the leaf spot.

Causal organism: *Ascomata* epiphyllous, immersed, visible as a slightly raised, darkened area with a central, black slot-like ostiole, which becomes erumpent through the stomata, single and separate or loosely gregarious, 150–220 mm diam; in section globose with a wide slot-like, periphysate, papillate ostiole, reduced clypeus consisting of brown fungal hyphae within the host cells surrounding the ostiole, approximately 270 µm high, 255 µm diam. *Peridium* consisting of three strata, an outer stratum of thick-walled, brown compressed *textura angularis,* becoming thin-walled, with an inner layer of thin-walled, hyaline *textura angularis,* with thick walled, large-lumened *textura angularis* to *textura globulosa* at the ostiole; peridium slightly thinner at the base, 14–27 µm diam. *Pseudoparaphyses* cellular, hyaline, tapering from the base to a slightly narrower apex, longer than the asci, embedded in a gelatinous matrix, 1.5–2 µm wide. *Asci* clavate to cylindro-clavate, bitunicate, attached to the base and sides of the peridium, pedicellate, apex rounded, with an inconspicuous ocular chamber, 8-spored, 60–83 × 6.5–8.5 µm. *Ascospores* fusiform, 1-septate, with two guttules in each cell, constricted at the septum, upper cell broader and widest at the base, hyaline, smooth, obtuse apices with a small mucilaginous appendage at the tip of each cell, 12–15.5 × 4–5 µm.

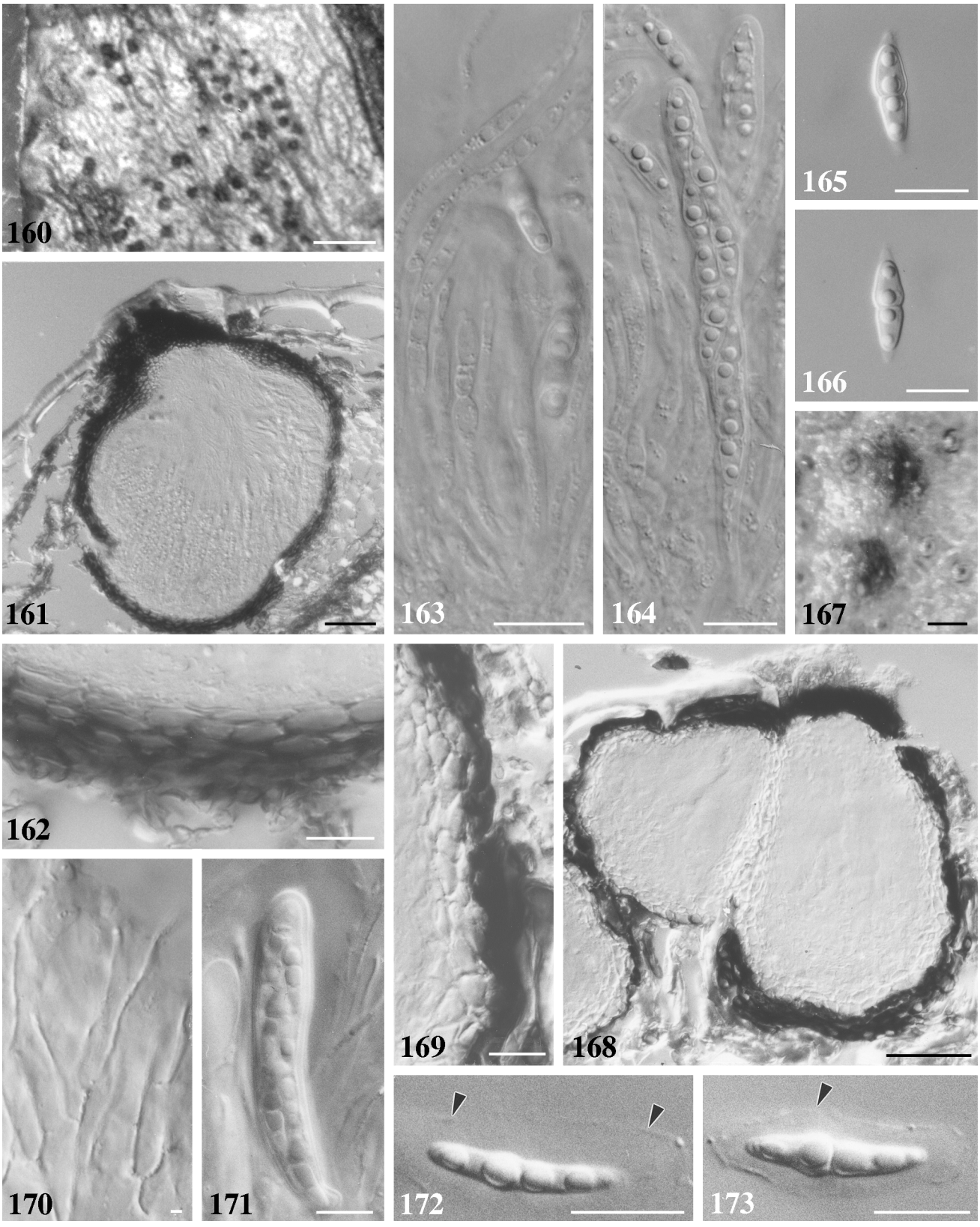
Cultural characteristics: Colonies circular with entire margins, centre pale mouse-grey (17””’d), with a wide honey (9”b) periphery, the same in reverse; aerial mycelium moderately dense, felty, broadly concentrically zoned; 25 mm diam after 1 month, and producing ascomata after 2 months on CLA (no anamorph produced).

Host range and geographic distribution: *Leucospermum* ‘Pink Star’*, P. cynaroides, P. susanne, P. laurifolia* × *neriifolia, Protea* sp. (**South Africa**); *P. cynaroides* (**USA, Hawaii, California**), *Lsp.* ‘High Gold’, *Lsp.* ‘Hybrid 24’, *Leucospermum* sp., *P.* ‘Lady Di’, *P. neriifolia* (**USA, Hawaii**).

Lophiostoma rubi (Fuckel) Liew, Aptroot & K.D. Hyde, *Fungal Diversity Research Series* **7**: 107 (2002). Figs 167–172.
≡ *Massarina rubi* (Fuckel) Sacc., *Sylloge Fungorum* **2**: 155 (1883).

Symptoms: *Leaf spots* necrotic, grey-brown, covering the whole leaf or concentrated at the leaf tips, ascomata visible as black dots.

Causal organism: *Ascomata* mainly epiphyllous, immersed, subepidermal, substomatal, visible as a slightly raised, darkened area with a central, black, rounded or slot-like ostiole, which becomes erumpent through the stomata, mainly gregarious, or sometimes single and separate, 100–160 µm diam; in section globose with a round or wide slot-like aperiphysate, papillate ostiole, reduced clypeus consisting of brown fungal hyphae within the host cells surrounding the ostiole, approximately 160–175 µm high, 115–150 µm wide. *Peridium* of equal width at the base and sides, 10–32 µm thick, and gradually widening at the ostiole, consisting of two strata, an outer stratum of thick-walled, large-lumened, brown, slightly compressed *textura angularis*, becoming melanised, with occluded lumina surrounding the ostiole; inner stratum consisting of thin-walled, hyaline *textura angularis,* asci attached to the base of the peridium. *Pseudoparaphyses* cellular, hyaline, anastomosing above and sometimes between the asci, embedded in a gelatinous matrix, 1–2 µm wide. *Asci* cylindrical to cylindro-clavate, bitunicate, short-pedicellate, rounded at the apex with an indistinct ocular chamber; asci 8-spored, 54–70 × 8–10 µm. *Ascospores* fusiform, 1-septate with 2–3 guttules in each cell, constricted at the septum, upper cell bulging above the septum, hyaline, smooth, with obtuse apices, (15–)17–18.5(–22) × (3–)4–4.5(–5.5) µm, surrounded by a uniform, 2–5 µm thick mucilaginous sheath.



Figs 160–173. *Lophiostoma* spp. Figs 160–167. *Lophiostoma fuckelii*. Fig. 160. Leaf spot with ascomata on *Protea cynaroides*. Fig. 161. Vertical section through a pseudothecium. Fig. 162. Pseudothecium wall layers. Fig. 163. Pseudoparaphyses. Fig. 164. Asci. Figs 165–166. Ascospores. Fig. 167. Ascomata on host substrate. Figs 168–173. *Lophiostoma rubi*. Fig. 168. Vertical section through a pseudothecium. Fig. 169. Pseudothecium wall. Fig. 170. Pseudoparaphyses. Fig. 171. Asci. Figs 172–173. Ascospores with sheath (arrows). Scale bars: 160 = 1 mm, 161, 167 = 100 µm, 168 = 50 µm, 170 = 20 µm, 162–166, 169, 171–173 = 10 µm. (From Taylor & Crous, 2000, 2001b).

Cultural characteristics: *Colonies* circular with entire margins, centre dark brown to black (7’’’’k), with a pale brown periphery (4’’’i), the same in reverse; with moderately dense, aerial mycelium; 7 mm diam after 8 days.

Host range and geographic distribution: *Leucospermum* 'Hybrid 24', *Lsp.* 'Hybrid 31', *P.* 'Pink Ice', *P. neriifolia* (**USA, Hawaii**).

Mycosphaerella leaf spot

Mycosphaerella spp. are widespread and cause leaf spot diseases on numerous plant hosts. Thirteen species have thus far been recorded on *Proteaceae*. Several were known from South Africa and have subsequently been recorded on proteas cultivated in other countries (*M. bellula* and *M. jonkershoekensis*). Others have been associated with leaf spots of proteas outside of South Africa, but not within South Africa. These include *M. leucospermi* in California and a *Mycosphaerella* sp. that was associated with necrotic leaf spots of *Lsp. cordifolium, P. eximia* and *P. cynaroides* (Taylor *et al*., 2001b). *M. holualoana*, *M. konae* and *M. waimeana* are known only from Hawaii, and *M. xylomeli* on *Xylomelum pyriforme* from Australia (Sivanesan & Shivas, 2002). *M. telopeae* has been associated with leaf spots of *Telopea* spp. in New Zealand (Swart *et al*., 1998), while *M. buckinghamiae* was described from leaf spots on *Buckinghamia* sp. in Australia (Crous *et al*., 2000b). The *Dissoconium dekkeri* anamorph of the widespread *M. lateralis* has been associated with leaf spots of *Protea* spp. and *Leucadendron* sp. in Australia (Crous *et al*., 2000b). The latter fungus is known to have a wide host range (Crous, 1998; Crous *et al*., 1999).

In general, *Mycosphaerella* species on proteas do not cause problems of commercial significance, although aesthetic damage can occur on plants cultivated in humid environments. *Mycosphaerella jonkershoekensis* produces necrotic lesions that can coalesce and become blight-like. This species and *M. bellula* are important phytosanitary organisms and have been regularly intercepted on *Protea* spp. at quarantine inspection points.

Mycosphaerella bellula Crous & M.J. Wingf., *Mycotaxon* **46**: 20 (1993). Figs 173–181, 238.

Symptoms: *Leaf spots* circular, mainly discrete, necrotic, parchment-like, pale grey-brown, sunken with a raised dark brown margin, regular in outline, with small, barely visible black ascomata, 3–5 mm diam.

Causal organism: *Ascomata* amphigenous, immersed, substomatal, black, singular, gregarious; in section intra-epidermal, pyriform or globose, with a papillate non-periphysate ostiole, becoming erumpent through the stomatal pore, (80–)90–114(–130) µm high, (62–)77–117(–125) µm diam. *Peridium* of two strata of 3–4 layers of compressed *textura angularis*, an outer stratum of dark brown, thick-walled cells with large lumina, becoming hyaline and thin-walled in the inner stratum, together 10–15 µm thick. *Paraphyses* absent. *Asci* obclavate to cylindrical, pedicel short, straight, tapering to a narrow rounded apex with an indistinct ocular chamber, 8-spored, bitunicate with fissitunicate dehiscence, (32.5–)40–46.5(–57.5) × (9.5–)10.8–12.3(–15) µm. *Ascospores* overlapping mono- to multiseriate, 1-septate, fusiform to ellipsoidal, with obtuse ends, widest in the middle of the upper cell with the lower cell narrower and longer, straight, hyaline, constricted at the septum, guttulate, (7.5–)9.5–10.5(–12.5) × (2.5–)3–3.5(–4) µm in water mounts, (8–)9–10(–11) × (2–)2.5–3(–3.5) µm in lactophenol, surrounded by an inconspicuous mucilaginous sheath, 4–6 µm.

Cultural characteristics: *Colonies* dark brown, moderately slow growing, with many thick-walled chlamydospore-like cells at the ends of the hyphae in the aerial mycelium; sterile.

Host range and geographic distribution: *Protea* sp. (**Australia**); *Leucospermum* spp., *P. cynaroides, P. neriifolia, P. repens, Protea* spp. (**South Africa, EC, NC, WC**) (Crous & Wingfield, 1993; Taylor & Crous, 1998e); *Protea* spp. (**USA, Hawaii**).

Notes: No anamorph has been observed. Germinating ascospores become brown and verruculose, and one to several germ tubes grow at irregular angles to the long axis of the spore. *Mycosphaerella bellula* resembles *M. jonkershoekensis* in the symptoms it causes, and both species are often found on the same leaf. The two species can easily be distinguished, however, in that *M. bellula* has smaller, guttulate ascospores, which are strongly constricted at the septum and surrounded by a sheath.

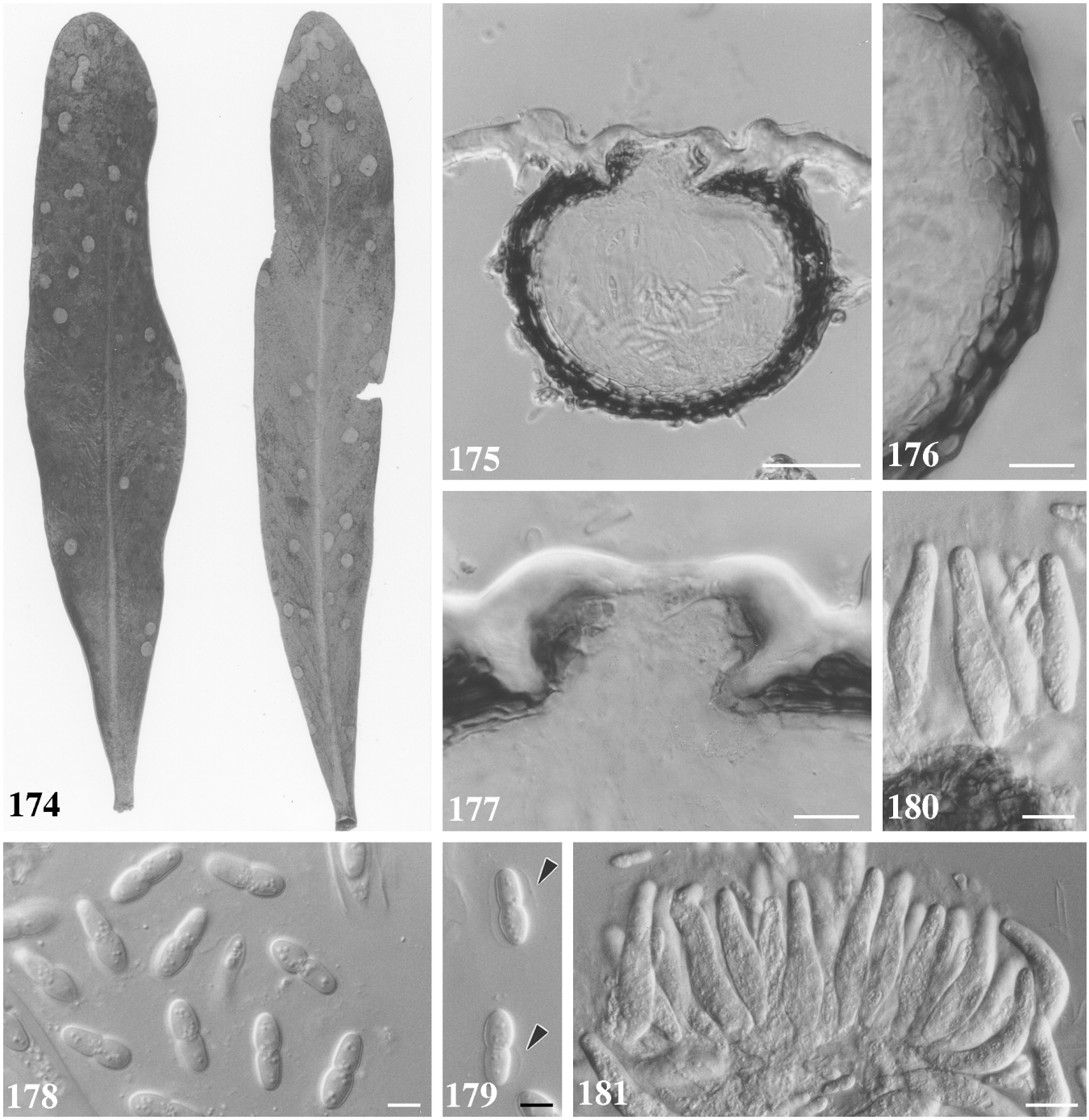
Mycosphaerella fibrillosa (Syd. & P. Syd.) Joanne E. Taylor & Crous, *Mycological Research* **107**: 657 (2003). Figs 182–185, Plate 5.

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

Figs 174–181. *Mycosphaerella bellula*. Fig. 174. Leaf spots on a *Protea* sp. Fig. 175. Vertical section through a pseudothecium. Fig. 176. Pseudothecial wall layer. Fig. 177. Ostiolar area. Figs 178–179. Ascospores with mucous sheath. Figs 180–181. Asci. Scale bars: 175 = 50 µm, 176–177, 180–181 = 10 µm, 178–179 = 5 µm. (From Taylor & Crous, 1998).

Symptoms: *Leaf spots* discrete, irregular in outline, comprising radially arranged, irregularly branched lines of immersed, black ascomata, non-necrotic, but the host tissue in direct contact with the ascomata often sharing a raised, 1–10 mm wide border.

Causal organism: *Ascomata* amphigenous, immersed, not erumpent, black, ostiolate, non-papillate, gregarious, 105–210 µm diam; in section subepidermal, globose, with a clypeus-like structure extending beyond the ascomatal walls, clypeus and stroma in which ascomata are immersed comprising host mesophyll cells, sparsely filled with pale brown fungal hyphae or with an amber-coloured crystalline matrix, periphysoids lining the ostiole and the upper ascomatal wall, 160–188 µm high, 100–170 µm diam. *Peridium* comprising two strata of compressed *textura angularis*, the outer stratum com-

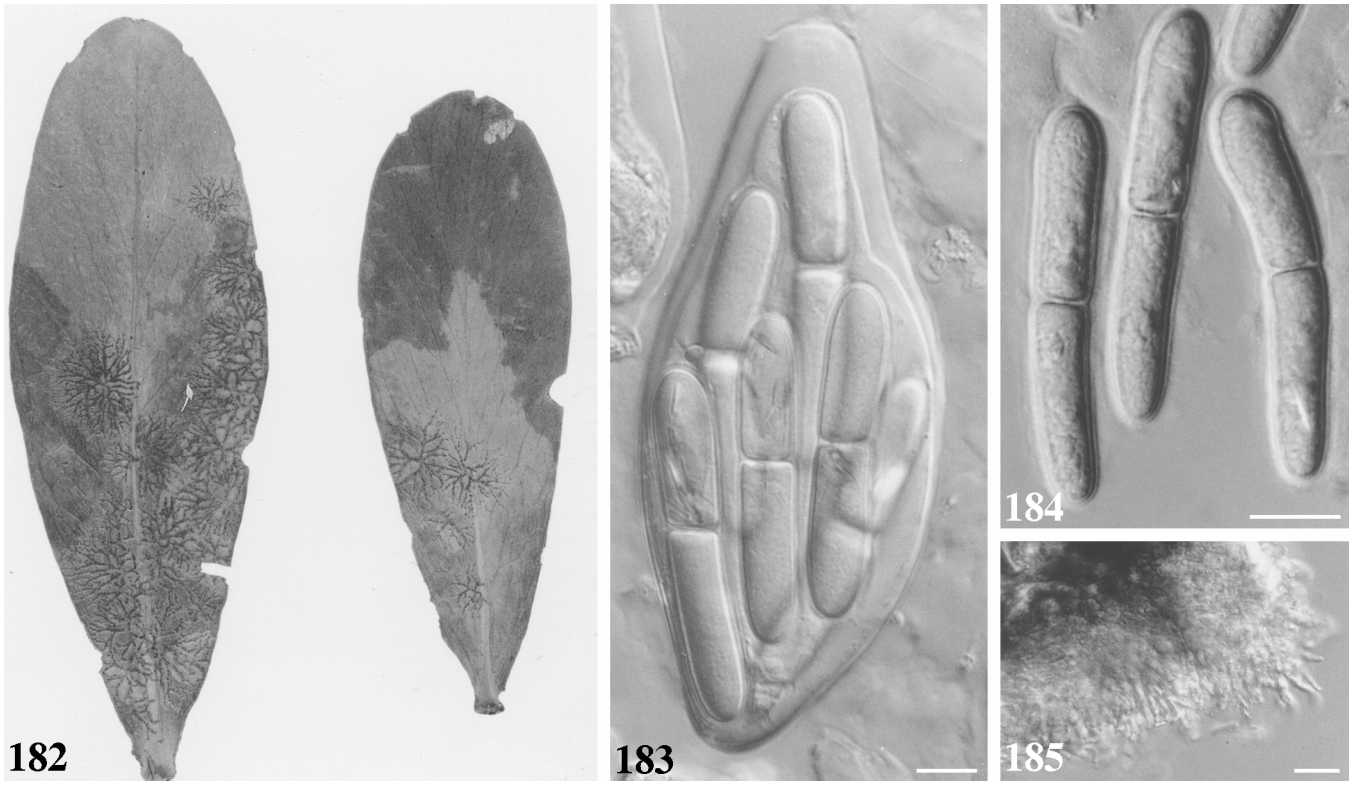


Figs 182–185. *Mycosphaerella fibrillosa*. Fig. 182. Leaf spots on *Protea nitida*. Fig. 183. Ascus. Fig. 184. Ascospores. Fig. 185. Periphysoids. Scale bars = 10 µm. (From Taylor & Crous, 2000).

posed of brown, thick-walled cells, becoming hyaline in the inner stratum, (10–)13–17(–20) µm. *Asci* obclavate to clavate, sessile or with a small pedicel, mainly straight, narrowing to a rounded apex, 4–8-spored, bitunicate with fissitunicate dehiscence, (83–)96–104(–118) × (26.5–)33.5–37(–42.5) µm. *Ascospores* bi- to multiseriate, cylindrical with rounded ends, straight, 1-septate, sepum median and slightly constricted, with a slightly longer upper cell, hyaline, becoming pale yellow-brown and finely verrucose with maturity, (33.5–)40.5–43(–51.5) × (6–)7.5–10(–12) µm (in water and lactophenol). *Spermogonia* intermixed with ascomata and possessing the same general structure, containing a hymenium of spermatiophores which produce unicellular, hyaline, bacillar spermatia, 2–5 × 0.5–1 µm (Taylor & Crous, 1998g).

Cultural characteristics: *Colonies* sooty to pink grey, black in reverse, moderately fast growing, dense, felty, with an entire margin. Cultures were sterile.

Host range and geographic distribution: *P. caffra, P. gaguedi, P. magnifica* hybrids, *P. nitida*, *Protea* spp. (**South Africa**).



Figs 182–185. *Mycosphaerella fibrillosa*. Fig. 182. Leaf spots on *Protea nitida*. Fig. 183. Ascus. Fig. 184. Ascospores. Fig. 185. Periphysoids. Scale bars = 10 µm. (From Taylor & Crous, 2000).

Notes: Mycosphaerella fibrillosa is not an important pathogen as it appears to be restricted to a few hosts with no commercial value (Van Wyk, 1973a).

Mycosphaerella holualoana Crous, Joanne E. Taylor & M.E. Palm, *Mycotaxon* **78**: 458 (2001). Figs 186–188, 214–215.

Symptoms: *Leaf spots* inconspicuous, pseudothecia dense, aggregated in round clusters, fruiting on necrotic leaves attached to bushes.

Causal organism: *Ascomata* amphigenous, black, subepidermal, substomatal, becoming erumpent; in section globose to subglobose, 50–90 µm diam, and 60–90 µm high, apical ostiole 5–10 µm diam; wall consisting of 2–3 layers of medium brown *textura angularis*. *Asci* aparaphysate, fasciculate, bitunicate, subsessile, narrowly ellipsoidal, straight to slightly curved, narrowing to a rounded apex, lacking an ocular chamber, 8-spored, 30–45 × 8–10 µm. *Ascospores* tri- to multiseriate, hyaline, guttulate, thin-walled, straight to slightly curved, fusoid–ellipsoidal with obtuse ends, widest in middle of apical cell, medianly 1-septate, not constricted at the septum, tapering towards both ends, but more prominently towards the lower end, 12–15 × 2.5–3 µm; frequently surrounded by a mucous sheath.

Cultural characteristics: *Colonies* erumpent, uneven, with lobed margins; aerial mycelium sparse, surface olivaceous-grey (23’’’’l), reverse green-black (33’’’’k); 27.5 mm diam after 14 days. No anamorph was produced in culture. Red crystals developed frequently in the agar.

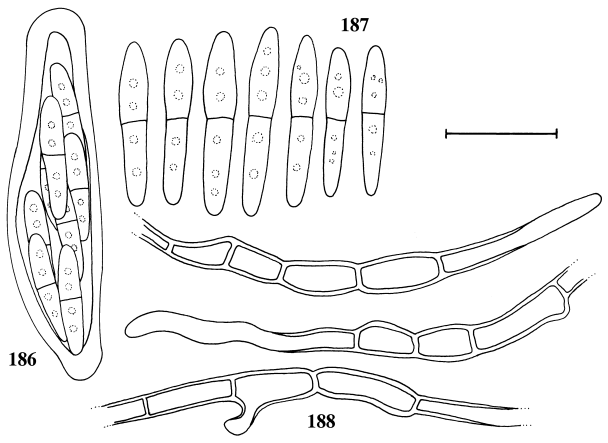
Host range and geographic distribution: *Leucospermum* spp. (**USA, Hawaii**).

Notes: Ascospores germinated after 24 h from both ends, with tubes parallel to the long axis of the spore, becoming slightly constricted and distorted after 24 h, with additional lateral branches appearing thereafter; germinating ascospores hyaline, 3–4 µm wide.

Mycosphaerella jonkershoekensis P.S. van Wyk, Marasas & Knox-Dav., *Journal of South African Botany* **41**: 234 (1975). Figs 189–193, 239.

Symptoms: *Leaf spots* circular, necrotic, parchment-like, pale grey-brown, sunken with a raised dark brown margin, discrete or confluent, regular in outline except where confluent, with small, barely visible black ascomata often clustered towards the centre of the leaf spot, 3–5(–9) mm diam.

Causal organism: *Ascomata* amphigenous, immersed, substomatal, black, singular, gregarious; in section, intra-epidermal, becoming erumpent through the stomatal pore, pyriform or globose with a papillate periphysate ostiole, immersed in a sparse stroma consisting of deteriorated host mesophyll cells containing pale brown fungal hyphae, (100–)129–143.5(–155) µm high, (97.5–)102.5–120(–130) µm diam. *Peridium*



Figs 186–188. *Mycosphaerella holualoana*. Fig. 186. Ascus. Fig. 187. Ascospores. Fig. 188. Germinating ascospores on MEA. Scale bar = 10 µm. (From Taylor & Crous, 2001a).

comprising two strata of 2–4 layers of compressed *textura angularis*, an outer stratum of dark brown, thick-walled cells with large lumina, becoming hyaline and thin-walled in the inner layer, together (12.5–)14.5–19.5(–28) µm thick. *Asci* obclavate, straight, subsessile or with a small pedicel, narrowing slightly to a rounded apex with a distinctive ocular chamber, 8-spored, bitunicate with fissitunicate dehiscense, (46.5–)53.5–56.5(–62.5) µm × (7–)13.5–15(–19) µm. *Ascospores* bi- to multiseriate, fusiform, tapering gradually to the rounded ends, widest in the middle of the upper cell, with the lower cell slightly narrower and longer, straight, hyaline, becoming pale brown with age, medianly septate, constricted or not, (10.5–)18–19.5(–24) × (4–)5–5.5(–7.5) µm in water mounts, (15.5–)18–19.5(–23) × (4–)4.5–5(–6) µm in lactophenol.

Cultural characteristics: *Colonies* slow-growing, grey, felty, raised, with irregular margins.

Host range and geographic distribution: *P. lepidocarpodendron*, *P. longifolia, P.* 'Kurrajong Carnival', *P. magnifica*, *P.* 'Pink Ice', *Protea* spp. (**Australia, NSW**); *P. longifolia, P. magnifica, P. repens, Protea* spp. (**South Africa, EC, NC, WC**).

Notes: No anamorph has been observed (Van Wyk *et al*., 1975b). Germ tubes grow parallel to the long axis of the spore, but after 48 h several germ tubes have been produced and germination is irregular. Germinating ascospores become brown, verruculose and constricted at the septum. Germination can take place at 25°C but isolates die unless the plates are incubated at 15°C for 1–2 weeks. After this initial phase, *M. jonkershoekensis* will grow at most temperatures and it is hypothesised that this low temperature requirement is necessary for successful germination and infection of leaf tissue (Swart *et al.*, 1998; Taylor & Crous, 1998d).

Mycosphaerella konae Crous, Joanne E. Taylor & M.E. Palm, *Mycotaxon* **78**: 459 (2001).

Figs 206–208, 216–220.

Anamorph: ***Pseudocercospora*** sp.

Symptoms: *Leaf spots* inconspicuous, pseudothecia associated with tip blight, and necrotic leaves still attached to bushes.

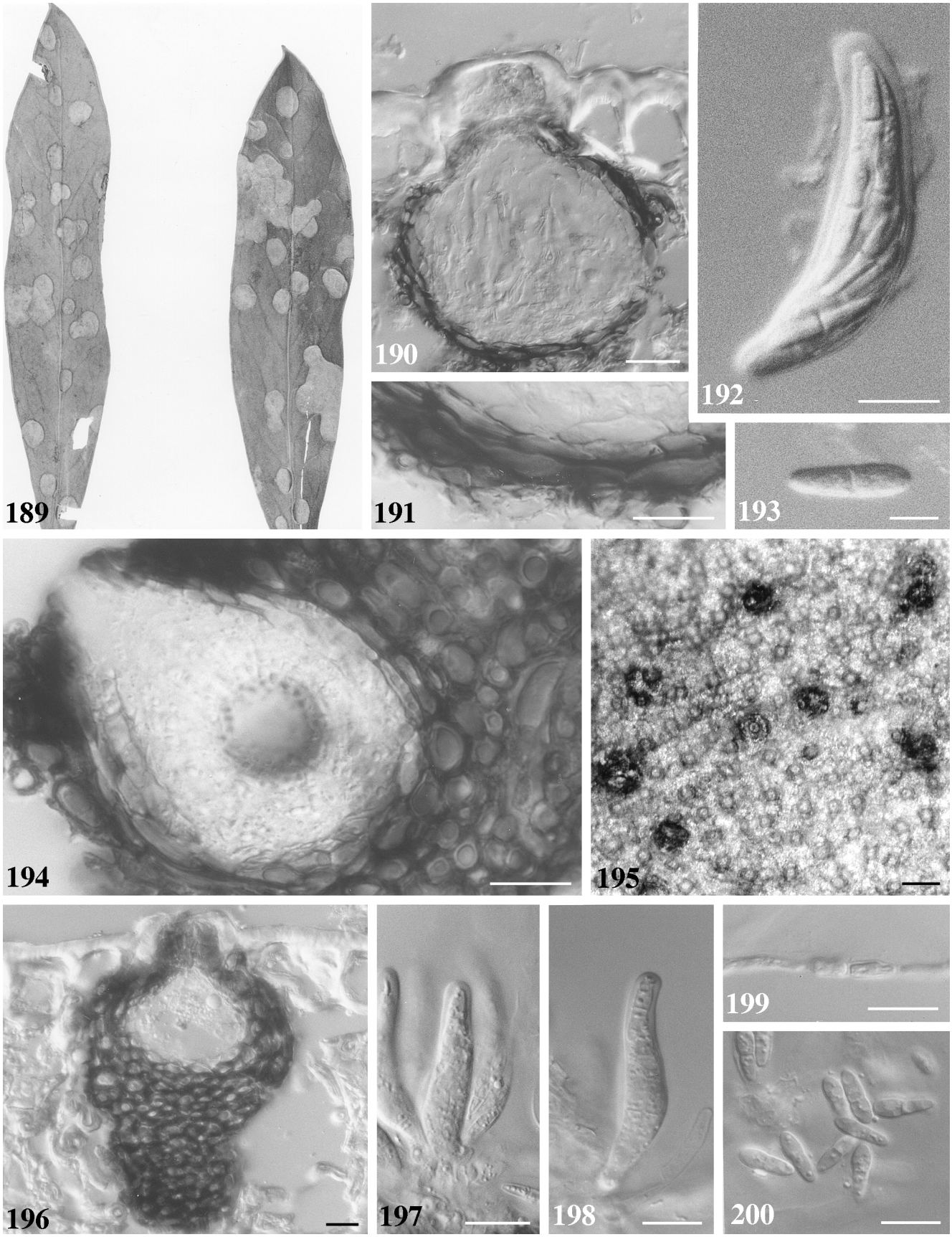
Causal organism: *Ascomata* amphigenous, black, subepidermal, substomatal, becoming erumpent, aggregated in dense clusters; in section globose, up to 100 µm high and 90 µm diam, apical ostiole 5–15 µm diam; wall consisting of 2–3 layers of medium brown *textura angularis*; older pseudothecia developing extensive stromatic tissue (apical and lateral). *Asci* aparaphysate, fasciculate, bitunicate, subsessile, narrowly ellipsoidal to cylindrical, straight to slightly curved, narrowing to a rounded apex, lacking an ocular chamber, 8-spored, 25–40 × 6–8 µm. *Ascospores* tri- to multiseriate, hyaline, guttulate, thin-walled, straight, fusoid–ellipsoidal with obtuse ends, widest in middle of the apical cell, medianly 1-septate, not constricted at the septum, tapering towards both ends, but more prominently towards the lower end, (7–)8–10(–12) × (2.5–)3 µm *in vivo*; 7–9 × 3 µm *in vitro*.

Mycelium of anamorph internal and external, hyphae branched, septate, smooth, olivaceous to pale brown, hyphae 1.5–3 µm wide. *Conidiogenous cells* integrated with mycelium, smooth, pale brown, loci up to 3 µm wide, 5 µm tall. *Conidia* solitary, smooth, pale brown, guttulate, straight or strongly curved, frequently constricted at the septa, subcylindrical, apex obtuse to subobtuse, base truncate, rarely narrowly obclavate, 1–6 septate, 15–60 × 2–3 µm.

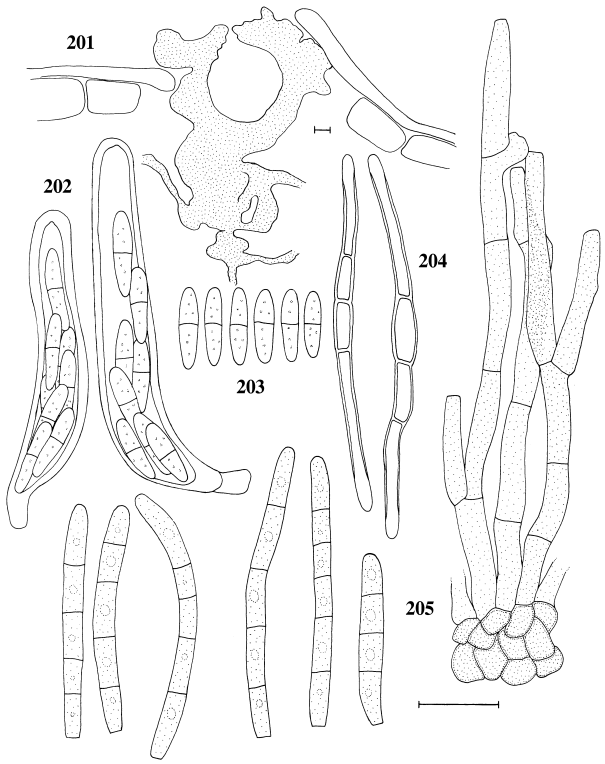
Cultural characteristics: *Colonies* circular with smooth, uneven margins, colonies forming radiating striations in the agar; aerial mycelium sparse, surface iron-grey (23''''k); 21 mm diam after 14 days. Anamorph produced in culture, but not observed on the host.

Host range and geographic distribution: *B. dryandroides, Banksia* sp., *Lcd.* 'Safari Sunset', *Lsp.* 'Yellow Bird', *Lsp.* 'Hybrid 24', *Leucospermum* spp., *P.* 'Mink', *Telopea* sp. (**USA, Hawaii**).

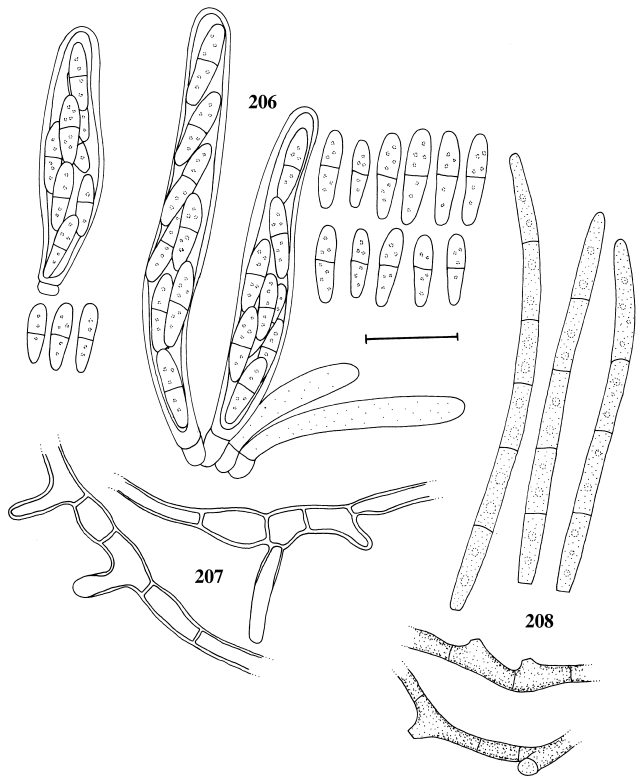
Notes: Ascospores germinate from both ends, with tubes parallel to the long axis of the spore, becoming constricted and distorted, with additional lateral branches appearing after 24 h; germinating ascospores hyaline, 3–4 µm wide.



Figs 189–200. *Mycosphaerella* spp. Figs 189–193. *Mycosphaerella jonkershoekensis*. Fig. 189. Leaf spot on a *Protea* sp. Fig. 190. Vertical section through a pseudothecium. Fig. 191. Pseudothecial wall layer. Fig. 192. Ascus. Fig. 193. Ascospore. Figs 194–200. *Mycosphaerella stromatosa*. Figs 194, 196. Vertical sections through ascomata. Fig. 195. Immersed ascomata on host substrate. Figs 197–198. Asci. Fig. 199. Germinating ascospore on MEA. Fig. 200. Ascospores. Scale bars: 190 = 30 µm, 191–194, 196–200 = 10 µm, 195 = 100 µm. (From Taylor & Crous, 1998, 2000).



Figs 201–205. *Mycosphaerella stromatosa* and its anamorph *Pseudocercospora stromatosa*. Fig. 201. Vertical section through an ascoma. Fig. 202. Asci. Fig. 203. Ascospores. Fig. 204. Germinating ascospores on MEA. Fig. 205. Conidiophores and conidia. Scale bars = 10 µm. (From Taylor & Crous, 2000).



Figs 206–208. *Mycosphaerella konae* and its *Pseudocercospora* anamorph. Fig. 206. Asci and ascospores. Fig. 207. Ascospores germinating on MEA. Fig. 208. Conidia and conidiogenous cells. Scale bar = 10 µm. (From Taylor *et al.*, 2001b).

Mycosphaerella lateralis Crous & M.J. Wingf., *Mycologia* **88**: 454 (1996). Figs 221–222.

Anamorph: ***Dissoconium dekkeri*** de Hoog & Hijwegen, *Mycological Research* **95**: 679 (1991).

= *Uwebraunia lateralis* Crous & M.J. Wingf., *Mycologia* **88**: 454 (1996).

Symptoms: *Leaf spots* amphigenous, subcircular, variable, grey-brown, surrounded by raised borders, medium brown on the adaxial surface, concolorous on the abaxial surface.

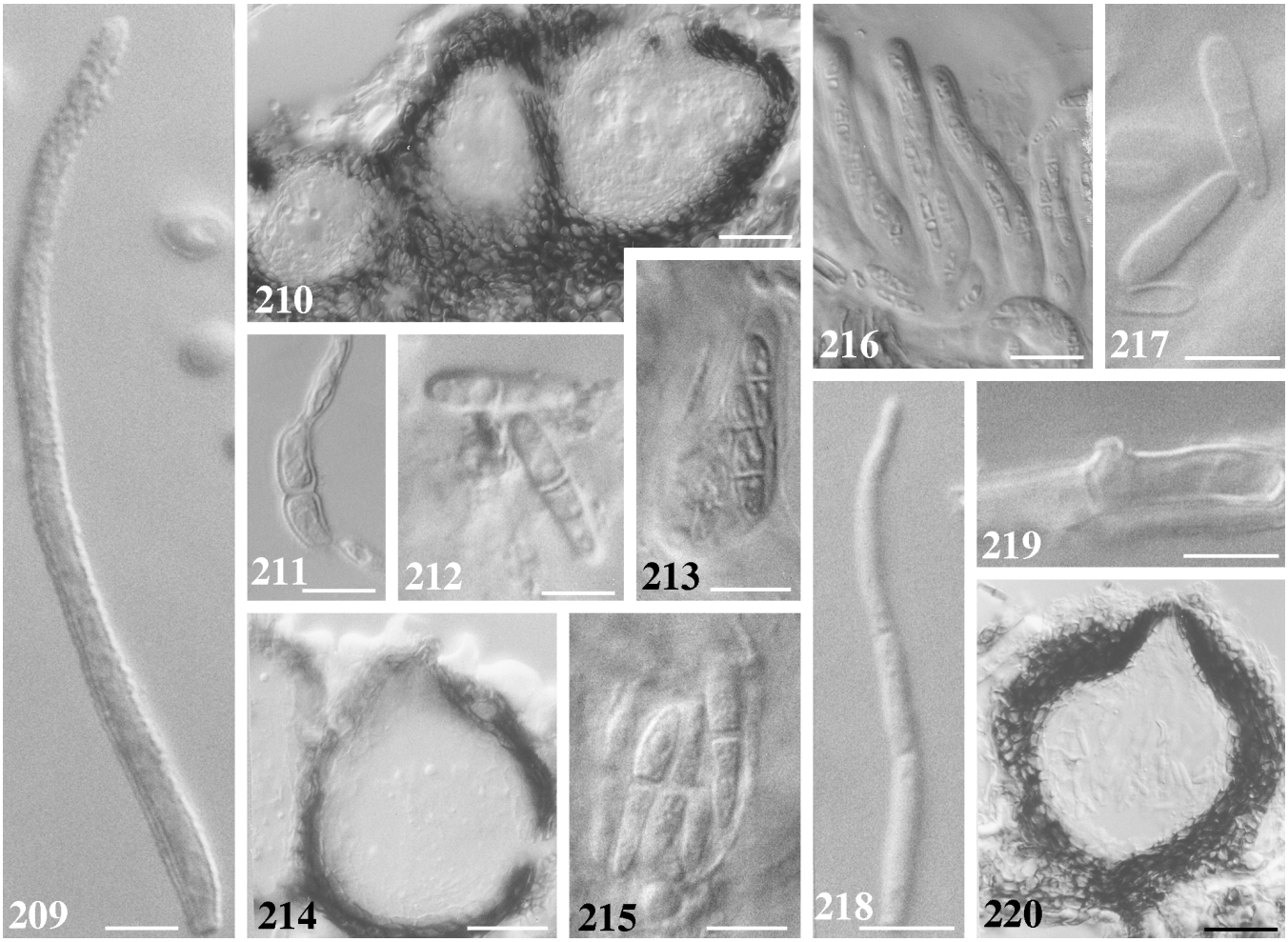
Causal organism: *Ascomata* amphigenous, mainly epiphyllous, single, inconspicuous, sparse on lesions, black, subepidermal, becoming erumpent, globose, 40–60 µm diam, 50–70 µm high; apical papillate ostioles 10–15 µm diam; walls of 3–4 layers of medium brown *textura angularis*, subhymenium of 1–2 layers of colourless cells. *Asci* cylindrical, straight or incurved, 8-spored, 30–50 × 6–10 µm. *Ascospores* multiseriate, overlapping, colourless, guttulate, thin-walled, straight or slightly curved, fusoid–ellipsoidal with an obtuse apex, widest in the middle of the apical cell, medianly 1-septate, not constricted at the septum, tapering towards both ends, (7–)8–14(–16) × 2–2.5(–3) µm.

Mycelium of anamorph internal and external, hyphae consisting of branched, septate, smooth, colourless to pale brown hyphae, 1.5–3 µm wide, giving rise to single conidiophores. *Conidiophores* separate, arising from mycelium, subcylindrical, tapering to a bluntly rounded apex, straight or geniculate-sinuous, smooth, medium brown, 0(–2)-septate, 20–70 × 3–5 µm. *Conidiogenous cells* smooth, pale to medium brown, subcylindrical, subulate, rarely lageniform, straight to sinuous, holoblastic, proliferating percurrently, with up to 4 annellations at the subtruncate apex, 20–55 × 3–5 µm. *Conidia* terminal, solitary, pale olivaceous, smooth, obclavate, apex obtuse, base obconic-truncate, medianly 1-septate, (15–)17–21(–35) × (2–)3.5–4(–4.5) µm; hilum subtruncate, unthickened, not darkened or refractive, flattened, 1.5–2 µm wide. *Secondary conidia* developing from primary conidia, pale olivaceous, one-celled, pyriform, with a truncate base, 3–4 × 2–3 µm.

Cultural characteristics: Colonies with even margin, aerial mycelium profuse, grey olivaceous (23””i), reverse the same; 53 mm diam on MEA after 1 month at 25°C in the dark. Anamorph produced in culture.

Host range and geographic distribution: *Leucadendron* sp., *P.* ‘Kurrajong Carnival’, *P. magnifica, Protea* sp. (**Australia, NSW**).

Disease cycle and epidemiology: Wind-borne ascospores and actively discharged conidia.



Figs 209–220. *Mycosphaerella* spp. Figs 209–213. *Mycosphaerella waimeana* and its *Stenella* anamorph. Fig. 209. Conidium. Fig. 210. Vertical section through pseudothecia. Fig. 211. Germinating ascospore. Fig. 212. Ascospores. Fig. 213. Ascus. Figs 214–215. *Mycosphaerella holualoana*. Fig. 214. Vertical section through a pseudothecium. Fig. 215. Ascus. Figs 216–220. *Mycosphaerella konae* and its *Pseudocercospora* anamorph. Fig. 216. Asci. Fig. 217. Ascospores. Fig. 218. Conidium. Fig. 219. Conidiogenous cell *in vitro*. Fig. 220. Vertical section through a pseudothecium. Scale bars: 209, 212 = 5 µm, 210, 214, 220 = 20 µm, 211, 213, 215–119 = 10 µm. (From Taylor *et al.*, 2001b).

Notes: This species has been recorded from a wide range of hosts, including conifers and dicotyledonous angiosperms. Although this species has been associated with leaf spots on some hosts, it appears to be a hyperparasite of other species of *Mycosphaerella*, which are primary pathogens. Its status as a plant pathogen remains to be proven. From recent molecular data (Crous, unpubl.) we concluded *M. lateralis* is a species complex, and that the species on *Protea* is probably distinct from *M. lateralis*. Further studies are required, however, to fully resolve this complex.

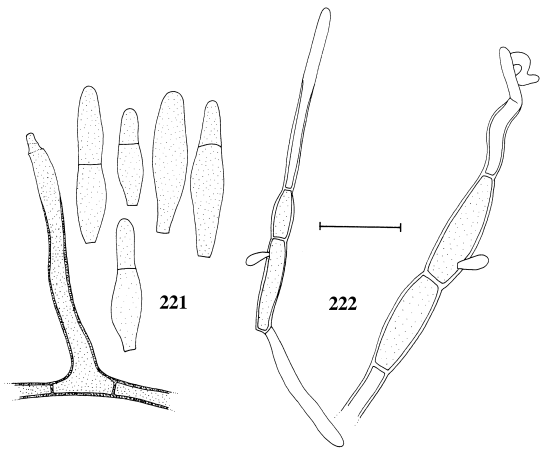
Mycosphaerella leucospermi Crous & Joanne E. Taylor, *Mycotaxon* **78**: 87 (2001). Figs 223–225.

Symptoms: *Leaf spots* amphigenous, subcircular to irregular, 3–10 mm diam, grey, surrounded by a thin, raised, brown border.

Causal organism: *Mycelium* internal and external, consisting of branched, septate hyphae, medium brown, finely verruculose, up to 5 µm wide. *Ascomata* amphigenous, sparsely distributed, intra to subepidermal, substomatal, becoming erumpent, globose to subglobose, up to 150 µm diam and 130 µm high; apical ostiole up to 20 µm diam; wall consisting of 2–3 layers of medium brown *textura angularis*. *Asci* 8-spored, aparaphysate, fasciculate, subsessile, obovoid, straight to incurved, apical chamber inconspicuous or minute, 40–64 × 8–20 µm. *Ascospores* bi- to multiseriate, guttulate, thick-walled, straight to slightly curved, fusoid–ellipsoidal with obtuse ends, widest in the middle of apical cell, tapering to both ends but more prominently to the lower end, becoming pale brown and constricted at the septum with age, (15–)17–20 × (4–)5–6(–7) µm; ascospores 1-septate, become brown and finely verruculose after discharge.

Host range and geographic distribution: *Lsp. cordifolium, P. cynaroides, P. laurifolia* (**USA, California**).

Disease cycle and epidemiology: Wind-borne ascospores.



Figs 221–222. *Dissoconium* anamorph of *Mycosphaerella lateralis*. Fig. 221. Conidiophore and primary conidia. Fig. 222. Primary and anastomosing secondary conidia. Scale bar = 10 µm. (From Crous *et al*., 2000).

Mycosphaerella microspora (Joanne E. Taylor & Crous) Joanne E. Taylor & Crous, *Mycological Research* **107**: 657 (2003). Figs 226–228.

≡ *Teratosphaeria microspora* Joanne E. Taylor & Crous, *Mycological Research* **104**: 631 (2000).

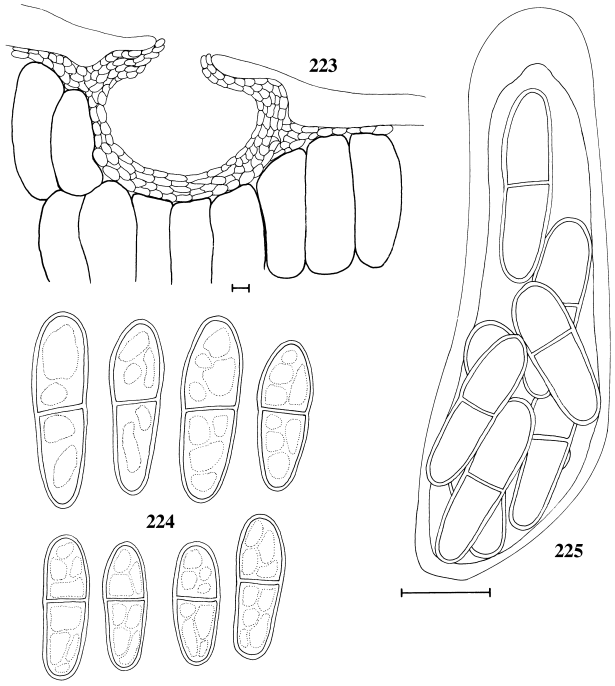
Anamorph: ***Trimmatostroma microsporum*** Joanne E. Taylor & Crous, *Mycological Research* **104**: 631 (2000).

Symptoms: *Leaf spots* associated with *Mycosphaerella proteae*, circular, non-necrotic red spots.

Causal organism: *Mycelium* immersed, pale brown, septate, smooth, 3–5.5 µm diam. *Ascomata* amphigenous, immersed, raising the host surface, substomatal, subepidermal, with small or no papilla, occurring singly but gregarious; in section globose to slightly subglobose, with periphysate central ostiole emerging through the stomata, asci attached at the periphery, stroma lacking, up to 150 µm high, 140 µm diam. *Peridium* comprising two strata, the outer stratum of thick-walled, medium brown small-celled *textura angularis,* becoming thinner-walled and hyaline in the inner stratum, altogether (15–)17–23(–27) µm thick. *Asci* oboclavate to globose, bitunicate, sessile, narrowing to a rounded apex, lacking an ocular chamber, 8-spored, sparse, (22–)30–36(–42) × (9–)11–13(–15) µm. *Ascospores* broadly fusiform, medianly 1-septate, not constricted at the septum, upper cell slightly broader, hyaline, to pale brown, smooth, eguttulate, with obtuse apices, apparently to be surrounded by a mucilaginous sheath, (7.5–)9–9.5(–12) × (3–)3.5–4(–4.5) µm.

Colonies sporodochial, pulvinate, dry, dark brown to black. *Stromata* in substomatal cavity, of brown walled pseudoparenchymatous cells. *Conidiophores* macronematous, mainly straight, caespitose, closely packed, emerging through stomata forming the sporodochia, short, smooth, olivaceous-brown. *Conidiogenous cells* irregularly cylindrical, terminal, holoblastic, delimitation of conidium by a single septum, with retrogressive delimitation of next conidium, giving an unconnected chain of conidia, secession schizolytic, 6–8 × 3–4.5 µm. *Conidia* variously shaped, cylindrical, y-shaped, ellipsoidal, straight or curved, with rounded or truncated apices, catenate in branched basipetal chains, which are schizogenous, olivaceous to red-brown, multiseptate, with transverse and often longitudinal or oblique septa, 1-septate conidia predominating on the host, thick-walled, verrucose, (8–)13–15(–21) × (3.5–)5.5–6(–8) µm.

Sporodochia not formed *in vitro*. *Conidiophores* micronematous, branched, flexuous, smooth, pale to medium brown, 3–4.5 µm wide. *Conidiogenous cells* mainly cylindrical, integrated, conidiogenesis as for that of fungi on the host, but forming an unconnected branched chain of conidia, *ca.* 4–12 × 3.5–4 µm. *Conidia* variously shaped, cylindrical, y-shaped, ellipsoidal, straight or curved, rounded or truncated apices,



Figs 223–225. *Mycosphaerella leucospermi*. Fig. 223. Vertical section through a pseudothecium. Fig. 224. Ascospores. Fig. 225. Ascus. Scale bar = 10 µm. (From Taylor *et al.*, 2001a).

catenate in branched basipetal chains which are schizogenous, olivaceous-brown, multiseptate, with transverse and often longitudinal or oblique septa, multicelled conidia predominating, thick-walled, verrucose, (5–)7.5–9(–16) × (3–)4.5–5.5(–7) µm.

Cultural characteristics: *Colonies* circular, flat with entire margins, dark mouse-grey (13”””k) to olivaceous-black (27”””k), the same in reverse; aerial mycelium moderately dense, felty; 15 mm diam after 14 days; anamorph produced in culture after 2–3 days.

Host range and geographic distribution: *P. cynaroides* (**South Africa, WC**).

Notes: Ascospores germinate from both cells and germ tubes grow parallel to the long axis of the spore. There is no constriction at the septum and the spores do not darken or become verruculose upon germination.

Mycosphaerella proteae (Syd.) Arx, *Die Gattungen der Didymosporen Pyrenomyceten. Beiträge zur Kryptogamenflora der Schweiz* **11(2):** 357 (1962).
Figs 229–237, 240, Plates 5, 6.

≡ *Oligostroma proteae* Syd., Annals of Mycology **12**: 265 (1914).

= *Didymella maculiformis* G. Winter, Hedwigia **23**: 169 (1884).

≡ *Oligostroma maculiformis* (G. Winter) Doidge, Bothalia **1**: 31 (1921).

≡ *Teratosphaeria maculiformis* (G. Winter) Joanne E. Taylor & Crous, IMI Descriptions of Fungi and Bacteria No. **1346** (1998).

= *Euryachora maculiformis* Nel, *Annals of the University of Stellenbosch* **20 Ser. A (2):** 11 (1942).

Symptoms: *Leaf spots* variable depending on host, discrete to confluent, bright purple-red to red-brown, becoming grey or black, roughened, irregular in outline, but with a clearly delineated red-brown margin, mainly non-necrotic, raised or dome-shaped with a corresponding concave reaction of the abaxial leaf surface, 4–10(–30) mm diam.

Causal organism: *Ascomata* amphigenous, globose, ostiolate, non-papillate, black, singular, gregarious; in section substomatal, subepidermal, globose to slightly pyriform, periphysoids lining the ostiole and the upper ascoma wall, stroma reduced to an epidermal clypeus consisting of host cells and sparse pale brown fungal hyphae, often becoming confluent with those of adjacent ascomata to form an almost continuous stromatal plate, host cells surrounding ascomata often filled with amber-coloured crystaline matrix, (158–)163–190(–216) µm high, (152–)165–187(–200) µm diam. *Peridium* consisting of three strata of compressed *textura angularis*, the outer stratum formed of hyaline cells, which become brown and thicker-walled in the middle stratum, and hyaline and thin-walled in the inner stratum, (16–)19–25.6(–32) µm. *Paraphyses* reduced to periphysoids. *Asci* obclavate, tapering abruptly to a small pedicel, narrowing to a rounded apex with an indistinct ocular chamber, mainly straight, 8-spored, fasciculate, bitunicate with fissitunicate dehiscence, (72.5–)81.5–87(–97.5) × (15–)20–22(–28) µm. *Ascospores* bi- to triseriate, clavate, straight, 1-septate, with septum slightly supra-median and mainly unconstricted, broadest in the middle of the apical cell and tapering to the lower end, hyaline, (17.5–)23.8–25(–33) × (6.5–)7.5–8(–10) µm in water (slightly narrower in lactophenol, (19–)25–27(–31.5) × (5.5–)6–7(–8) µm) (Taylor & Crous, 1998f).

Cultural characteristics: *Colonies* black and erumpent, devoid of aerial mycelium, slow-growing; 5 mm diam after 6 months at 25ºC.

Host range and geographic distribution: *P. amplexicaulis, P. burchellii, P. caffra, P. compacta, P. cordata, P. cynaroides, P. eximia, P. gaguedi, P. grandiceps, P. laurifolia, P. magnifica, P.* 'Susara', *P. neriifolia, P. nitida, P. obtusifolia, P.* 'Pink Duke', *P. punctata, P. repens, P. roupelliae, P.* 'Shelia', *P. simplex, P. speciosa, P. stokoei, P.* 'Sylvia', *Protea* spp. (**South Africa, EC, WC, GT, KN**); *P. magnifica, P. neriifolia, Protea* sp. (**Zimbabwe**) (Masuka *et al*., 1998).

Notes: Ascospores become brown at germination, and produce germ tubes parallel to the long axis of the ascospore, but the spore wall does not become verruculose. Ascospores were observed to disarticulate at the septum, with each cell frequently becoming 1-septate and germinating. Ascospores often adhere to leaf hairs, with the resulting hyphae growing superficially until they infect the host. Ascospores on the leaf surface are frequently hyperparasitised by a species of *Cladosporium*.

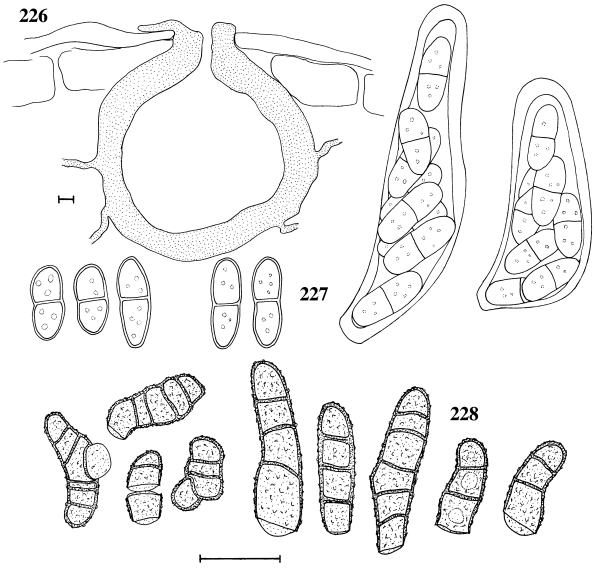
Mycosphaerella proteae is the most common causal agent of leaf spots recorded on proteas and is specific to *Protea* spp. (Von Broembsen, 1989). It is not as significant as some other protea diseases such as *Leptosphaeria protearum,* as it is not as destructive to young plants (Von Broembsen, 1989).

Mycosphaerella proteae-arboreae (P.S. Van Wyk, Marasas & Knox-Dav.) Joanne E. Taylor & Crous, *Mycological Research* **107**: 657 (2003).
Figs 241–251, Plate 7.

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

Symptoms: *Leaf spots* circular in outline, discrete, raised and black in the centre due to development of ascomata, 1–5 mm diam, surrounded by a narrow pinkish margin which may enlarge to form a necrotic zone.

Causal organism: *Ascomata* amphigenous, immersed, black, ostiolate, non-papillate, gregarious; in section subcuticular, intra-epidermal, globose or with flattened sides due to pressure from adjacent ascomata; periphysoids lining the ostiole and the upper ascomatal wall, embedded in a sparse stroma consisting of host cells and sparse pale brown fungal hyphae, host mesophyll cells often filled with amber-coloured crystalline matrix, 120–240 µm high, 115–220 µm diam. *Peridium*



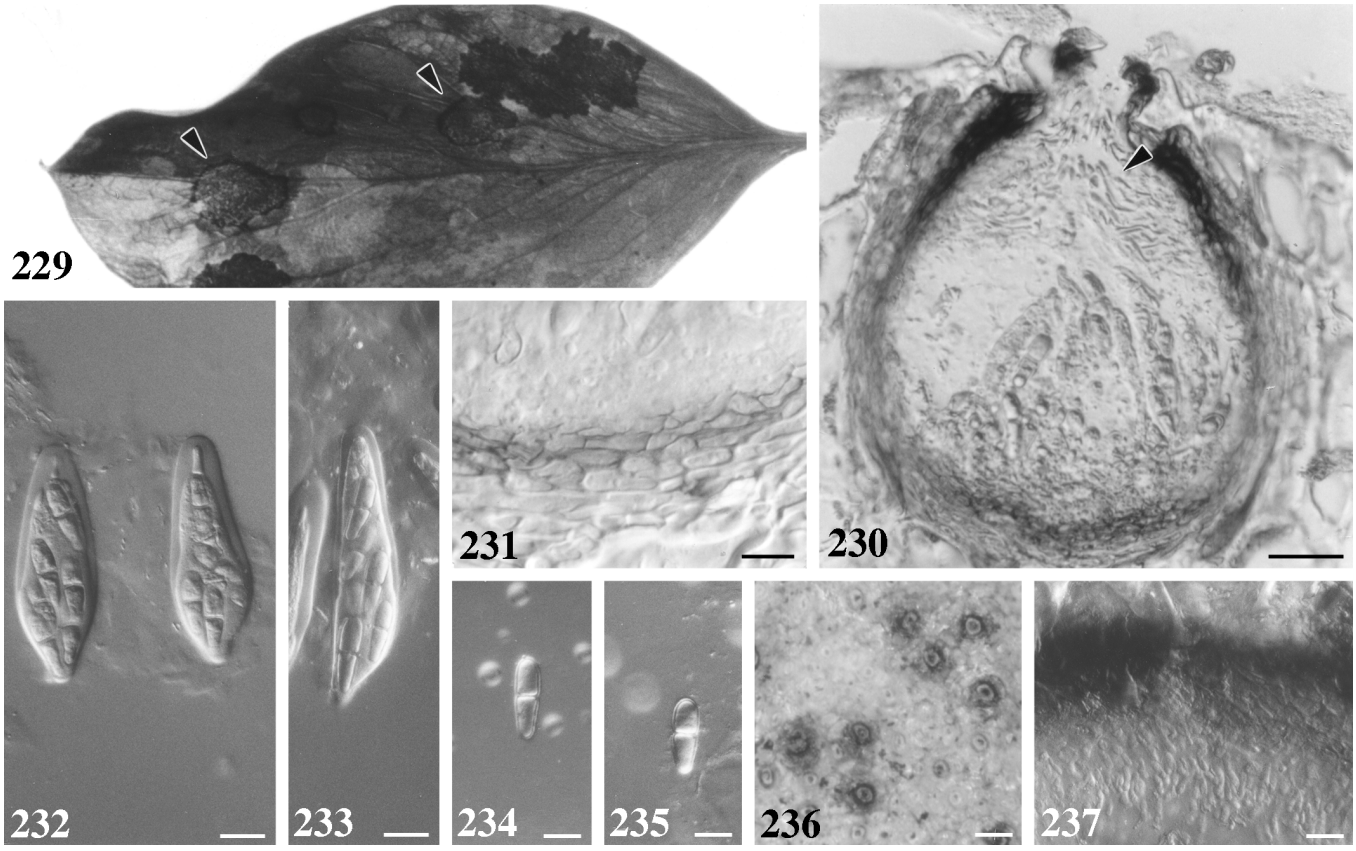
Figs 226–228. *Mycosphaerella microspora* and its anamorph *Trimmatostroma microsporum*. Fig. 226. Vertical section through a pseudothecium. Fig. 227. Asci and ascospores. Fig. 228. Conidia. Scale bars = 10 µm. (From Taylor & Crous, 2000).

consisting of two strata of compressed *textura angularis*, the outer stratum of brown melanised thick-walled cells, becoming hyaline and thinner walled in the inner layer, together 16–28 µm thick. *Asci* obclavate or lageniform, sessile or with a small pedicel, mainly straight, narrowing to a rounded apex with a distinct ocular chamber, 8–spored, bitunicate with fissitunicate dehiscence, (60–)75–82(–95) × (20–)22–24.5(–33) µm. *Ascospores* bi- to multiseriate, cylindrical with rounded ends, straight or slightly curved, medianly 1-septate, and slightly constricted with a slightly wider upper cell, hyaline, becoming pale yellow-brown at maturity, (19–)24.5–26(–32) × (4.5–)6–8(–9) µm in water (in lactophenol, (21–)25–26(–28) × (5–)6–6.5(–7.5) µm). *Spermogonia* intermixed with ascomata and possessing the same general structure; hymenium of spermatiophores which produce unicellular, hyaline, bacillar spermatia, 1.5–4 × 1 µm (Taylor & Crous, 1998h).

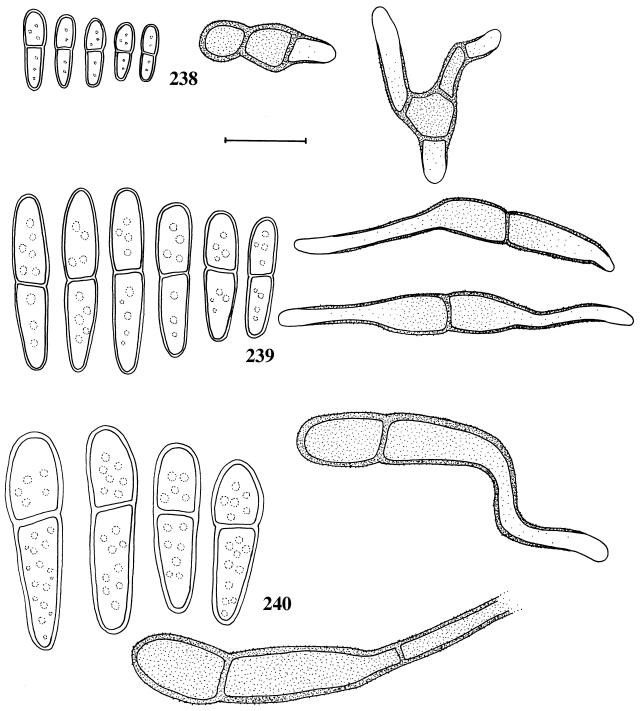
Host range and geographic distribution: *P. neriifolia, P. nitida, P. magnifica* hybrid (**South Africa, WC**).

Notes: M. proteae-arboreae causes a leaf spot, but is not an economically significant pathogen as it is most commonly recorded on *Protea nitida*, a host of no present commercial importance.

Mycosphaerella stromatosa Joanne E. Taylor & Crous, *Mycological Research* **104**: 625 (2000).
Figs 194–205.
Anamorph: ***Pseudocercospora stromatosa*** Joanne E.



Figs 229–237. *Mycosphaerella proteae*. Fig. 229. Leaf spots on *Protea cynaroides*. Fig. 230. Vertical section through a pseudothecium. Fig. 231. Pseudothecial wall layer. Figs 232–233. Asci. Figs 234–235. Ascospores. Fig. 236. Pseudothecia on host substrate. Fig. 237. Periphysoids. Scale bars: 230 = 30 µm, 231–235, 237 = 10 µm, 236 = 200 µm. (From Taylor & Crous, 1998).



Figs 238–240. Ascospores of *Mycosphaerella* spp. *in vivo*, and germinating *in vitro* on MEA. Fig. 238. *M. bellula*. Fig. 239. *M. jonkershoekensis*. Fig. 240. *M. proteae*. Scale bar = 10 µm. (From Swart *et al*., 1998).

Taylor & Crous, *Mycological Research* **104**: 625 (2000).

Symptoms: *Leaf spots* necrotic, sunken, pale brown, with a raised dark brown margin, causing tip dieback; ascomata visible as densely scattered blackened dots within the leaf spots.

Causal organism: *Ascomata* mainly epiphyllous, immersed, becoming erumpent, substomatal and initially subepidermal, visible as a raised, darkened area, with or without a small papilla, occurring singly but gregarious; in section globose surrounded by an extensive and irregularly shaped stromata, with a well developed, non-periphysate, central ostiolar pore, (95–)121–170(–180) µm high, (88–)100–119(–125) µm diam. *Peridium* not easy to differentiate from the stromata. *Stromata* composed of thick-walled, large-lumened dark brown cells, becoming paler, thin-walled and more compressed in the inner layer, melanised around the ostiole, together (15–)18–28(–40) µm thick, embedded in pseudostroma comprising host cells loosely filled with thick-walled, smooth, brown fungal hyphae. *Asci* obclavate to cylindro-clavate, bitunicate, sessile, narrowing to a rounded apex, lacking an ocular chamber, 8-spored, (29–)33–36(–40) × (6–)7–8(–10) µm. *Ascospores* broadly fusiform, 1-septate, unconstricted at the septum, upper cell broader and widest at the middle, hyaline, smooth, eguttulate, with obtuse apices, (8.5–)10.5–11(–13) × (2.5–)3–3.5(–4) µm.

Mycelium of anamorph internal and external, hyphae branched, septate, smooth, olivaceous to pale brown, 1.5–3 µm wide. *Conidiomata* fasciculate, amphigenous, medium brown, approximately 120 µm diam, 90 µm high. *Conidiophores* aggregated in dense fascicles arising from upper cells of a brown stroma, approximately 120 µm diam, 60 µm high; conidiophores pale brown, smooth, 0–6 septate, cylindrical, straight to curved, prominently branched below or above, 20–70 × 2–3 µm. *Conidiogenous cells* terminal or intercalary, unbranched, olivaceous, smooth, tapering to flat-tipped apical loci, holoblastic, proliferating sympodially, 10–20 × 2–3 µm. *Conidia* solitary, olivaceous, smooth, guttulate, cylindrical, apex obtuse and base truncate to long obconically truncate, straight to curved, 3–6-septate, 25–40 × 2.5–3 µm.

Cultural characteristics: *Colonies* circular, convex with crenated margins; an uneven mixture of dark grey and pale grey with some red patches; dense, woolly aerial and immersed mycelium; 16 mm diam after 30 days. Cultures sterile.

Host range and geographic distribution: *Protea* sp. (**South Africa**).

Mycosphaerella waimeana Crous, Joanne E. Taylor & M.E. Palm, *Mycotaxon* **78**: 463 (2001).

Figs 209–213, 252–254, Plate 8.

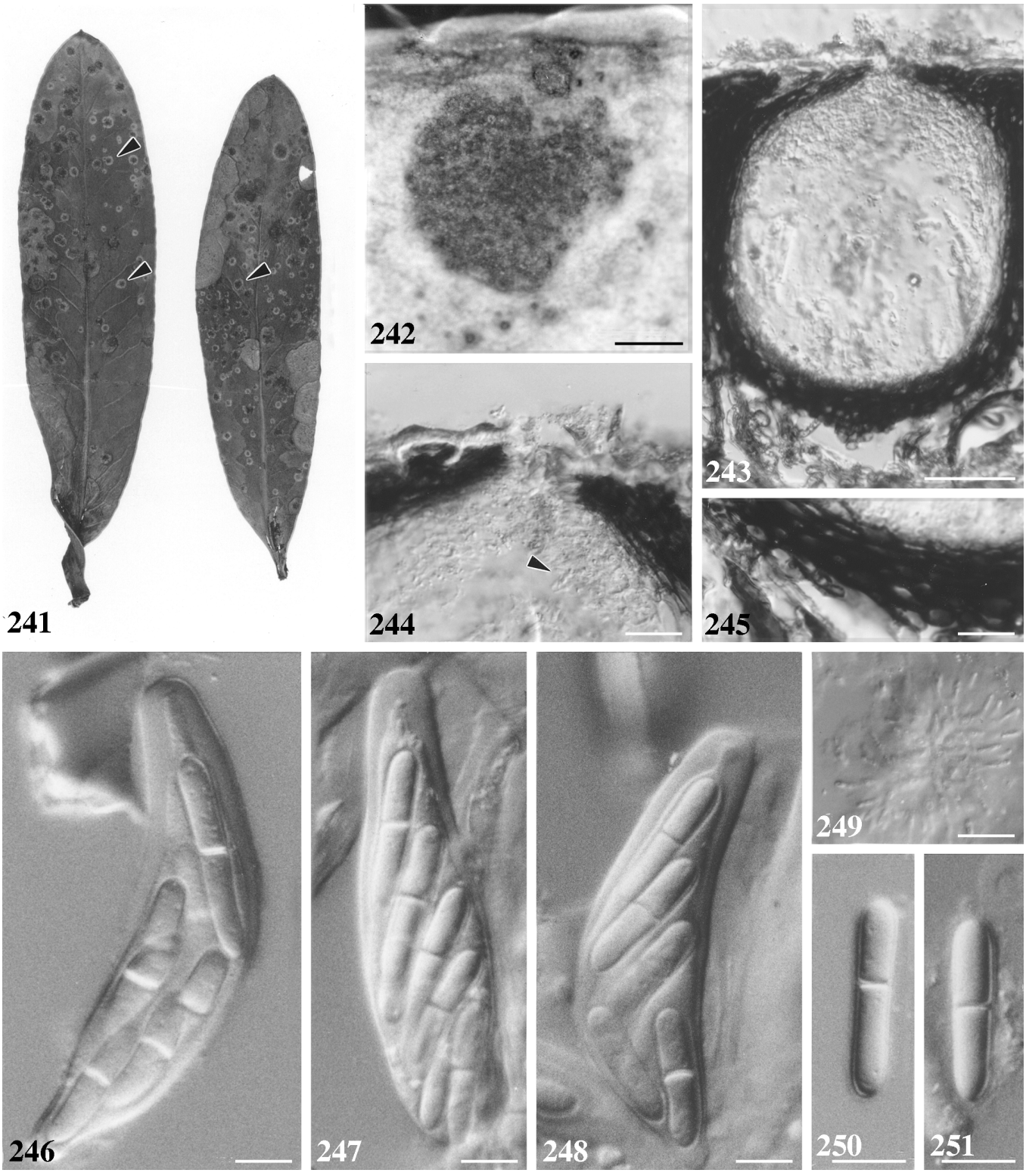
Anamorph: ***Stenella*** sp.

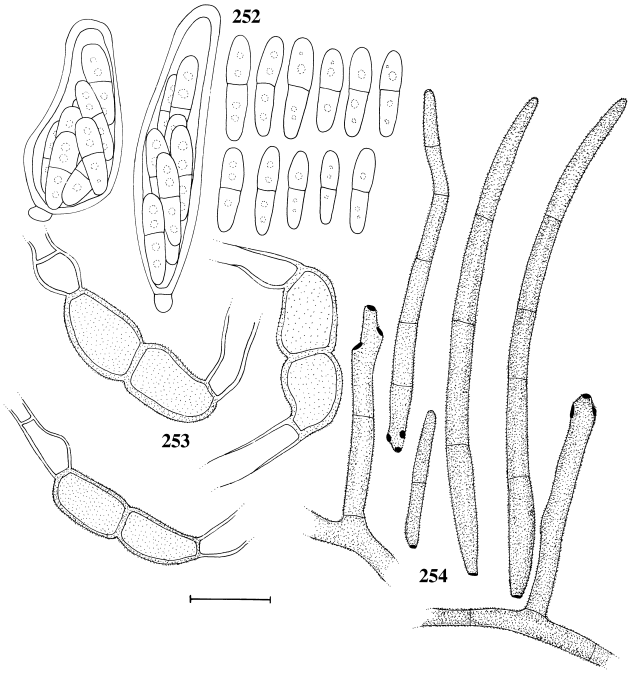
Symptoms: *Leaf spots* amphigenous, irregular to subcircular, 1–5 mm diam, grey to pale brown, with raised borders and purple margins; pseudothecia sporulating on necrotic leaves attached to bushes.

Causal organism: *Ascomata* amphigenous, black, subepidermal, substomatal, becoming erumpent, frequently aggregated in dense clusters (on necrotic leaves); in section globose to subglobose, 40–90 µm wide, 50–90 µm high, apical ostiole 5–15 µm wide; wall consisting of 2–3 layers of medium brown *textura angularis*; older pseudothecia developing additional stromatic tissue around the ostiolar area. *Asci* aparaphysate, fasciculate, bitunicate, subsessile, obovoid to broadly ellipsoidal, straight to slightly curved, narrowing to a rounded apex, lacking an ocular chamber, 8-spored, 25–35 × 7–9 µm. *Ascospores* tri- to multiseriate, hyaline, guttulate, mostly with two large guttules per cell, thin-walled, straight to slightly curved, fusoid–ellipsoidal with obtuse ends, widest in middle of apical cell, medianly 1-septate, slightly constricted at the septum, tapering towards both ends, but more prominently towards the lower end, (9–)10–11(–12) × (2.5–)3 µm.

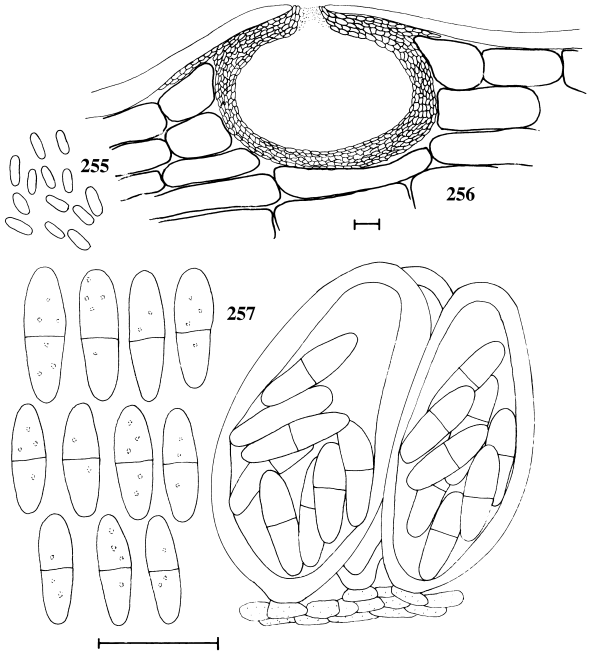
Mycelium of anamorph internal and external, hyphae branched, septate, medium brown, verruculose, hyphae 2–3 µm wide. *Conidiophores* arising singly from superficial mycelium, 25–40 × 3–4 µm, 0–2-septate, subcylindrical, straight to variously curved, unbranched or branched above. *Conidiogenous cells* terminal, unbranched, medium brown, verruculose, tapering to flat-tipped or rounded apical loci, proliferating sympodially, 10–30 × 3–4 µm; loci thickened, darkened and refractive. *Conidia* solitary, verruculose, medium brown, straight or curved, narrowly obclavate, apex subobtuse, base long obconically subtruncate, 1–11-septate, 25–100 × 3–3.5 µm; hila thickened, darkened and refractive. *Cultural characteristics*: *Colonies* circular with smooth but uneven margins, erumpent, forming radiating striations in agar; aerial mycelium sparse, surface olivaceous-grey (23’’’’b), olivaceous-black (25’’’’m) in reverse; 20 mm diam after 14 days. The anamorph was produced in culture and also observed on the host. No pseudothecia formed in culture.

Host range and geographic distribution: *Lsp.* ‘Hybrid 24’, *Leucospermum* sp. (**USA, Hawaii**).





Figs 252–254. *Mycosphaerella waimeana* and its *Stenella* anamorph. Fig. 252. Asci and ascospores. Fig. 253. Germinating ascospores on MEA. Fig. 254. Conidiophores and conidia. Scale bar = 10 µm. (From Taylor *et al*., 2001b).



Figs 255–257. *Mycosphaerella* sp. Fig. 255. Spermatia. Fig. 256. Vertical section through a pseudothecium. Fig. 257. Asci and ascospores. Scale bars = 10 µm. (From Taylor *et al*., 2001a).

Notes: Ascospores germinate from both ends of the spore, not parallel to the long axis of the spore, but at various angles, mostly 90º, becoming pale brown after 24 h, spore becoming verruculose and distorted, 6–7 µm wide.

Mycosphaerella sp., *Mycotaxon* **78**: 89 (2001)
Figs 255–257.

Symptoms: *Lesions* caulicolous and foliicolous, inconspicuous on stems, but circular and amphigenous on leaves, 1–8 mm diam, surrounded by a dark brown, raised border, and a thin, chlorotic margin.

Causal organism: *Mycelium* internal and external, consisting of branched, septate, medium brown, finely verruculose hyphae, up to 5 µm wide. *Ascomata* amphigenous, evenly distributed, black, intra- to subepidermal, becoming slightly erumpent, substomatal, subglobose, up to 100 µm wide and 60 µm high; apical ostiole 10–15 µm wide; wall consisting of 3–4 layers of brown *textura angularis*, together up to 10 µm thick. *Asci* aparaphysate, fasciculate, bitunicate, subsessile, obovoid to ellipsoid, straight to incurved, 8-spored, 28–50 × 8–11 µm. *Ascospores* tri- to multiseriate, hyaline, not to sparsely guttulate, fusoid–ellipsoidal with obtuse ends, widest just above the septum, tapering more prominently towards the lower end, 1-septate, not constricted at the septum, thick-walled, (9–)10–11(–12) × 3–3.5 µm.

Host range and geographic distribution: *Lsp. cordifolium*, *P. cynaroides, P. eximia* (**USA, California**).

Disease cycle and epidemiology: Ascospores are wind-borne.

Control: Unknown.

Figs 241–251. *Mycosphaerella proteae-arboreae*. Fig. 241. Leaf spots on *Protea magnifica*. Fig. 242. Leaf spot with ascomata. Fig. 243. Vertical section through a pseudothecium. Fig. 244. Ostiolar area. Fig. 245. Pseudothecial wall layer. Figs 246–248. Asci. Fig. 249. Periphysoids. Figs 250–251. Ascospores. Scale bars: 242 = 2 mm, 243 = 50 µm, 244–251 = 10 µm. (From Taylor & Crous, 1998).

KEY TO MYCOSPHAERELLA *SPECIES ON* PROTEACEAE

1 Ascospores mostly > 13 µm long **2**
1 Ascospores mostly < 13 µm long **8**
2 Ascospores up to 16 × 3 µm **3**
2 Ascospores longer than 16 µm **4**
3 Ascospores frequently with a mucous sheath; no anamorph known from culture ..... ***M. holuoloana***
3 Ascospores without mucous sheath; forming *Dissoconium dekkeri* in culture ..... ***M. lateralis***
4 Ascospores > 25 µm long **5**
4 Ascospores < 25 µm long **7**
5 Lesions comprising radiating lines of immersed black pseudothecia, spots non-necrotic; ascospores (33.5–)40.5–43(–51.5) × (6–)7.5–10(–12) µm ..... ***M. fibrillosa***
5 Lesions different from above; leaf spots discrete..... **6**
6 Leaf spots bright red-purple to red-brown, becoming grey or black, ascospores (17.5–)23.8–25 (–33) × (6.5–)7.5–8(–10) µm; no anamorph known from culture ..... ***M. proteae***
6 Leaf spots black, 1–5 mm diam, raised, due to aggregated ascomata; ascospores (19–)24.5–26 (–32) × (4.5–)6–8(–9) µm ..... ***M. proteae-arboreae***
7 Ascospores fusiform, up to 25 µm long, 3.5–4(–5) µm wide, with slightly longer basal cell ..... ***M. jonkershoekensis***
7 Ascospores fusoid–ellipsoidal, up to 20 µm long, (4–)5–6(–7) µm wide, medianly septate ..... ***M. leucospermi***
8 Ascospores producing *Trimmatostroma microsporum* in culture ..... ***M. microspora***
8 Ascospores not producing a *Trimmatostroma* anamorph in culture **9**
9 Ascospores surrounded by a mucilaginous sheath (4–6 µm thick) when viewed in water ..... ***M. bellula***
9 Ascospores not surrounded by a sheath when viewed in water **10**
10 Ascomata formed in a well-developed stroma; ascospores up to 4 µm wide; anamorph *Pseudocercospora* ..... ***M. stromatosa***
10 Ascomata not formed in a stroma; ascospores less than 3.5 µm wide **11**
11 Ascospores widest just above the septum, thick-walled; on *Protea* and *Leucospermum* ..... ***Mycosphaerella* sp.**
11 Ascospores widest in middle of the apical cell, thin-walled **12**
12 Ascospores slightly constricted at the septum, becoming pale brown at germination, germ tubes not parallel to the long axis of the spore; anamorph *Stenella* ..... ***M. waimeana***
12 Ascospores not constricted at septum, remaining hyaline at germination; germ tubes parallel to the long axis of the spore; anamorph *Pseudocercospora* ..... ***M. konae***

KEY TO MYCOSPHAERELLA *ANAMORPHS ON* PROTEACEAE

1 Conidiomata consisting of single conidiophores on superficial hyphae, sporodochia or fasciculate tufts of conidiophores **2**
1 Conidiomata pycnidial **3**
2 Conidiophores hyaline, conidial scars conspicuous, thickened, darkened and refractive; conidia catenate, subcylindrical–fusoid, 5–8(–10) × 1–1.5(–2) µm, 0(–1)-septate, hyaline, smooth; hila slightly thickened, darkened and refractive ..... ***Ramularia proteae***
2 Conidiophores and conidia pigmented **9**
3 Conidiophores and conidia hyaline **4**
3 Conidiophores and conidia pigmented **5**
4 Conidia narrowly cylindrical, straight or curved, with a rounded apex and a truncate base, up to 7-euseptate, (33–)43–46(–57) × 2–3 µm ..... ***Septoria grandicipis***
4 Conidia subcylindrical to narrowly obclavate, apex subobtuse, base narrow obconically truncate to truncate, straight to curved, (0–)1–3(–4)-euseptate, (6–)15–22(–30) × 1.5–2 µm ..... ***Septoria protearum***
5 Conidia > 4-euseptate; conidia subcylindrical or narrowly ellipsoidal, medium brown, verruculose, (1–)4–7-euseptate (30–)35–45(–50) × (5–)6–7 µm ..... ***Phaeophleospora protearum***
5 Conidia < 4-euseptate **6**
6 Conidia < 25 µm long **7**

6 Conidia > 25 µm long **8**

7 Conidia narrowly fusiform, (0–)3(–4)-euseptate, (12–)15–16.5(–19) × (4–)4.5–5(–6) µm ..... ***Phaeophleospora capensis***

7 Conidia cylindrical, (0–)1(–3)-euseptate, (10–)12–22(–25) × 3–4(–4.5) µm ..... ***Phaeophleospora congesta***

8 Conidia narrowly obclavate to subcylindrical, pale brown, (0–)1–3-euseptate, (17–)22–32(–38) × (2.5–)3–3.5 µm ..... ***Phaeophleospora abyssinicae***

8 Conidia subcylindrical to narrowly obclavate, medium yellow-brown, (0–)3(–4)-euseptate, (32–)39–42(–52) × (3.5–)4(–4.5) µm ..... ***Phaeophleospora concentrica***

9 Conidiophore loci thickened, darkened and refractive; conidia solitary, verruculose, medium brown, straight or curved, narrowly obclavate, 1–11-septate, 25–100 × 3–3.5 µm ..... ***Stenella*** anamorph of ***Mycosphaerella waimeana***

9 Conidiophore loci not thickened, darkened or refractive **10**

10 Conidia < 15 µm long **11**

10 Conidia > 15 µm long **13**

11 Conidia less than 10 µm long, mainly aseptate; leaf spots large, up to 25 mm diam, usually with a reddish tinge ..... ***Batcheloromyces proteae***

11 Conidia commonly more than 10 µm long, occurring on smaller leaf spots of up to 8 mm diam, often in fragile chains composed of single-celled conidia and multi-celled structures **12**

12 Conidial outer wall layers very roughly verrucose, often appearing as uneven plates; conidia often remaining in chains of up to eight; conidiogenous cells cylindrical ..... ***Batcheloromyces leucospermi***

12 Conidial outer wall layers roughly verrucose; conidia remaining in chains of up to three; conidiogenous cells doliiform to calyciform ..... ***Batcheloromyces leucadendri***

13 Conidia 1-septate; pale olivaceous, smooth, obclavate, (15–)17–21(–35) × (2–)3.5–4(–4.5) µm; secondary conidia developing from primary conidia, pale olivaceous, one-celled, pyriform, 3–4 × 2–3 µm ..... ***Dissoconium dekkeri*** anamorph of ***M. lateralis***

13 Conidia multiseptate **14**

14 Conidia distoseptate **15**

14 Conidia euseptate **19**

15 Conidia < 7 µm wide; conidia cylindrical, fusiform or obclavate, straight, yellow-brown, finely verrucose, 0–3 distoseptate, (11–)14–16(–19) × (3–)5–5.5(–7) µm ..... ***Stigmina californiae***

15 Conidia > 7 µm wide **16**

16 Conidia > 27 µm long; conidia variable in shape, frequently constricted at the septa, medium brown, verruculose, with one to several horizontal distosepta; vertical and oblique distosepta frequently present (12–)25–35(–45) × (7–)10–15(–25) µm ..... ***Trimmatostroma protearum***

16 Conidia < 27 µm long **17**

17 Leaf spots bright purple-red to red-brown, becoming grey or black, similar to those of *M. proteae*; conidia olivaceous to red-brown, multiseptate, with transverse and often longitudinal or oblique septa, with bicelled conidia predominating on the host, thick-walled, verrucose ..... ***Trimmatostroma microsporum*** anamorph of ***M. microspora***

17 Leaf spots different than above **18**

18 Leaf spots pale brown, circular; conidia mainly cylindrical, ellipsoidal, straight or curved, olivaceous-brown, thick-walled, finely verrucose to smooth ..... ***Trimmatostroma elginense***

18 Leaf spots circular, discrete or confluent, sometimes covering the whole leaf surface, spots non-necrotic, tissue becoming yellow-brown, dry, speckled, sooty; conidia ellipsoidal, cylindrical, clavate, y-shaped, curved or straight, 1- to multiseptate, pale to dark red-brown or olivaceous -brown, verrucose ..... ***Trimmatostroma macowanii***

19 Conidia medium brown, verruculose, 1–3(–6)-septate **20**

19 Conidia olivaceous to pale brown, smooth **21**

20 Conidia subcylindrical to obclavate, (5–)6–7(–9) µm wide ..... ***Pseudocercospora protearum*** var. ***protearum***

20 Conidia subcylindrical, 4.5–5(–7) µm wide ..... ***Pseudocercospora protearum*** var. ***leucadendri***

21 Conidia subcylindrical, apex obtuse to subobtuse, base truncate, rarely narrowly obclavate, 1–6 septate, 15–60 × 2–3 µm ..... ***Pseudocercospora*** anamorph of ***Mycosphaerella konae***

21 Conidia cylindrical, apex obtuse and base truncate to long obconically truncate, straight to curved, 3–6-septate, 25–40 × 2.5–3 µm ..... ***Pseudocercospora*** anamorph of ***Mycosphaerella stromatosa***

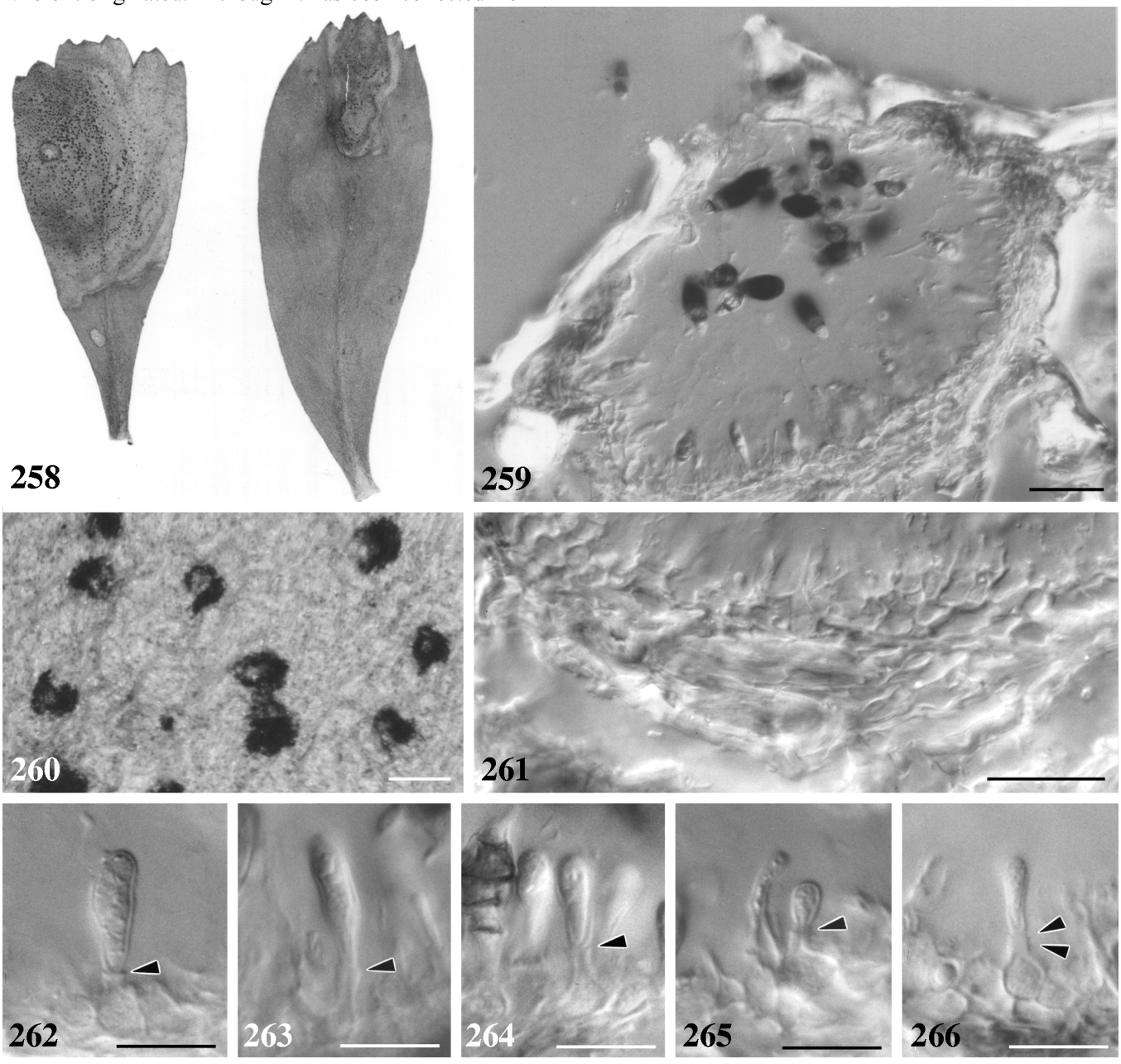
Pestalotiopsis leaf spot

Pestalotiopsis spp. are widespread, occur on many hosts, and generally regarded to be saprobic or weakly pathogenic. Two species of *Pestalotiopsis* have been described from *Proteaceae*. *Pestalotiopsis montellicoides* was isolated from *Protea cynaroides* leaves from South Africa (Mordue, 1986), and a *Pestalotiopsis* sp., the anamorph of *Pestalosphaeria leucospermi*, was described from living leaves of a *Leucospermum* sp. in New Zealand (Samuels *et al.,* 1987).

In Zimbabwe, a species of *Pestalotiopsis* was recorded causing leaf spots on several *Protea* and *Leucospermum* hosts (Swart *et al*., 1999b). This species subsequently was recorded in Australia, which is possibly where it originated. Although it has been collected from *Proteaceae* in the Western Cape Province of South Africa, it has thus far not been associated with any disease symptoms in this region. This species of *Pestalotiopsis* is probably only a problem of commercial importance in summer rainfall areas. It has been intercepted at quarantine inspection points (Taylor, 2001).

Pestalotiopsis sp., *South African Journal of Botany* **65**: 239 (1999). Figs 258–267, Plate 9.

Symptoms: *Leaf spots* irregular, necrotic, associated with leaf margins or causing tip dieback; slightly sunken, pale brown with red-brown margins that are mostly raised and distinct, rarely diffuse, 2–35 mm diam.



Figs 258–266. *Pestalotiopsis* sp. Fig. 258. Leaf spot symptoms on a *Leucospermum* sp. Fig. 259. Vertical section through a conidioma. Fig. 260. Conidiomata on the leaf surface. Fig. 261. Conidiomatal wall layer. Figs 262–266. Conidiogenous cells with percurrent proliferations (arrowed). Scale bars: 259 = 20 µm, 260 = 200 µm, 261–266 = 10 µm. (From Swart *et al*., 1999).

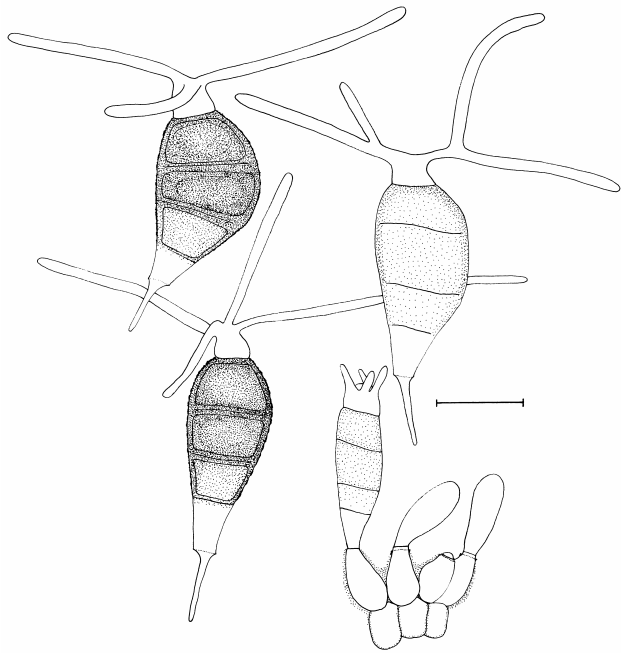


Fig. 267. Conidia and conidiogenous cells of a *Pestalotiopsis* sp. pathogenic to *Leucospermum*. Scale bar = 10 µm. (From Swart *et al*., 1999).

Causal organism: *Conidiomata* amphigenous, pycnidioid to acervular, immersed, becoming erumpent, unilocular, dark brown to black, dehiscing by irregular splits in the apical wall and the overlying host tissue, scattered, (100–)195–240(–400) µm; in section pyriform or conical, with applanate base, intraepidermal in origin (125–)138–165(–180) µm wide, and (125–)138–165(–180) µm diam. *Peridium* comprising two strata of *textura angularis*, an outer stratum of pale brown, thick-walled cells becoming hyaline in the inner layer, apical and lateral walls composed of slightly compressed, thinner-walled cells; basal wall (13–)17–21(–23) µm, apical wall (7–)11–17(–19) µm thick. *Conidiophores* peripheral, reduced to conidiogenous cells, invested in mucus. *Conidiogenous cells* discrete, ampulliform, hyaline, smooth, (4–)5.5–6.5(–8) × (2–)4–5(–6) µm; conidiogenesis initially holoblastic, with up to two enteroblastic, percurrent proliferations to produce additional conidia at slightly higher levels. *Conidia* ellipsoidal to obovoid, 4-euseptate, 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) × 4–4.5(–5) µm; second cell from base subcylindrical, pale brown, verruculose, (4–)5–5.5(–6) µm, third and fourth cells doliiform to subcylindrical, dark red-brown, verruculose, (4–)5–6(–7) µm and (4–)5.5–6(–7) µm long respectively, combined dimensions of 3 median cells (14–)16–17(–18) × (6.5–)8–9(–10) µm; apical cell subconical, hyaline, collapsed at maturity, thin-walled, smooth, (3–)3.5–4.5(–6) × (3–)3.5–4(–5) µm; 2–4 appendages arising apically, tubular, branched or not, straight to flexuous, tip rounded, (15–)26–32(–43) µm long; basal appendage occasionally absent, filiform, flexuous, slender, centric, (2–)4.5–6(–9) µm.

Conidia in vitro ellipsoidal to obovoid, 4-euseptate, 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, (4–)5–6(–8) × 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) × (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) × 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: *Colonies* circular with undulate margins; mycelium of medium density, woolly, with white aerial mycelium, white in reverse; 69 mm diam after 7 days at 25ºC (minimum ≥ 10ºC, optimum 25ºC, maximum ≤ 30ºC). Conidiomata fertile after 12 days at 25ºC under white light, with conidiomata developing over the entire surface of the colony and producing black, wet spore masses.

Host range and geographic distribution: *P.* 'Pink Ice', *T. speciosissima*, *Telopea* spp. (**Australia, NSW**); *Protea* sp. (**South Africa, WC**); *Lsp.* 'Sunbird', *Lsp.* 'Scarlet Ribbon', *Lsp. vestitum*, *P. eximia, Protea* sp. (**Zimbabwe**).

Notes: *Pestalotiopsis montellicoides,* when compared to the *Pestalotiopsis* sp. from Zimbabwe reported on here, differs mainly in the larger dimensions of its conidia (e.g. three median cells, 26–35 × 7.5–10.5 µm). The *Pestalotiopsis* anamorph of *Pestalosphaeria leucospermi* from *Leucospermum,* however (Samuels *et al*., 1987), has conidia of similar dimensions to the Zimbabwe collection, but possesses concolourous median cells, and more cylindrical conidiogenous cells (11–18 × 2–2.5 µm). In culture, it becomes green-yellow (also noted for some Zimbabwean isolates, 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* species in the key provided by Nag Raj (1993), the species from Zimbabwe does not correspond to any previously de-

scribed species, although it is most similar in dimensions and morphology to *P. macrospora.* 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 reassessed before new taxa can be described in this genus.

It should be noted that, as observed by Samuels *et al.* (1987), the conidia produced in culture differ somewhat from those produced on host material. It was observed here that, in culture, the third and fourth cells of the conidia of this species were medium brown rather than dark red-brown, 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.

In a recent study of *Pestalotiopsis* and allied genera, several isolates from *Proteaceae* were included, not including the species discussed here (Jeewon, 2002). Isolates were identified as follows: *Pestalotiopsis longisetula*, *Leucospermum* sp. (STE-U 1771) (**Sicily**); *Pestalotiopsis aquatica*, *Leucospermum* sp. (**USA, Hawaii**) (JT 615); *Pestalotiopsis leucothoës, Telopea* sp., (JT 551) (**USA, Hawaii**); *Pestalotiopsis sydowiana,* endophyte from *P. neriifolia*, (JT 258) (**South Africa**); *Pestalotiopsis theae,* endophyte from *Protea neriifolia*, (JT 258) (**South Africa**); *Pestalotiopsis vismiae*, *Leucospermum* sp., (JT 694) (**USA, Hawaii**). Clearly, many *Pestalotiopsis* species occur on *Proteaceae*, and further research is required to elucidate their taxonomy and pathology.

Control: In vitro, benomyl (Benlate), iprodione (Rovral) and prochloraz manganese chloride (Octave) reduced mycelium growth more than other products tested (Denman, unpubl. data). Tebuconazole (Folicur) was not as effective as these three, but was more effective than other products tested. All three effective fungicides are registered for ornamental plants in South Africa but not specifically for proteas, and tebuconazole is not registered either in South Africa or for proteas (Nel *et al*., 1999).

Phaeophleospora leaf spot

Species of *Phaeophleospora* are commonly associated with leaf spot diseases of numerous hosts (Taylor & Crous, 1999). Of the 14 known species of *Phaeophleospora*, six have been described from *Proteaceae*, being distributed on the African continent from the south (South Africa) to the north (Ethiopia). The known teleomorphs of species of *Phaeophleospora* are *Mycosphaerella* (Crous, 1998). Although *Phaeophleospora faureae* occurs on *Proteaceae* (*Faurea*), it has never been recorded on any of the host genera treated here, and is therefore excluded. A full account of this species can, however, be found in Taylor & Crous (1999).

Little is known about the pathogenicity of *Phaeophleospora* spp. on *Proteaceae*. They currently are not regarded as a problem of commercial importance as they have thus far only been recorded on species of *Protea* that are not important in commercial cultivation. There are no records of interception of *Phaeophleospora* spp. at quarantine inspection points (Taylor, 2001).

Phaeophleospora abyssinicae Crous & M.E. Palm, *Mycological Research* **103**: 1300 (1999). Fig. 268.

≡ *Septoria proteae* Ciccar., *Mycopathologia et Mycologia Applicata* **5**: 229 (1951).

Symptoms: *Leaf spots* amphigenous, circular to subcircular, 3–4 mm diam, grey to pale brown, with a raised, brown border.

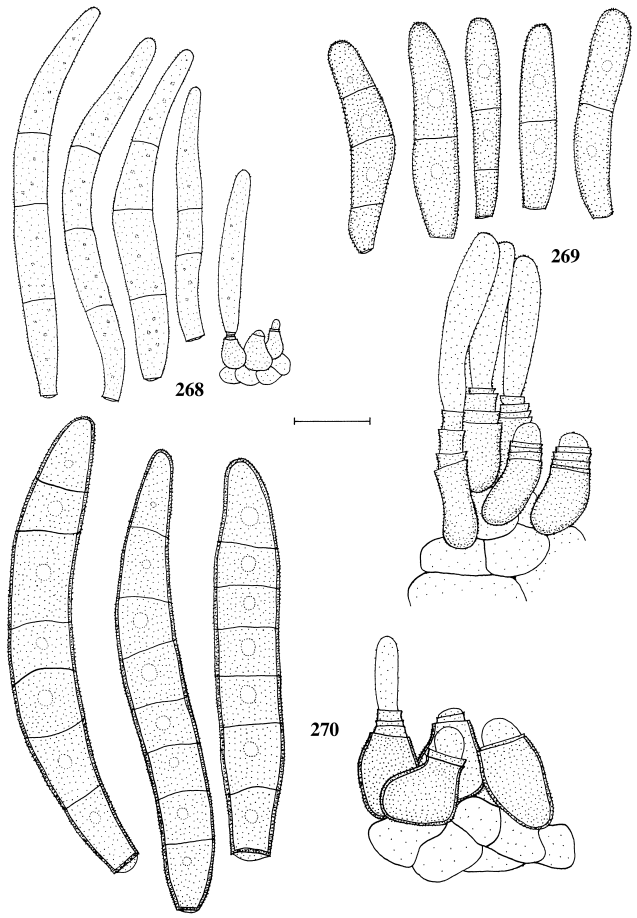
Causal organism: *Mycelium* internal, hyphae consisting of branched, septate, smooth, pale brown hyphae, 2–3 µm wide. *Conidiomata* pycnidial, amphigenous, substomatal, scattered, black, globose to subglobose, up to 100 µm diam; wall consisting of 2–3 layers of brown *textura angularis*. *Conidiogenous cells* lining the cavity, discrete, ampulliform to doliiform, pale brown, finely verruculose, holoblastic and proliferating percurrently resulting in 1–3 annellations, 2.5–3.5 x 2–3 µm. *Conidia* solitary, narrowly obclavate to subcylindrical, apex subobtuse, base truncate to long obconic-truncate, straight to flexuous, pale brown, verruculose, not prominently guttulate, (0–)1–3-euseptate, (17–)22–32(–38) × (2.5–)3–3.5 µm; minute basal marginal frill present.

Host range and geographic distribution: *P. gaguedi* (**Ethiopia**).

Phaeophleospora capensis Joanne E. Taylor & Crous, *South African Journal of Botany* **67**: 39 (2001). Figs 171, 278–281.

Symptoms: *Leaf spots* amphigenous, approximately 4 mm diam, circular, or larger and coalescing, necrotic, parchment-like, grey-brown, with a raised medium brown margin.

Causal organism: *Mycelium* internal, forming a sparse stroma surrounding the conidiomata, consisting of host cells and branched, septate, pale brown, smooth, 2–3 µm wide hyphae. *Conidiomata* pycnidial, hypophyllous, singular, scattered to aggregated, black, immersed, raising the host surface but not becoming erumpent, up to 160 µm diam; in section subglobose, unilocular, subepidermal, substomatal, non-papillate, with an ostiolar pore, approximately 140 µm high, 155 µm wide. *Peridium* consisting of 1–2 strata of *textura intricata,* the outer stratum dark brown, thick-walled, becoming thinner-walled and hyaline in the inner



Figs 268–270. Conidia and conidiogenous cells of *Phaeophleospora* spp. Fig. 268. *P. abyssinicae*. Fig. 269. *P. congesta*. Fig. 270. *P. protearum*. Scale bar = 10 µm. (From Crous & Palm, 1999).

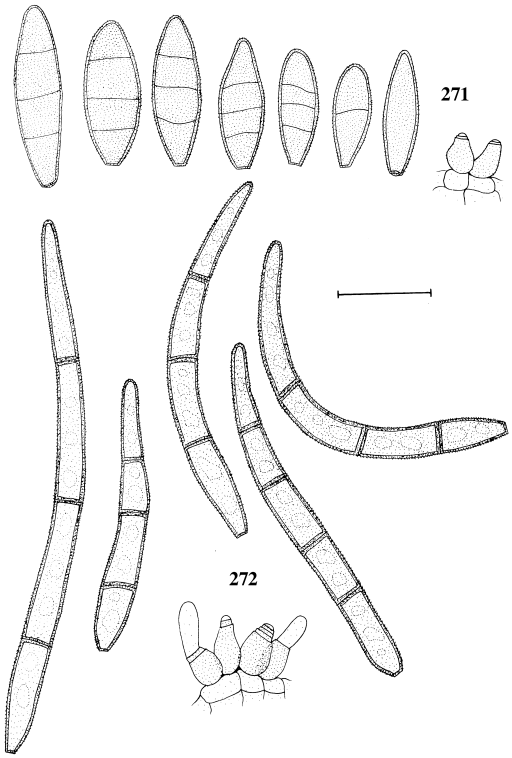
layer, together 12–16 µm thick. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* ampulliform, pale brown, finely verruculose, holoblastic, proliferating percurrently, with 1–3 annellations, 4–5 × 2.5–3 µm. *Conidia* solitary, narrowly fusiform, tapering slightly to a truncate, often slightly extended base, straight with a narrowly rounded apex, pale to medium yellow-brown, finely verruculose (more pronounced in lactophenol), (0–)3(–4)-euseptate, (12–)15–16.5(–19) × (4–)4.5–5(–6) µm.

Host range and geographic distribution: *Protea* sp. (**South Africa, WC**).

Phaeophleospora concentrica P.F. Cannon & J.C. David, *South African Journal of Botany* **67**: 41 (2001). Figs 172–277.

Symptoms: *Leaf spots* amphigenous, approximately 2–15 mm diam, circular or slightly irregularly shaped, necrotic, with concentric rings of development which alternate in colour from pale to dark yellow-brown, conidiomata visible as green-black dots.
Causal organism: *Mycelium* internal, forming a very sparse stroma surrounding the conidiomata, consisting of host cells and branched, septate, pale brown, smooth, 3–4 µm wide hyphae. *Conidiomata* pycnidial, amphigenous, singular, scattered or aggregated, dark green-black, immersed, raising the host surface and causing bleaching of the adjacent host tissue, forming on the tops of the ridges of the concentric rings, 90–170 µm diam; in section subglobose to irregular, unilocular, subepidermal, substomatal, non-papillate, with an ostiolar pore, 90–130 µm high, 80–155 µm diam. *Peridium* consisting of 2 strata, an outer stratum of thick-walled, medium brown cells of compressed *textura angularis*, becoming paler in the inner layer, together 8–20 µm thick. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* ampulliform to doliiform, pale brown, finely verruculose, holoblastic, proliferating percurrently resulting in 1–4 annellations, 5–7 × 4–5 µm. *Conidia* solitary, scolecosporous, subcylindrical to narrowly obclavate, narrowing slightly to a truncate base and tapering gradually to a narrowly rounded apex, straight or curved to flexuous, medium yellow-brown, verruculose, (0–)3(–4)-euseptate, (32–)39–42(–52) × (3.5–)4(–4.5) µm.

Cultural characteristics: *Colonies* circular with fimbriate margins; mycelium of medium density, woolly, with a buff (19”d) centre and margins, sepia (13”k) in between, brown-vinaceous (84’”m) in reverse; relatively slow-growing, 13 mm in 45 days on PCA at 25ºC. Sterile.



Figs 271–272. Conidia and conidiogenous cells of *Phaeophleospora* spp. Fig. 271. *P. capensis*. Fig. 272. *P. concentrica*. Scale bar = 10 µm. (From Taylor *et al*., 2000).

Host range and geographic distribution: *P. caffra* (**Kenya**).

Phaeophleospora congesta (Syd.) Crous & M.E. Palm, *Mycological Research* **103**: 1301 (1999)
Fig. 269.

≡ *Didymosporium congestum* Syd., *Annales Mycologici* **10**: 45 (1912).
= *Coniothyrium proteae-abyssinicae* Bacc., *Annals of Botany* **14**: 139 (1917).
= *Kirramyces proteae* B. Sutton, *Mycological Papers* **167**: 35 (1993).
≡ *Phaeophleospora proteae* (B. Sutton) Crous, F.A. Ferreira & B. Sutton, *South African Journal of Botany* **63**: 115 (1997).

Symptoms: *Leaf spots* amphigenous, subcircular, up to 8 mm diam, medium brown with a pale brown, slightly raised, sinuate border.

Causal organism: *Mycelium* internal, consisting of branched, septate, smooth, pale brown hyphae, 2–4 µm wide. *Conidiomata* pycnidial, ostiolate, subepidermal, amphigenous, scattered, dark brown to black, globose, unilocular, up to 150 µm diam; wall consisting of 2–3 layers of brown *textura angularis*. *Conidiogenous cells* discrete, cylindrical to lageniform, pale to medium brown, verruculose, holoblastic, proliferating percurrently, with 1–6 annellations, 5–10 x 3–6 µm. *Macroconidia* solitary, cylindrical, straight to curved, (0–)1(–3)-euseptate, rarely guttulate, medium brown, verruculose, apex obtuse, with truncate to subtruncate base with a marginal frill, (10–)12–22(–25) × 3–4(–4.5) µm. *Microconidia* (Sutton, 1993) not observed on the type.

Host range and geographic distribution: *P. caffra* (**South Africa, MP**); *P. gaguedi* (**Ethiopia**).

Phaeophleospora protearum (Wakef.) Crous & M.E. Palm, *Mycological Research* **104**: 627 (2000).
Fig. 270.

≡ *Hendersonia protearum* Wakef., *Kew Bulletin of Miscellaneous Information* **1918**: 210 (1918).

Symptoms: *Leaf spots* indistinct; pycnidia produced abundantly in necrotic leaf tissue.

Causal organism: *Mycelium* internal, consisting of branched, septate, smooth, hyaline to pale brown, 2.5–4 µm wide hyphae. *Conidiomata* pycnidial, substomatal, black, ostiolate, amphigenous, scattered, black, globose to subglobose, unilocular, up to 150 µm diam; wall consisting of 2–3 layers of medium brown *textura angularis*; inner layer pale brown. *Conidiogenous cells* holoblastic, discrete, ampulliform or doliiform to subcylindrical, pale brown, verruculose, with 1–5 percurrent proliferations, 5.5–11 × 5–7 µm. *Conidia* solitary, exuded in cirri, subcylindrical or narrowly ellipsoidal, straight to flexuous, tapering from the middle towards a narrowly truncate base, with subobtuse to obtuse apex, medium brown, verruculose, guttulate, (1–)4–7-euseptate (30–)35–45(–50) × (5–)6–7 µm; base with a minute marginal frill.

Host range and geographic distribution: *P. madiensis* (**Uganda**).

KEY TO PHAEOPHLEOSPORA *SPECIES ON* PROTEACEAE

See key to *Mycosphaerella* anamorphs.

Phyllosticta – see Guignardia leaf spot

Physalospora leaf spot

Physalospora is a widespread genus of approximately 30 species (Hsieh *et al*., 1998) in the *Hyponectriaceae* (Kirk *et al*., 2001).

Physalospora hawaiiensis Joanne E. Taylor, Crous & M.E. Palm, *Mycotaxon* **78**: 471 (2001)
Figs 282–286.

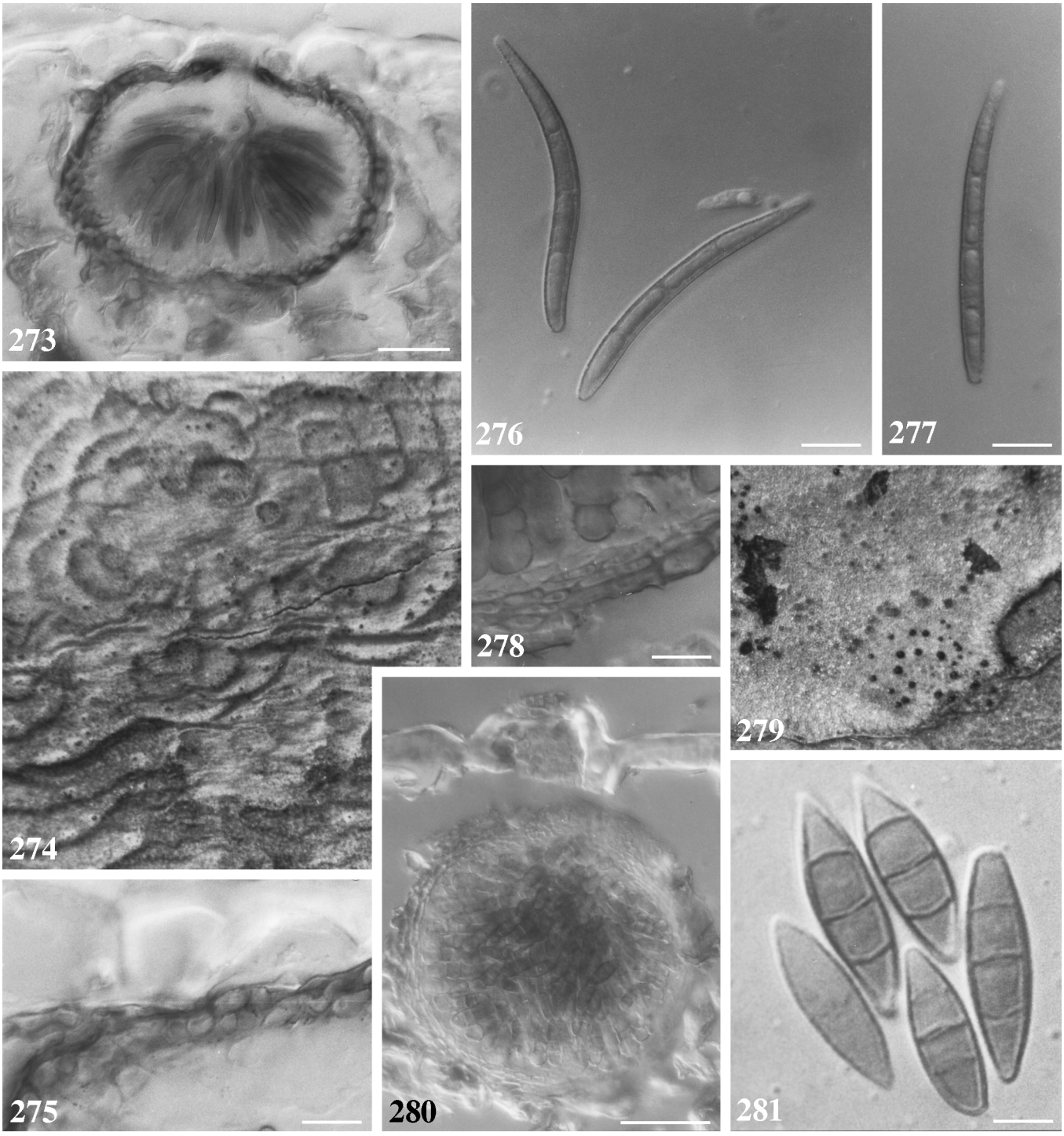
Symptoms: *Leaf spots* necrotic, grey-brown with a raised dark brown margin, often situated at the leaf tips, ascomata visible as raised, slightly darkened spots, scattered and singular.

Causal organism: *Ascomata* amphigenous, immersed, non-erumpent, visible as a slightly raised, darkened area, apapillate, but host tissue often splitting above the apex of the ascomata, 250–320 µm diam; in section globose with a minutely papillate, sparsely periphysate, central ostiole, aclypeate, but with some brown fungal hyphae in the host cells surrounding the ostiole, asci attached to the basal peridium, approximately 197–300 µm high, 245–270 µm wide. *Peridium* comprising two strata, an outer stratum of thick-walled, brown compressed *textura angularis*, becoming thin-walled in the inner layer, with an inner layer of thin-walled, hyaline *textura angularis*, together 14–35 µm thick. *Paraphyses* numerous, hyaline, tapering from base to the apex, longer than the asci, dissolving at maturity, 4–7 µm wide. *Asci* cylindrical, unitunicate, short-pedicellate, with rounded apex, lacking an apical apparatus, 8-spored, 52–125 × 12–22 µm. *Ascospores* ellipsoidal with apex slightly pointed, aseptate, hyaline to yellowish, thick-walled, smooth, refractive with a large central guttule, overlapping uni- to biseriate, (14–)17.5–19(–23) × (7–)9–9.5(–11) µm, with a mucilaginous sheath which can sometimes be slightly thicker at the poles, 1.5–7 µm thick. *Cultural characteristics*: *Colonies* circular with entire margins, cream

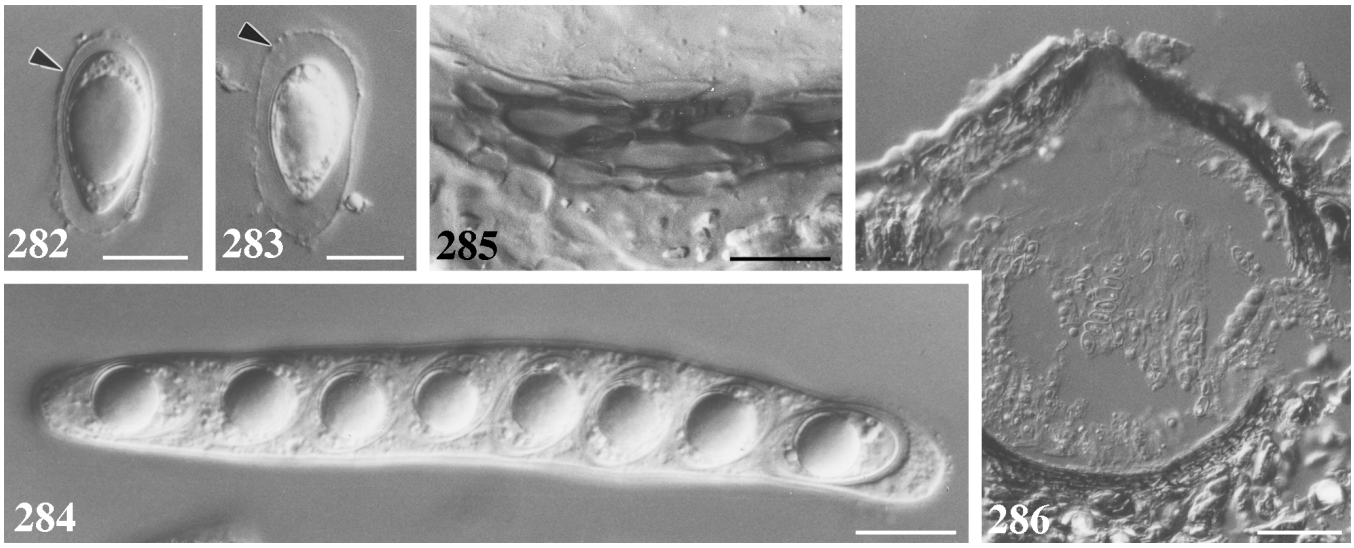
(19”d) with a narrow, pale brown margin (19”’i), in reverse, fine concentric rings of pale brown and cream; aerial mycelium moderately dense, felty; 25 mm diam after 7 days; sterile fruiting bodies produced after 7 days.

Host range and geographic distribution: *Leucospermum* ‘Hybrid 24’, *Lsp.* ‘High Gold’, *P. cynaroides*, *P. neriifolia, P. repens* (**USA, Hawaii**).

Notes: Several species of *Physalospora* have similar-sized ascospores surrounded by sheaths, such as *Physalospora vitis-idaeae* (18–20 × 9–10.5 µm) (Barr, 1970), and *Physalospora hyalospora* (16–22 × 8–11 µm) (von Arx & Müller, 1954). However, both generally have smaller asci and ascomata than *P. hawaiiensis*. *Physalospora palmicola* is similar to *P. hawaiiensis* in that it lacks an apical ring and in ascospore size (17–20 × 9–10 µm), but *P. palmicola* has distinctly verrucose ascospores (Hsieh *et al*., 1998)



Figs 273–281. *Phaeophleospora* spp. Fig. 273–277. *P. concentrica*. Fig. 273. Vertical section through a pycnidium. Fig. 274. Leaf spot on *Protea caffra* with pycnidia. Fig. 275. Pycnidial wall layer. Figs 276–277. Conidia. Figs 278–281. *P. capensis*. Fig. 278. Pycnidial wall layer. Fig. 279. Leaf spot on a *Protea* sp. with pycnidia. Fig. 280. Vertical section through a pycnidium. Fig. 281. Conidia. Scale bars: 273 = 30 µm, 275–278 = 10 µm, 280 = 50 µm, 281 = 5 µm. (From Taylor *et al*., 2000).



Figs 282–286. *Physalospora hawaiiensis*. Figs 282–283. Ascospores with mucilaginous sheaths (arrowed). Fig. 284. Ascus. Fig. 285. Ascomatal wall layer. Fig. 286. Ascoma in cross section. Scale bars: 282–284 = 10 µm, 285 = 20 µm, 286 = 50 µm. (From Taylor *et al*., 2001b).

Pseudocercospora 'Stigmina' leaf spots

These "Stigmina" leaf spot organisms, originally described in that genus, actually are species of *Pseudocercospora* and are treated as such below. Three varieties of *Pseudocercospora protearum* are associated with "Stigmina" leaf spots and the two most common varieties are treated here. They are part of a complex within the teleomorph genus *Mycosphaerella,* but are treated separately here because of their well-known disease common names and field symptoms. These varieties are included in the key to anamorphs of *Mycosphaerella*.

The var. *hakeae* occurring on *Hakea* and *Grevillea* (Crous & Braun, 1994) is more easily distinguishable from the other two varieties in that its conidia are up to 100 µm long and have up to 12 septa.

Stigmina californiae, a true species of *Stigmina,* which occurs on *Proteaceae*, is treated below under 'Stigmina leaf spot.'

Pseudocercospora protearum (Cooke) U. Braun & Crous var. ***protearum***, *Mycological Progress* **1**: 22 (2002). Figs 287–291, Plate 8.

≡ *Cercospora protearum* Cooke, *Grevillea* **12**: 39 (1883).

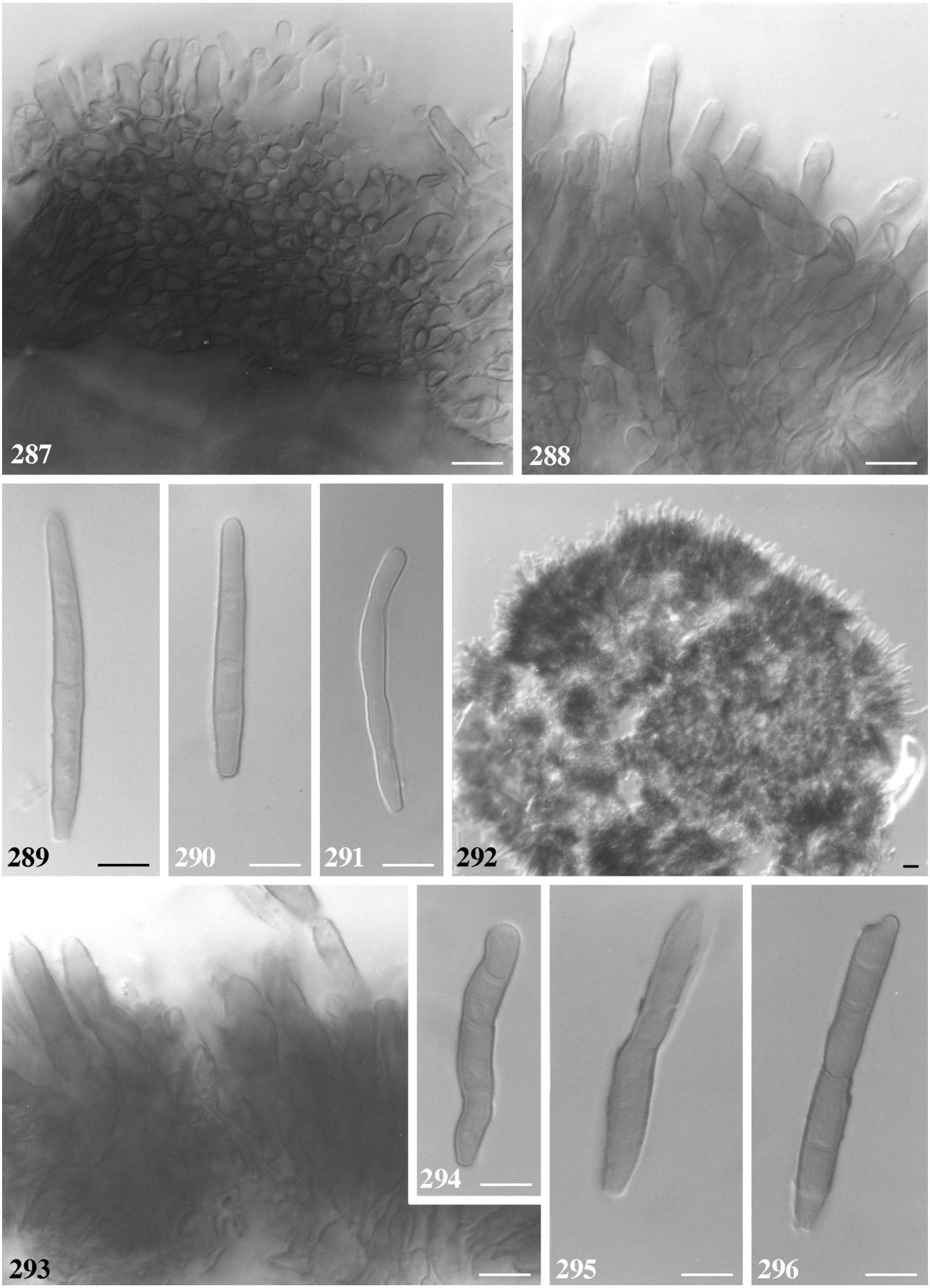
≡ *Cercospora protearum* Cooke var. *leucospermi* Cooke, *Grevillea* **12**: 39 (1883).

≡ *Stigmina protearum* (Cooke) M.B. Ellis var. *protearum*, *Mycological Papers* **131**: 6 (1972).

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

Symptoms: *Leaf spots* amphigenous, irregular or subcircular to circular, 6–25 µm diam, pale to medium brown, with a raised, dark brown border; symptoms varing depending on the host. On *Lsp. conocarpodendron*, the lesions appear as a dead, parchment-like spot with black, raised structures, 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 smaller (1–3 mm) than that on *Lsp. conocarpodendron* (5–20 mm) (Van Wyk, 1973a). The areas surrounding the blotches become chlorotic and die. Young shoot tips can be severely damaged (Von Broembsen, 1989).

Causal organism: *Mycelium* predominantly internal, medium brown, consisting of septate, branched, smooth, 3–4.5 µm wide hyphae. *Conidiomata* acervular to sporodochial, amphigenous, medium to dark brown on leaves, up to 500 µm diam and 300 µm high. *Conidiophores* aggregated in dense fascicles arising from the upper cells of a brown stroma up to 400 µm diam and 250 µm high; conidiophores medium brown, verruculose, 1–3-septate, subcylindrical, straight to slightly curved, unbranched or rarely branched below, 30–50 × 5–8 µm. *Conidiogenous cells* holoblastic, terminal, medium brown, verruculose, tapering to the truncate apical loci, proliferating percurrently near the apex, with 1–5 irregular annellations, 20–35 × 5–7 µm. *Conidia* solitary, medium brown, verruculose, guttulate, subcylindrical to obclavate, with obtuse apex, and an obconically truncate base with a minute marginal frill, straight to slightly curved, 1–3(–6)-septate, (30–)40–65(–80) × (5–)6–7(–9) µm.



Figs 287–296. *Pseudocercospora* spp. Figs 287–291. *P. protearum* var. *protearum.* Fig. 287. Sporodochium with conidiophores. Fig. 288. Conidiophores. Figs 289–291. Conidia. Figs 292–296. *P. protearum* var. *leucadendri*. Fig. 292. Sporodochium with conidiophores. Fig. 293. Conidiophores. Figs 294–296. Conidia. Scale bars = 10 µm.

Host range and geographic distribution: *Lsp. conocarpodendron* subsp. *conocarpodendron* (= *Lsp. conocarpum*)*, Lsp. cordifolium, Lsp. reflexum* (**South Africa, EC, NC, WC**) (Saccardo, 1886; Chupp & Doidge, 1948; Doidge, 1950; Chupp, 1953; Ellis, 1972, 1976; Van Wyk, 1973a; Crous & Braun, 1996). Although this fungus was reported from *Protea* spp. (Ellis, 1976; Masuka *et al*., 1998), we have been unable to recollect it from this host genus and the record remains unconfirmed.

Notes: This species has a complicated and rather confused taxonomic history. It was initially described by Cooke as *Cercospora protearum* var. *leucospermi* (1883). When Ellis (1972) treated this taxon, he changed it to var. *protearum*. Ellis also placed the fungus in *Stigmina*, because of its percurrently proliferating conidiogenous cells, and rough walled, brown conidia. Apart from the Ellis record on *Protea*, this species has never been collected on a host other than *Leucospermum*, and we suspect that he mistakenly identified one collection as being *Protea*. Braun (1993) redefined this complex and erected the genus *Cercostigmina* for species with scolecosporous conidia and predominantly percurrently proliferating conidiogenous cells. This variety was therefore transferred to *Cercostigmina*. In later molecular studies, however (Crous *et al.*, 2001), it was shown that *Cercostigmina* is not clearly distinguishable from *Pseudocercospora*, and hence a new combination in *Pseudocercospora* was warranted for this taxon.

Pseudocercospora protearum var. ***leucadendri*** (Cooke) U. Braun & Crous, *Mycological Progress* **1**: 22 (2002). Figs 292–296, Plates 7, 8.

≡ *Cercospora protearum* Cooke var. *leucadendri* Cooke, *Grevillea* **12**: 39 (1883).

≡ *Stigmina protearum* var. *leucadendri* (Cooke) M.B. Ellis, *Mycological Papers* **131**: 7 (1972).

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

≡ *Passalora protearum* Kalchbr. & Cooke, *Grevillea* **19**: 6 (1890).

Symptoms: *Leaf spots* amphigenous, irregular to subcircular or circular, 3–10 µm diam, medium brown, with an indistinct, raised, dark brown border.

Causal organism: *Mycelium* predominantly internal, medium brown, consisting of septate, branched, smooth, 3–4.5 µm wide hyphae. *Caespituli* fasciculate to sporodochial, amphigenous, medium to dark brown, up to 350 µm diam and 170 µm high. *Conidiophores* aggregated in dense fascicles arising from the upper cells of a brown stroma up to 250 µm diam and 150 µm high; conidiophores medium brown, verruculose, 1–3-septate, subcylindrical, straight to slightly curved, unbranched or rarely branched below, 30–60 × 4–7 µm. *Conidiogenous cells* terminal, medium brown, verruculose, tapering to truncate apical loci, proliferating percurrently near the apex, with 1–5 irregular annellations, 10–30 × 4–6 µm. *Conidia* solitary, medium brown, verruculose, guttulate, subcylindrical, with a subobtuse apex and a long obconically truncate base with a minute marginal frill, straight to slightly curved, 1–3(–6)-septate, (25–)40–65(–80) × 4.5–5(–7) µm.

Host range and geographic distribution: *Lcd. argenteum, Lcd.* 'Chameleon', *Lcd. discolor*, *Lcd.* 'Jester', *Lcd.* 'Safari Sunset' (**South Africa, EC, NC, WC**) (Chupp & Doidge, 1948; Ellis, 1976; Knox-Davies *et al.,* 1987; Crous & Braun, 1996).

Notes: The variety *leucadendri* is confined to species of *Leucadendron*. Ellis (1972, 1976) distinguished var. *leucadendri* from var. *protearum* by the former having 1–3-septate conidia that are only up to 50 µm long and 7 µm wide. An examination of numerous fresh collections showed, however, that both varieties could have conidia that were 1–3(–6)-septate and up to 80 µm long. On average, however, conidia of var. *protearum* were wider, (5–)6–7(–9) µm, while those of var. *leucadendri* were only 4.5–5(–7) µm wide. In var. *leucadendri*, however, conidiomata were fasciculate to sporodochial, while conidiomata were subepidermal sporodochia (almost acervular) in var. *protearum*, thus possibly explaining the distorted, wider conidia. The two varieties are very similar and molecular studies may well show them to represent the same taxon.

The name *Passalora protearum* represents a fungus described from '*Protea argentea*' collected in the Cape region of South Africa. The type specimen, however, could not be traced at IMI, Kew, B or BRA (Crous & Braun, 2003). *Passalora protearum* is described as having fasciculate conidiomata, and subfusoid, brown, 1-septate conidia, 35–40 x 7 µm. The conidial dimensions closely match those reported for *Pseudocercospora protearum* var. *leucandendri* (Ellis, 1976). Furthermore, there is no host such as *Protea argentea* and the fungus was probably collected on *Leucadendron argenteum.* We consider this name to be a synonym of *Pseudocercospora protearum* var. *leucandendri*.

Disease cycle and epidemiology: Conidia are splash-dispersed and all varieties of *Pseudocercospora protearum* sporulate profusely on young leaves under moist conditions. Once lesions mature, conidia cease to germinate on MEA, suggesting that these pathogens have a relatively small window to establish new infections, and that infections occur predominantly by means of conidia forming on lesions on young growth. Older lesions are frequently colonised by a white, powdery growth of an unidentified, hyperparasitic hyphomycete.

Control: Conditions of prolonged high humidity, especially at mild temperatures, favour disease outbreaks. Stressed plants are particularly susceptible. Management practices that improve air circulation between plants, such as wide plant spacing, would help to avoid or reduce infection. Overhead or mist irrigation is to be avoided. Irrigation is to be used only as necessary, and ground cover (grass or weeds) must be mown. Further research is needed on fungicide control since there is great potential to prevent losses through the use of fungicides.

KEY TO PSEUDOCERCOSPORA *SPECIES ON* PROTEACEAE

See key to *Mycosphaerella* anamorphs.

Pseudocladosporium leaf spot

Pseudocladosporium proteae (Viljoen & Crous) Crous, **comb. nov.** Fig. 297.

≡ *Cladophialophora proteae* Viljoen & Crous, *South African Journal of Botany* **64**: 137 (1998).

Cladophialophora and *Pseudocladosporium* are morphologically similar, and therefore several species of *Pseudocladosporium* were originally placed in *Cladophialophora*. The two genera are, however, ecologically and phylogenetically clearly distinct. Species of *Pseudocladosporium* are assumed to be saprobes, usually isolated from leaf litter, and anamorphs of *Caproventuria*, while species of *Cladophialophora* are human pathogens and anamorphs of *Capronia* (*Herpotrichiellaceae*).

Symptoms: Isolated from red leaf spots, associated with *Batcheloromyces proteae*; pathogenicity has not been confirmed.

Causal organism: *Mycelium in vitro* consisting of branched, septate hyphae, 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. *Conidiogenous cells* holoblastic, integrated, forming short, truncate protuberances, 2–3 × 1.5–2 µm, concolorous with the mycelium, subcylindrical. *Conidia* in long acropetal chains of up to 20 conidia, simple or branched, subcylindrical to oblong-doliiform, 0–1(–2)-septate, pale brown to pale olivaceous, smooth, with subtruncate to truncate, not thickened but somewhat refractive hila, (9–)13–17(–22) × 2.5–3(–4) µm.

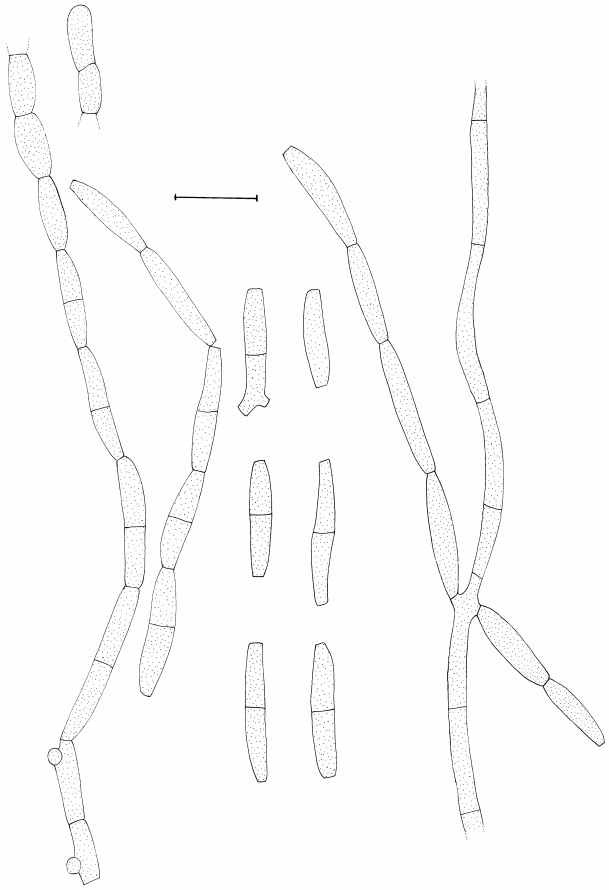


Fig. 297. Septate and aseptate conidial chains of *Pseudocladosporium proteae*. Scale bar = 10 µm. (From Swart *et al*., 1998).

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

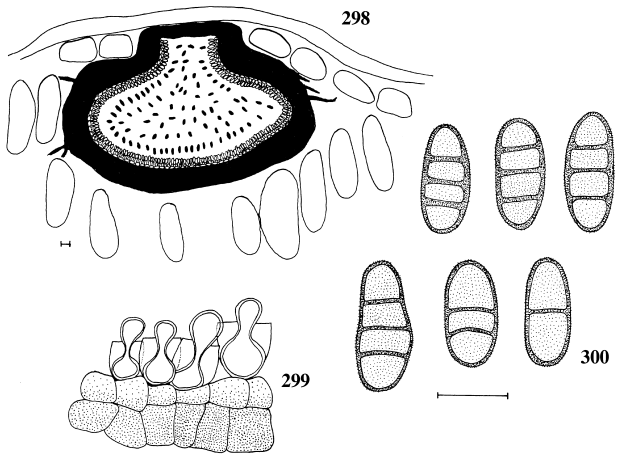
Host range and geographic distribution: *P. cynaroides* (**South Africa, WC**).

Disease cycle and epidemiology: Isolates of this fungus were obtained from red leaf spots attributed to *Batcheloromyces proteae* on *P. cynaroides*. It is assumed that *Pseudocladosporium proteae* is an endophyte, and infection studies are required to determine its potential as a plant pathogen.

Control: Unknown.

Pseudohendersonia leaf spot

The genus *Pseudohendersonia* is presently monotypic, based on *Pseudohendersonia proteae* which was described from leaves of a *Protea* sp. intercepted during a USDA quarantine inspection (Crous & Palm, 1999). The genus is characterised by having pycnidia in which pigmented, distoseptate conidia are produced from determinate conidiogenous cells with prominent periclinal thickening. Little is known about the patho-



Figs 298–300. *Pseudohendersonia proteae*. Fig. 298. Vertical section through a conidioma. Fig. 299. Conidiogenous cells with prominent periclinal thickening. Fig. 300. Distoseptate conidia. Scale bars = 10 µm. (From Crous & Palm, 1999).

genicity of this fungus, which rarely has been encountered to date.

Pseudohendersonia proteae Crous & M.E. Palm, *Mycological Research* **103**: 1303 (1999). Figs 298–300.

Symptoms: *Leaf spots* associated with tip blight symptoms of leaves, grey, with a raised, red-brown margin.

Causal organism: *Mycelium* internal, consisting of branched, septate, verruculose, medium brown, 2–3.5 µm wide hyphae. *Conidiomata* pycnidial, amphigenous, scattered, black, subcuticular, becoming erumpent, globose to subglobose, unilocular, up to 230 µm diam, 160 µm high, with a central ostiole of up to 60 µm diam; wall up to 30 µm thick, consisting of 3–4 layers of medium brown *textura angularis*. *Conidiogenous cells* hyaline, smooth, lining the inner layer of the wall, subcylindrical to cupulate, phialidic, with successive conidia liberated at the same level, periclinal thickening prominent, 3.5–7.5 × 2.5–5 µm. *Conidia* solitary, dry, broadly ellipsoidal, pale brown when immature, becoming dark brown at maturity, straight to slightly curved, rounded at both ends, with an inconspicuous dehiscence scar, verruculose, (1–)3-distoseptate, (9–)10–12 × 4–5(–6) µm.

Host range and geographic distribution: *Protea* spp. (**South Africa, WC**).

Disease cycle and epidemiology: Conidia are probably splash-dispersed.

Control: Unknown.

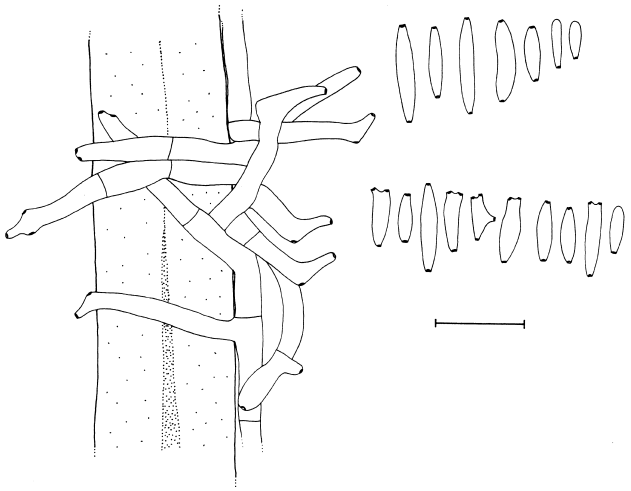


Fig. 301. Conidiophores and conidia of *Ramularia proteae*. Scale bar = 10 µm. (From Crous *et al*., 2000).

Ramularia leaf spot

Members of the genus *Ramularia* are leaf spot pathogens that are assumed to be host specific (Braun, 1998). Although *Ramularia proteae* is presently the only member of the genus known to occur on *Proteaceae*, *Ramularia* is an anamorph of *Mycosphaerella*, many of which are commonly associated with disease symptoms on this host family.

Ramularia proteae Crous & Summerell, *Australasian Plant Pathology* **29**: 277 (2000). Fig. 301.

Symptoms: *Leaf spots* amphigenous, circular to subcircular, 5–15 mm diam, pale to medium brown.

Causal organism: *Mycelium* internal and external, consisting of branched, septate, hyaline, 2–3.5 µm wide hyphae, on leaf hairs. *Caespituli* rarely observed, amphigenous, grey-white, reduced to a few conidiophores. *Conidiophores* separate, rarely aggregated into poorly developed, loose fascicles, erect, subcylindrical, straight to geniculate–sinuous, rarely branched, 3–20 × 2–4 µm, 0–2-septate, hyaline, smooth. *Conidiogenous cells* holoblastic, hyaline, smooth, straight to geniculate, 3–15 × 2–3 µm; scars thickened, darkened and refractive. *Conidia* catenate, chains simple or branched, subcylindrical–fusoid, 5–8(–10) × 1–1.5(–2) µm *in vivo*, 5–8(–11) × 2–2.5 µm *in vitro*, 0(–1)-septate, hyaline, smooth, with somewhat attenuated ends; hila slightly thickened, darkened and refractive.

Cultural characteristics: Margins uneven; colonies erumpent; aerial mycelium absent; colonies mouse-grey (15’’’’i), reverse greyish-sepia (17’’’i); 6 mm diam after 14 days at 25°C.

Host range and geographic distribution: *P. longifolia* (**Australia, Tasmania**).

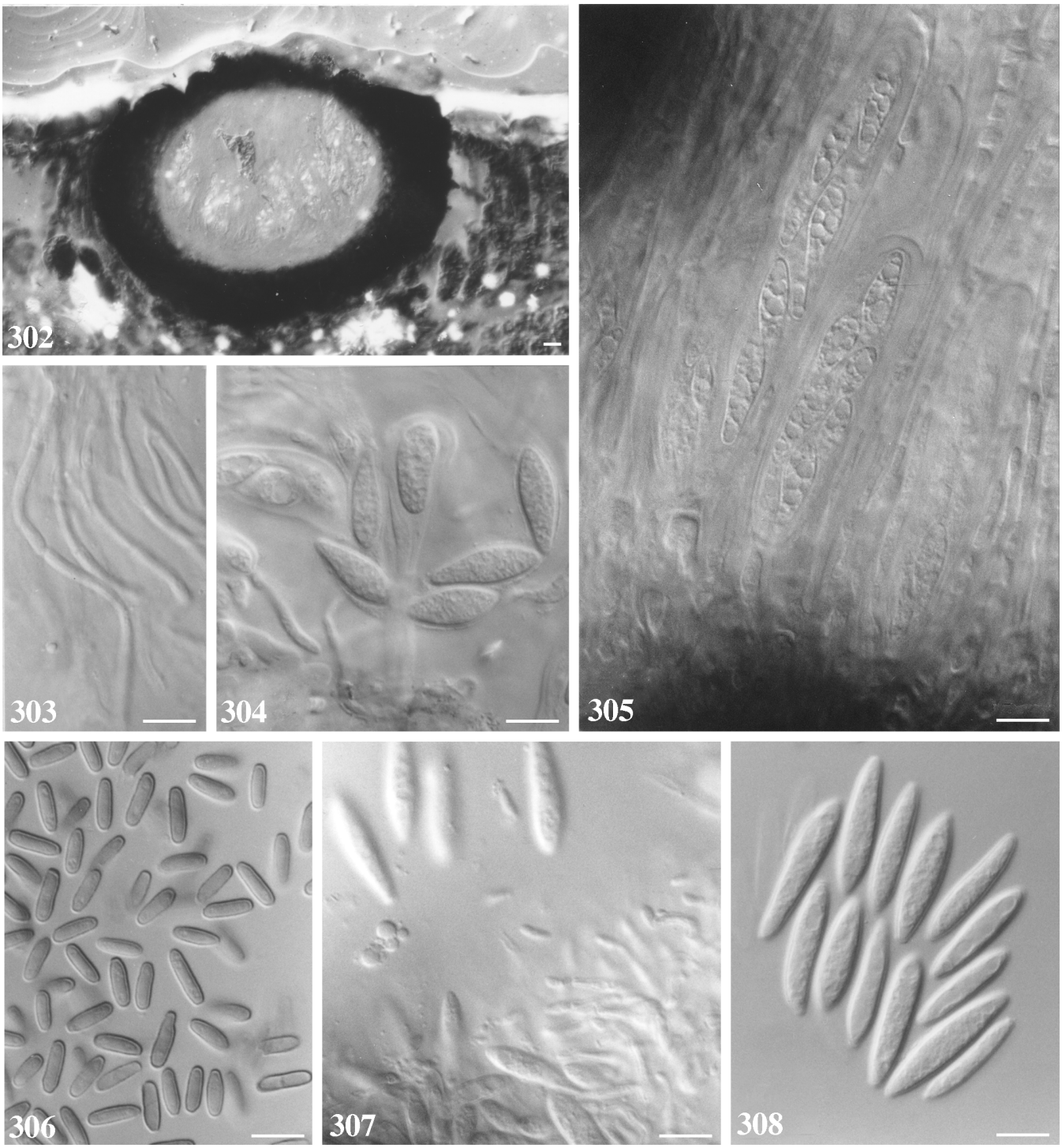
Disease cycle and epidemiology: Conidia are probably splash-dispersed.

Control: Unknown.

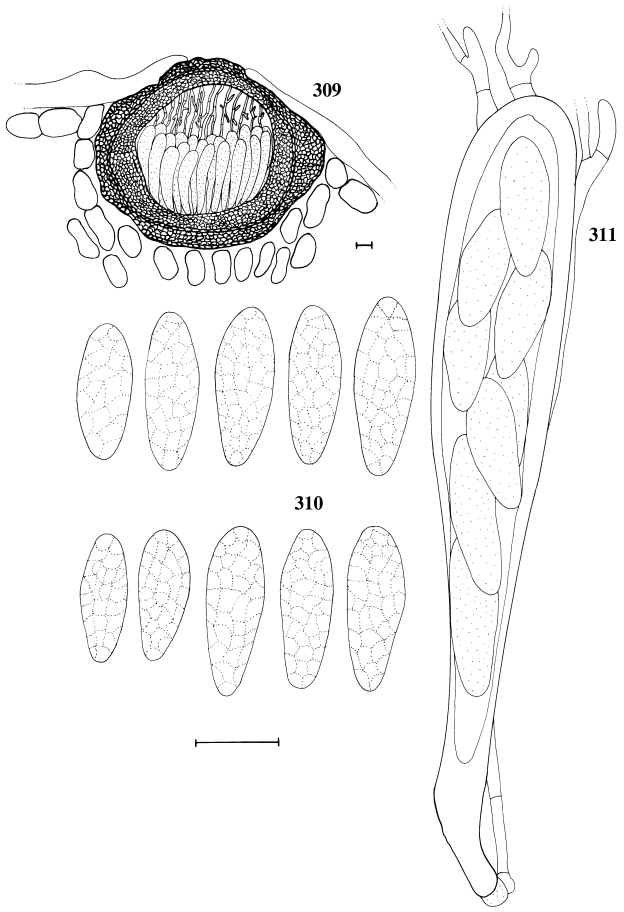
Saccharata leaf tip dieback

Saccharata proteae (≡ *Phyllachora proteae* ≡ *Botryosphaeria proteae*) is commonly associated with leaf tip die-back and spots of *Protea* in particular, but also of *Leucospermum* species. The fungus has only rarely been isolated from stem tissue where it usually was a secondary coloniser of wounded tissue. This is not a serious pathogen, but it causes unsightly damage to leaves. Its endophytic habit suggests that it is present in all regions where *Proteaceae* from South African germplasm are cultivated (Swart *et al*., 2000; Taylor *et al*., 2001c).

This fungus was described by Wakefield (1922) as having unilocular ascomata that develop under a very small epidermal clypeus, cylindrical asci, pseudoparaphyses and hyaline, aseptate, ellipsoidal ascospores, 19–



Figs 302–308. *Saccharata proteae* and its anamorph *Fusicoccum proteae*. Fig. 302. Section through a pseudothecium. Fig. 303. Pseudoparaphyses. Fig. 304. Ascospores. Fig. 305. Asci. Fig. 306. *Diplodia*-like synanamorph. Fig. 307. Spermatia, spermatiophores, and *Fusicoccum* conidia. Fig. 308. *Fusicoccum* conidia. Scale bars = 10 µm. (From Denman *et al*., 1999).



Figs 309–311. *Saccharata proteae*. Fig. 309. Vertical section through a pseudothecium. Fig. 310. Ascospores. Fig. 311. Ascus and pseudoparaphyses. Scale bars = 10 µm. (From Denman *et al*., 1999).

22 × 8–9 µm. In a re-examination of the type material, Doidge (1942) found the ascomatal wall to be continuous with and similar in structure to the clypeus. She noted, however, that the ascomatal stromata differed from those of other South African *Phyllachora* spp. In his study of leaf pathogens of *Protea*, *Leucadendron* and *Leucospermum* spp., van Wyk (1973a) commented that the ascocarps of *P. proteae* appeared to be unilocular with pseudoparaphyses, and that the fungus should probably be transferred to *Guignardia* or *Botryosphaeria*. Denman *et al*. (1999) observed a *Fusicoccum* anamorph in single-ascospore cultures, as well as a *Diplodia*-like synanamorph with narrowly ellipsoidal, brown, thick-walled conidia. Based on its bitunicate asci, as well as pseudothecial and ascospore morphology, a new combination for *P. proteae* was thus proposed in *Botryosphaeria*, as *B. proteae*. In a later phylogenetic study employing ITS DNA sequence data, Denman *et al*. (2000b) demonstrated that *B. proteae* was not congeneric with the two clades of *Botryosphaeria* correlating with *Diplodia* and *Fusicoccum* anamorphs respectively. From these data it was concluded that a new genus had to be introduced for *B. proteae*, distinguished from *Botryosphaeria* by its pseudothecial anatomy (unilocular ascomata with a clypeus-like thickening around the ostiolar area), and synanamorphs. No species of *Botryosphaeria* has both synanamorphs but the *Fusicoccum* and *Diplodia*-like synanamorphs are readily produced in cultures of *S. proteae*.

Saccharata Denman & Crous, **gen. nov.**

Mycelium immersum, e hyphis septatis, levibus, brunneis compositum. *Pseudothecia* epiphylla, singula, deinde aggregata, unilocularia, immersa, substomatalia, ostiolo centrali, applanato praedita, obovoidea, modice depressa, intumescentia clypei simili apicali praedita; paries e 8–11 stratis cellularum brunnearum pseudoparenchymatarum, *textura angulari*, composita. *Asci* clavati vel cylindrici, stipitati, bitunicati, fissitunicati; camera apicali conspicua indentata. *Ascosporae* uni. vel biseriatae, hyalinae, guttulatae, leves, ellipsoideae, clavatae vel fusiformes, saepe in tertio superiore latissimae, utrinque angustatae. *Pseudoparaphyses* hyalinae, septatae, ramosae, saepe in summo et ad basim cavitati pseudothecii affixae (*Botryosphaeriaceae*).

Typus: *Saccharata proteae* (Wakef.) Denman & Crous.

Mycelium immersed, consisting of branched, septate, smooth, medium brown hyphae. *Ascomata* epiphyllous, separate, becoming aggregated, unilocular, immersed, substomatal, with a central, flattened ostiole, surrounded by a continuous, clypeus-like apical thickening of the wall, obovoid, slightly depressed; wall consisting of 8–11 layers of brown pseudoparenchymatous *textura angularis*. *Asci* clavate to cylindrical, stipitate, bitunicate, fissitunicate; apical chamber visible as a notch-like indentation at the apex. *Pseudoparaphyses* hyaline, septate, branched, frequently attached to the top and base of the pseudothecial cavity. *Ascospores* uni- to biseriate, hyaline, guttulate, smooth, ellipsoidal, clavate to fusiform, frequently widest in the upper third of the ascospore, tapering to obtuse ends.

Etymology: Named after the common name for *Protea repens*, its primary host, which is called sugar bush (saccharum = sugar).

Type species: ***Saccharata proteae*** (Wakef.) Denman & Crous.

Specimens examined. South Africa, Western Cape Province, Klapmuts, on leaves of *Protea repens* (as *P. mellifera*), *P. Van Der Bijl,* No. 357 (holotype PREM 32915); Grabouw, Molteno Estate, *Protea grandiceps*, 5 Jun. 1997, *S. Denman* (PREM 55769, anamorph, culture ex-type STE-U 1694).

Saccharata proteae (Wakef.) Denman & Crous, **comb. nov.** Figs 302–315.

≡ *Phyllachora proteae* Wakef., *Kew Bulletin* **1922**: 164 (1922).

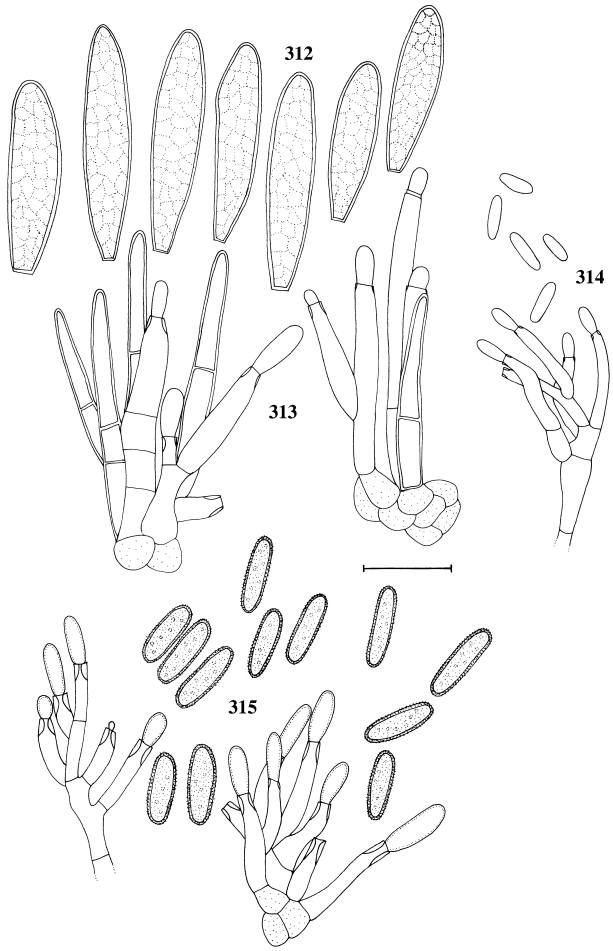
≡ *Botryosphaeria proteae* (Wakef.) Denman & Crous, *Mycologia* **91**: 511 (1999).

Anamorphs: *Fusicoccum proteae* Denman & Crous, *Mycologia* **91**: 511 (1999), and a *Diplodia*-like synanamorph.

Symptoms: *Leaf spots* and tip blight of pale to medium brown necrotic tissue, usually associated with insect wounds.

Causal organism: *Mycelium* immersed, consisting of branched, septate, smooth, medium brown, 2.5–5 µm wide hyphae. *Ascomata* epiphyllous, separate, unilocular, initially solitary and discrete, becoming aggregated, immersed, substomatal, with a central, flattened ostiole, obovoid, slightly depressed, 200–300 µm diam, 200–240 µm high; wall consisting of 8–11 layers of brown pseudoparenchymatous *textura angularis*, up to 65 µm thick in the upper, thickest part. *Asci* fissitunicate, clavate to cylindrical, stipitate, bitunicate, 90–150 × 12–15 µm; apical chamber visible as a notch-like indentation at the apex. *Pseudoparaphyses* hyaline, septate, branched, frequently attached to the top and base of the pseudothecial cavity, 2–3.5 µm wide. *Ascospores* uni- to biseriate, hyaline, guttulate, smooth, ellipsoidal, clavate to fusiform, frequently widest in the upper third of the ascospore, tapering to obtuse ends, (15–)17–20(–21) × (5–)6–8(–9) µm.

Conidiomata pycnidial, eustromatic, to 450 µm diam, immersed, subepidermal, separate, dark brown, uni- to multi-locular, walls consisting of dark brown *textura angularis*, ostiolate. *Fusicoccum* anamorph: *Conidiophores* hyaline, smooth, branched, subcylindrical, 1–3-septate, formed from the inner layer of the locule, 20–40 × 3–4.5 µm, intermingled with hyaline, septate paraphyses. *Conidiogenous cells* phialidic, discrete or integrated, hyaline, smooth, cylindrical, enteroblastic, proliferating percurrently with 1–2 indistinct annellations or with periclinal thickening (sensu Sutton 1980), 20–30 × 2.5–3.5 µm. *Conidia* hyaline, thin-walled, aseptate, smooth, clavate to fusiform, widest in the middle or upper third of the conidium, with a subobtuse apex, and a truncate base, (20–)22–25(–30) × (4.5–)5–6 µm. Microconidial state occurring in separate or the same conidiomata as the *Fusicoccum* anamorph. *Microconidiophores* hyaline, smooth, branched, cylindrical, 1–3-septate, formed from the inner layers of the locule, 15–25 × 2–3 µm. *Microconidiogenous cells* phialidic, discrete or integrated, hyaline, smooth, cylindrical, determinate, with prominent periclinal thickening, 6–10 × 2–3 µm. *Microconidia* medium brown, thick-walled, finely verruculose, guttulate, aseptate, subcylindrical to narrowly ellipsoid with rounded ends, (7–)8–11(–14) × 2.5–3.5 µm. The spermatial state occurs in conidiomata with the *Fusicoccum* anamorph or in separate spermogonia. *Spermatiophores* hyaline, smooth, branched, cylindrical, 1–3-septate, formed from the inner layer of the locule, 15–20 × 3–4 µm. *Spermatiogenous cells* discrete or integrated, hyaline, smooth, cylindrical, proliferating via determinate phialides with periclinal thickening, 10–12 × 2–3 µm. *Spermatia* hyaline, smooth, aseptate, rod-shaped with rounded ends, 5–7 × 1.5–2 µm.



Figs 312–315. *Fusicoccum proteae* and its *Diplodia*-like synanamorph. Fig. 312. *Fusicoccum* conidia. Fig. 313. Conidiophores and paraphyses. Fig. 314. Spermatia and spermatiophores. Fig. 315. *Diplodia*-like synanamorph and conidiophores. Scale bar = 10 µm. (From Denman *et al*., 1999).

Cultural characteristics: *Colonies* after one month on PDA in the dark at 25°C with crenate or irregular margins, moderate to sparse aerial mycelium, grey, occasionally sectored, with black conidiomata that occur over the entire surface, aggregated in dense masses along the outer colony margins; smoke-grey (19’’’’i), reverse ranging from buff (21”f) to olivaceous grey (23’’’’i) or iron grey (23’’’’k); 7 mm/d at 25°C in the dark. Temperature requirements for growth: minimum 5°C, optimum 25°C and maximum 35°C. Ascomata formed infrequently.

Host range and geographic distribution: *P. eximia, P. magnifica, P. obtusifolia* (**Australia, Tasmania**) (Crous *et al*., 2000b); *Lsp. cordifolium, Lsp. lineare* × *cordifolium*, *Leucospermum* sp., *P. acaulos, P. cynar-*

oides, *P. eximia*, *P. grandiceps*, *P.* 'Lady Di', *P. magnifica*, *P. obtusifolia, P.* 'Queen Mink', *P. repens, P.* 'Venus' (**South Africa**) (Denman *et al*., 1999; Taylor *et al*., 2001c); *P. cynaroides, P. eximia, P.* 'Queen Mink' (**USA, California**) (Taylor *et al*., 2001b), *P. cynaroides, P. neriifolia, P.* 'Carnival', *P.* 'Lady Di', *P.* 'Mink', *P.* 'Pink Ice', *P.* 'Rose Mink', *Protea* sp. (**USA, Hawaii**) (Taylor *et al*., 2001a).

Disease cycle and epidemiology: The disease cycle is unknown. The pathogen occurs endophytically and has been isolated consistently from symptomless leaves of *Protea* spp. and some species of *Leucospermum*. It has not been isolated as an endophyte or pathogen from *Leucadendron* spp. It is considered to be an opportunistic pathogen and frequently only forms lesions on damaged (leaves stung by insects and stems wounded by borer insects) or stressed tissues. Pseudothecia are commonly observed on senescent leaves during the winter.

Control: The most important control measure for leaf spot and tip dieback caused by *S. proteae* is control of insects that cause wounds to leaves and stems. *Protea repens* appears particularly susceptible to tip dieback caused by this pathogen in association with insect damage. Furthermore, *S. proteae* is often associated with cankers caused primarily by other pathogens such as *Phomopsis saccharata* (in the case of *P. repens*) or wounds caused by wood-boring insects. Sanitation pruning of stem cankers and insect-wounded wood will help control stem infections and reduce possible sources of inoculum. Leaf infections are difficult to control preventively with fungicides due to the endophytic nature of this fungus. The pathogen is very sensitive to fungicides such as benomyl (Benlate), iprodione (Rovral), prochloraz manganese chloride (Octave) and tebuconazole (Folicur) (Denman, unpubl. data). Applying fungicides to control this disease solely is not economically feasible but prophylactic fungicide spray programmes in commercial plantations implemented for control of other pathogens, should also control *S. proteae*.

Septoria leaf spot

Kirk *et al.* (2001) estimate that there are more than 1000 species of *Septoria*. *Septoria* is an anamorph of *Mycosphaerella* and is commonly associated with leaf spot symptoms of numerous hosts. Two species are presently known to occur on *Proteaceae*, namely *S. protearum* and *S. grandicipis* (Swart *et al.*, 1998). A third described species, *Septoria proteae*, is a synonym of *Phaeophleospora abyssinicae* (Crous & Palm, 1999).

Septoria grandicipis Joanne E. Taylor & Crous, *Mycological Research* **104**: 628 (2000). Figs 316–319, 321.

Symptoms: *Leaf spots* circular, necrotic, raised, grey-brown with a dark brown margin, 3 mm diam.

Causal organism: *Conidiomata* pycnidial, ostiolate, amphigenous, substomatal, single, visible only as pale brown areas surrounding the stoma; in section globose, subepidermal, with a central, non-papillate ostiole, with peripheral conidiogenous cells, 122 µm high, 100 µm diam. *Peridium* consisting of a stratum of thick-walled, hyaline, small-celled, compressed *textura angularis* to *textura intricata,* with medium brown, thick-walled *textura angularis* surrounding the ostiolar pore, becoming hyaline and more compressed in the inner layer, together (12–)13.5–18(–20) µm thick. *Conidiogenous cells* hyaline, irregularly doliiform, pyriform or ellipsoidal, with rounded or flattened apices, holoblastic, proliferating sympodially, (2.5–)5–6(–8) × (2–)3–4(–6) µm. *Conidia* narrowly cylindrical, straight or curved, with a rounded apex and a truncate base, thin-walled, smooth, hyaline, euseptate, with up to 7 septa, (33–)43–46(–57) × 2–3 µm.

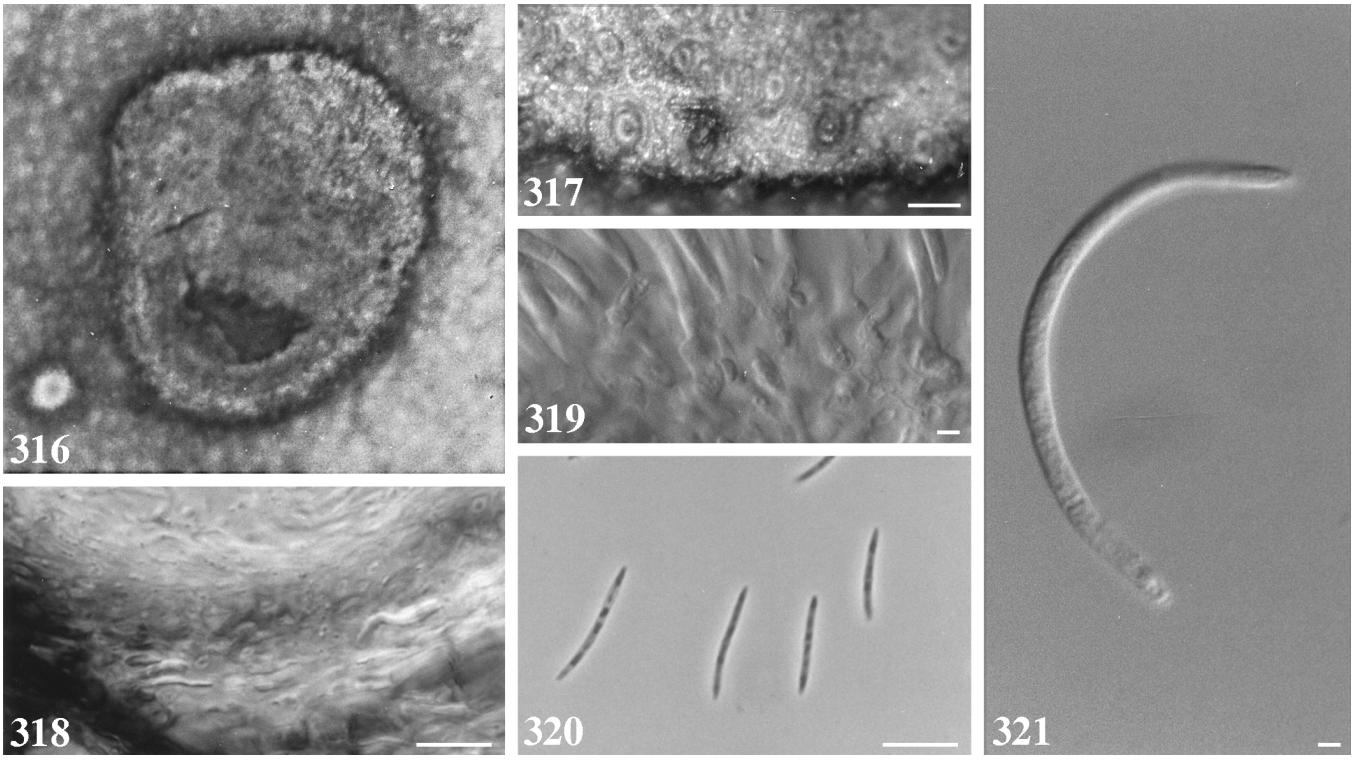
Cultural characteristics: *Colonies* circular, umbonate, with entire margins, ochreous (13'b) to pale luteous (19d), becoming slightly darker (19"d) in the centre, the same in reverse; aerial mycelium moderately dense, slimy, becoming woolly in the centre; 10 mm diam after 14 days.

Host range: *Protea grandiceps* (**South Africa, WC**).

Septoria protearum Viljoen & Crous, *South African Journal of Botany* **64**: 144 (1998). Figs 320, 322.

Symptoms: *Leaf spots* amphigenous, circular to irregular, 8–15 mm diam, medium brown with a red margin.

Causal organism: *Conidiomata* pycnidial, associated with leaf spots, amphigenous, black on surface, subepidermal, becoming erumpent, 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 diam. *Conidiophores* hyaline, smooth, subcylindrical, unbranched or branched above, 0–5-septate, 8–30 × 1.5–3.5 µm. *Conidiogenous cells* integrated, terminal and lateral, hyaline, subcylindrical, unbranched, tapering to rounded or flattened apices, proliferating sympodially, 4–12 × 1.5–3 µm diam. *Conidia in vitro* solitary, hyaline, smooth, guttulate, (0–)1–3(–4)-septate, subcylindrical to narrowly obclavate, with a subobtuse apex, and a narrow obconically truncate to truncate base, straight



Figs 316–321. *Septoria* spp. Figs 316–319, 321. *S. grandicipis*. Fig. 316. Leaf spot on *Protea grandiceps*. Fig. 317. Conidiomata on leaf. Fig. 318. Pycnidial wall layer. Fig. 319. Conidiogenous cells. Fig. 321. Conidium. Fig. 320. Conidia of *S. protearum*. Scale bars: 317 = 100 µm, 318–321 = 10 µm. (From Swart *et al*., 1998; Taylor & Crous, 2000).

to curved, (6–)15–22(–30) × 1.5–2 µm *in vitro*, and *in vivo*.

Cultural characteristics: *Colonies* with smooth margins, with moderate whitish aerial mycelium, reverse iron-grey 23’’’’k; 10.3 mm diam after 2 weeks in the dark at 25°C.

Host range: *Protea cynaroides* (**South Africa, GT**), *Protea* sp. (**Spain, Tenerife**) (Taylor *et al*., 2003).

Disease cycle and epidemiology: Conidia are probably splash-dispersed.

Control: Unknown.

Stictis leaf spot

This species, which is rarely encountered, is probably a saprobe. Further research is required to determine its potential as a plant pathogen (Taylor *et al*., 2001a).

Stictis leucospermi Joanne E. Taylor, Crous & M.E. Palm, *Mycotaxon* **78**: 474 (2001). Figs 323–331.

Symptoms: Unknown, isolated from brown, necrotic areas of living leaves.

Causal organism: *Ascomata* apothecial, opening broadly by a pore, partially erumpent, 350–500 µm diam, margin narrow to medium, white to apricot, lacerate, the disc slightly urceolate and pale apricot. *Margin* 37–92 µm wide, three-layered, without a prominent accessory thalline margin; wall 5–10 µm thick, of compressed, hyaline pseudoparenchymatous cells; the crystalline layer consisting of well-developed colourless, heterogeneous, non-rosettiform crystals and periphysoids, unbranched, gelatinous, 8–10 × 1.5–3 µm; subhymenium of small, colourless angular, cells, J-, 12–20 µm wide. *Paraphyses* numerous, filiform, simple or branched above, not enlarged at the apex but sometimes slightly circinate, J-, 1.5–2.5 µm wide. *Asci* cylindrical to slightly fusiform, narrowing to a truncate apex, with a medium-length pedicel, eight-spored, with a 2–3 µm thick cap, pierced by a narrow conical pore, J-, (65–)84–93(–112) × (6–)7–8(–10) µm. *Ascospores* filiform, hyaline, multiseptate, cells 7–13 µm long, unconstricted at the septa at maturity and not disarticulating, often with small mucilaginous appendages at the apices, (61–)68–72(–80) × (1.5–)2(–2.5) µm.

Host range and geographic distribution: *Lsp.* ‘T86-09-05’, *Lsp.* ‘High Gold’, *Lsp.* ‘Hybrid 31’, *Lsp.* ‘Hybrid 24’, *Leucospermum* sp. (**USA, Hawaii**).

Stigmina leaf spot

This species has been recorded infrequently on *Protea* spp., and its relative importance is presumed to be mi-

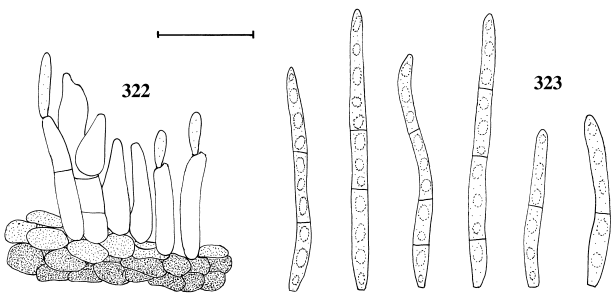


Fig. 322–323. *Septoria protearum.* 322. Conidiophores and conidiogenous cells. 323. Conidia formed *in vitro*. Scale bar = 10 µm. (From Swart *et al*., 1998).

nor. Species of *Stigmina* are anamorphs of *Mycosphaerella* (Crous *et al*., 2001) and this species is included in the key to *Mycosphaerella* anamorphs.

The two varieties of *Stigmina protearum* treated here are listed under *Pseudocercospora*, the appropriate name for those taxa.

Stigmina californiae Joanne E. Taylor & Crous, *Mycotaxon* **78**: 91 (2001). Figs 332–338.

Symptoms: *Leaf spots* yellow to pale brown, sunken, necrotic, with raised borders, 4–25 mm diam.

Causal organism: *Mycelium* superficial, red-brown, thick-walled, finely verrucose, constricted at the septa, reticulate and forming a crust-like layer beneath and between the sporodochia, 7–9(–10) µm wide. *Stromata* emergent from the stomata. *Sporodochia* amphigenous, numerous, dark brown to black, punctiform, (70–)80–140(–200) µm diam, forming superficial and often coalescing masses on stromata. *Conidiophores* cylindrical, tapering to the conidiogenous cells, in loose fascicles, arising from the stromata, macronematous, straight or flexuous, mainly simple, red-brown, finely verrucose and with striations, (8–)14–24(–60) × (5–)6–6.5(–7) µm. *Conidiogenous cells* cylindrical, medium red-brown, finely verrucose, indeterminate with 0–4 irregular, roughened annellations (5–)9.5–13(–17) × (4-)5–6 µm. *Conidia* cylindrical, fusiform or obclavate, with a truncate base and tapering to a rounded apex, straight, yellow-brown, finely verrucose, 0–3-distoseptate, (11–)14–16(–19) × (3–)5–5.5(–7) µm.

Host range and geographic distribution: *P. laurifolia, P. magnifica, P.* 'Pink Ice' (**USA, California**).

Stilbospora leaf spot

Stilbospora proteae is presently the only member of this genus known to occur on *Proteaceae*. The only other record of a species of *Stilbospora* occurring on a proteaceous host, *S. faurea*, has been transferred to *Phaeophleospora* (Taylor & Crous, 1999).

Stilbospora proteae Joanne E. Taylor & Crous, *Mycological Research* **104**: 629 (2000). Figs 339–348.

Symptoms: *Leaf spots* circular to irregular, necrotic, sunken, grey-brown, with a raised, dark brown, 2–15 mm broad margin.

Causal organism: *Conidiomata* epiphyllous, pycnidial, substomatal, non-erumpent but raising the host surface slightly, dark brown, (90–)111–151(–170) µm diam; in section globose, with a central, non-papillate ostiole; reduced stroma consisting of brown fungal hyphae in host cells surrounding the ostiole; with peripheral conidiogenous cells, 145–178 µm high, 135–182 µm diam. *Peridium* comprising an outer stratum of thick-walled, small-celled, dark brown *textura angularis* becoming paler in the inner layer; innermost cells thin-walled and hyaline, (11–)14–17(–20) µm thick. *Paraphyses* deliquescing early and remaining as brown amorphous material between and above the conidiophores. *Conidiophores* filiform, hyaline, smooth, straight, simple or branched above, 1–3-septate, 1–17 × 1.5–2 µm. *Conidiogenous cells* terminal, filiform, hyaline, proliferating percurrently, with up to three annellations, secession schizolytic, (3–)9–15(–26) × 1.5–2 µm. *Conidia* fusiform to ellipsoidal, base truncate with or without a small hilum, narrowing slightly to a rounded or slightly acute apex, thick-walled, verrucose, red-brown, 1–3-euseptate, (11–)14–15(–18) × (5–)6–6.5(–7.5) µm.

Cultural characteristics: *Colonies* circular with undulate margins, fuscous-black (7''''k–7''''k), often with white patches in the centre of younger colonies, the same in reverse; aerial mycelium moderately dense, smooth, becoming woolly in the centre, and faintly concentrically zoned; 29 mm diam after 40 days.

Host range and geographic distribution: *P. susannae* (**South Africa, WC**).

Disease cycle and epidemiology: Conidia are probably splash-dispersed.

Control: Unknown.

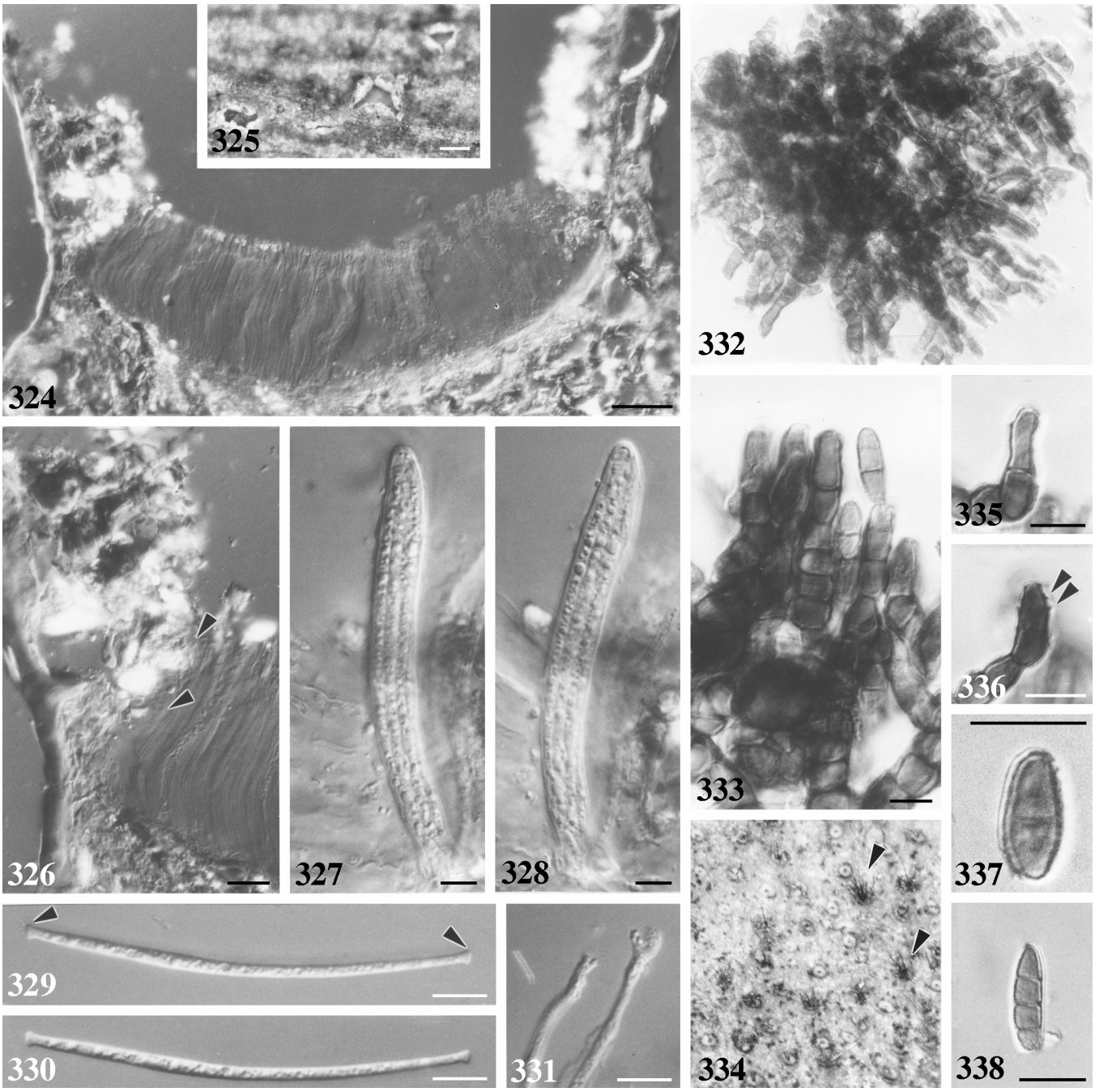
Teratosphaeria leaf spot

Teratosphaeria occurs only in the Southern Hemisphere and is mainly associated with *Proteaceae*. Four species cause distinctive leaf spots on *Protea* spp. Recent molecular studies (Taylor *et al*., 2003), have shown that *Teratosphaeria* is congeneric with *Mycosphaerella* and therefore these species are treated under Mycosphaerella leaf spot above.

Trimmatostroma leaf spot

Species of *Trimmatostroma* are widespread saprobes or parasites of several hosts including *Proteaceae*. Several species have recently been shown to be anamorphs of *Mycosphaerella* (de Hoog *et al.*, 1999; Taylor *et al*., 2003). *Trimmatostroma macowanii* is a fairly uncommon pathogen that attacks *Protea* spp. in South Africa and Malawi (Ellis, 1976; Sutton, 1993).

There are also unverified reports of *T. macowanii* occurring on *Leucospermum* and *Leucadendron* (Van Wyk, 1973a). It is generally regarded to be of little economic importance, as it does not affect commercially important hosts. Two other, less common species of *Trimmatostroma* occur on *Proteaceae*, namely *T. elginense* and *T. protearum,* both recorded on *Protea grandiceps*. Species of *Trimmatostroma* have been intercepted as quarantine organisms.

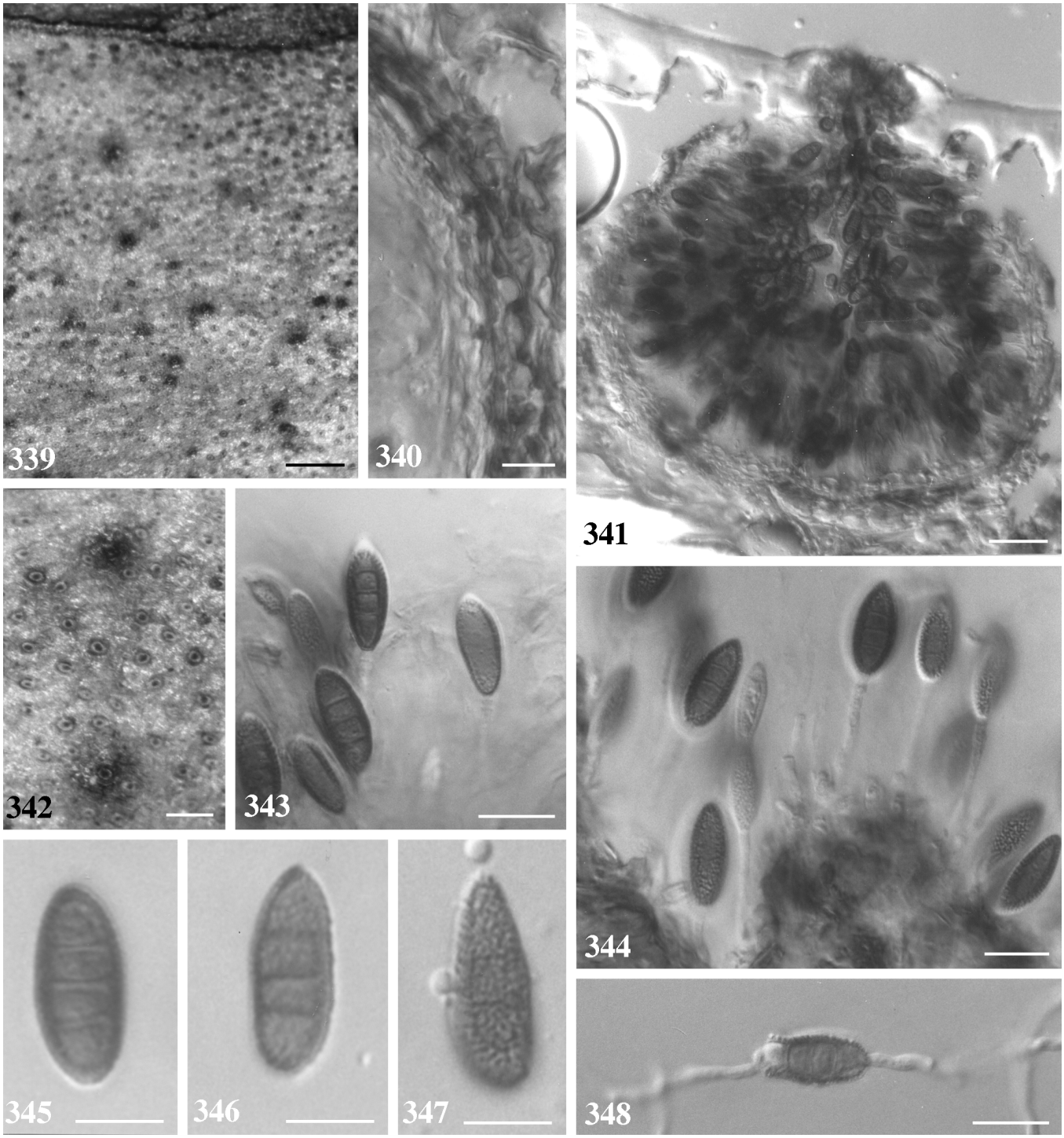


Figs 324–338. *Stictis leucospermi* and *Stigmina californiae*. Figs 324–331. *Stictis leucospermi.* Fig. 324. Vertical section through an apothecium. Fig. 325. Apothecia on host. Fig. 326. Margin, three-layered, without a prominent margin; comprising the wall, the crystaline layer and periphysoids (arrowed). Figs 327–328. Asci. Figs 329–330. Ascospores with mucous caps (arrowed). Fig. 331. Paraphyses. Figs 332–338. *Stigmina californiae*. Fig. 332. Sporodochium. Fig. 333. Conidiophores. Fig. 334. Sporodochial initials forming on host (arrowed). Figs 335–336. Conidiogenous cells with percurrent proliferations (arrowed). Figs 337, 338. Conidia. Scale bars: 323 = 50 µm, 324 = 200 µm, 325–331, 333, 325–338 = 10 µm, 332 = 30 µm. (From Taylor *et al*., 2001a, b).

Trimmatostroma elginense Joanne E. Taylor & Crous, *Mycological Research* **104**: 633 (2000).
Figs 349–355.

Symptoms: Occurring on the same leaf spots as *Septoria grandicipis.*

Causal organism: *Mycelium* immersed, hyphae pale brown, septate, smooth, 3–6 µm wide. *Conidiomata* s*porodochial* pulvinate, dry, dark brown to black, 90–100 × 39–82 µm. *Stroma* in substomatal cavity, of brown walled pseudoparenchymatous cells. *Conidiophores* macronematous, branched, fairly loosely packed, emergent through stomata, smooth, medium red-brown. *Conidiogenous cells* irregularly cylindrical to doliiform, terminal, holoblastic, delimitation of conidium by a single septum, with retrogressive delimitation of next conidium, giving an unconnected branched or simple chain of conidia, 10–18 × 6–7 µm. *Conidia* variously shaped, cylindrical, fusiform, ellip-



Figs 339–348. *Stilbospora proteae*. Fig. 339. Leaf spot on *Protea susannae*. Fig. 340. Conidiomatal wall layer. Fig. 341. Vertical section through a conidioma. Fig. 342. Conidiomata on host. Figs 343, 344. Conidiogenous cells with annellations. Figs 345–347. Conidia. Fig. 348. Germinating conidium. Scale bars: 339 = 1 mm, 340–341 = 20 µm, 342 = 100 µm, 343, 344, 348 = 10 µm, 345–347 = 6 µm. (From Taylor & Crous, 2000).

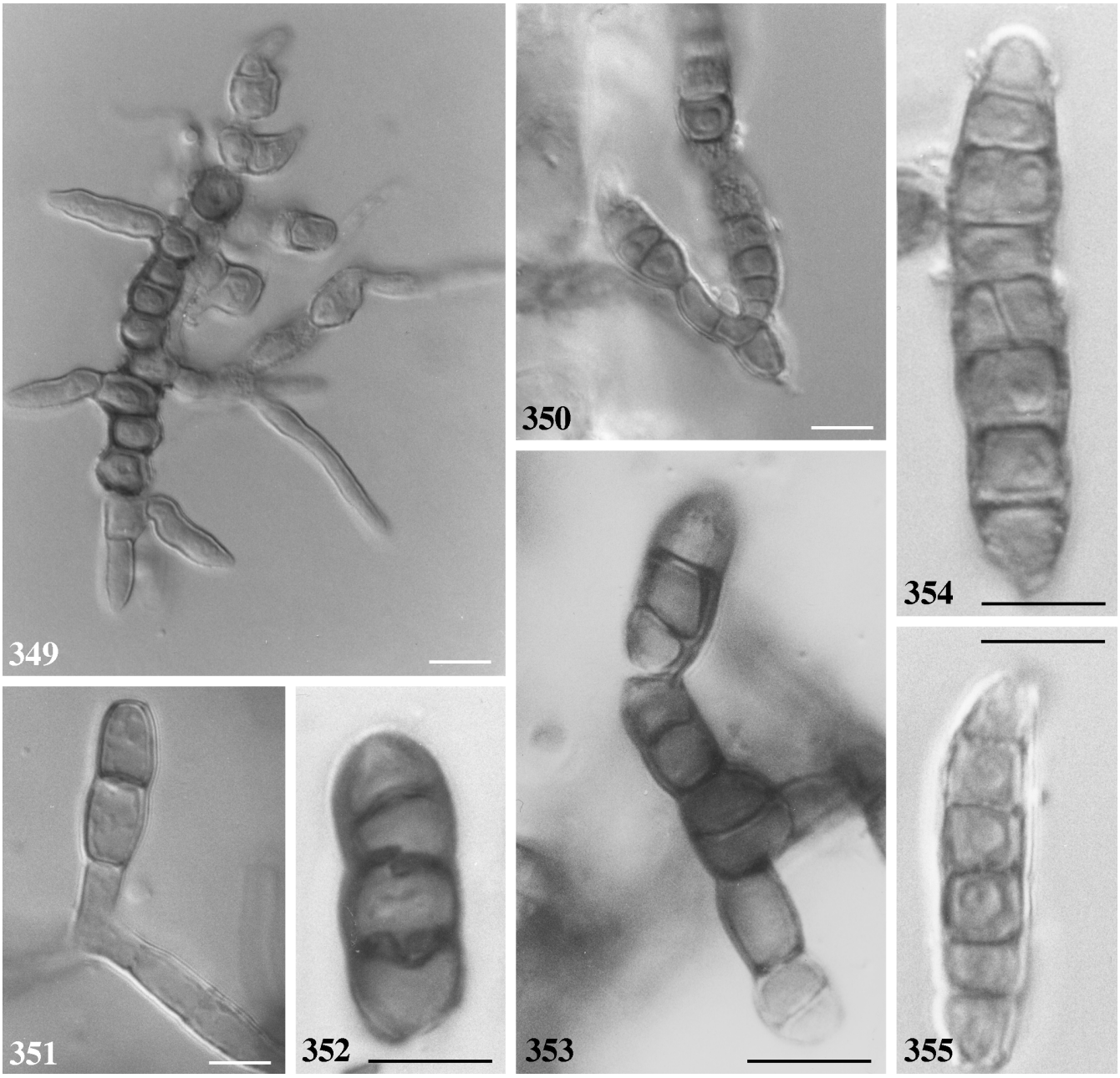
soidal, straight or curved, with apices rounded or truncated, often with a indistinct frill, catenate in branched basipetal chains, schizogenous, red-brown, multiseptate, transverse and often longitudinal or oblique septa, thick-walled, verrucose, (9–)16–20(–36) × (10–)14–18(–27) µm.

In culture sporodochia absent, but numerous, globose, multicellular sclerotia-like structures formed, pale brown, smooth, 15–49 µm diam. *Conidiophores* semi-macronematous, branched, flexuous, smooth, pale to medium red-brown, 5.5–7.5 µm long. *Conidiogenous cells* 8–16 × 6–8 µm, mainly cylindrical, integrated or terminal, conidiogenesis as on host tissue, with unconnected simple or branched chains of conidia. *Conidia* mainly cylindrical, ellipsoidal, straight or curved, rounded or truncated apices, catenate in branched basipetal chains, schizogenous, olivaceous-brown, multiseptate, with transverse and less often longitudinal or oblique septa, the multicelled conidia predominating, thick-walled, finely verrucose to smooth, (10–)14–18(–27) × (6–)7–8(–10) µm.

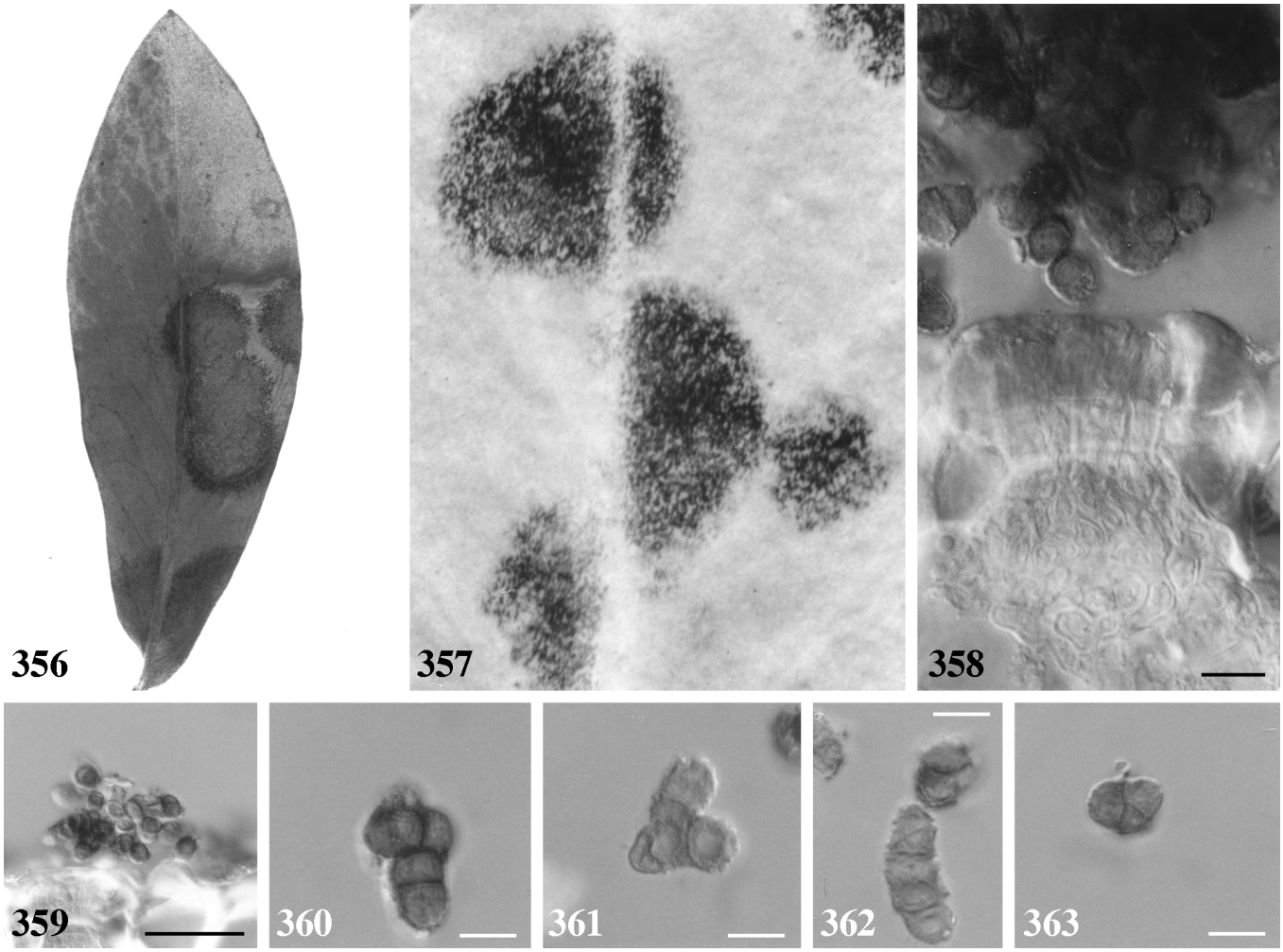
Cultural characteristics: *Colonies* circular, umbonate with entire margins, green-black (3’’’’k), the same in reverse; aerial mycelium dense, felty; 15 mm diam after 40 days; fertile after 7 days.

Host range and geographic distribution: *P. grandiceps* (**South Africa, WC**).

Notes: Germ tubes produced from individual conidial cells, perpendicular to the long axis of the conidia, irregular and branched.



Figs 349–355. *Trimmatostroma elginense*. Fig. 349. Germinating conidium. Figs 350–351, 353. Conidiogenous cells. Fig. 352, 354–355. Conidia. Scale bars: 349–351 = 10 µm, 352–355 = 8 µm. (From Taylor & Crous, 2000).



Figs 356–363. *Trimmatostroma macowanii*. Fig. 356. Leaf spot on a *Protea* sp. Fig. 357. Leaf spots. Fig. 358. Sporodochium with stroma in a substomatal cavity and conidiophores emerging in a fascicle through the stoma. Fig. 359. Sporodochium. Figs 360–363. Conidia. Scale bars: 358, 360–363 = 10 µm, 359 = 50 µm. (From Taylor & Crous, 1998).

Trimmatostroma macowanii (Sacc.) M.B. Ellis, *More Dematiacous Hyphomycetes*: 29 (1976). Figs 356–363, Plate 10.

≡ *Coniothecium macowanii* Sacc., *Sylloge Fungorum* **4**: 512 (1886).

= *Coniothecium punctiforme* G. Winter, *Hedwigia* **24**: 33, 1885, non *C. punctiforme* Corda, *Icones Fungorum* (Prague) **1**: 2 (1837).

Symptoms: *Leaf spots* amphigenous, circular, discrete or confluent, sometimes covering the whole leaf surface, non-necrotic, but causing discoloration of the host tissue which becomes yellow-brown, dry, speckled, sooty in appearance, 2–12 mm diam.

Causal organism: *Conidiomata* sporodochial, pulvinate, punctiform, brown-black, 40–80 µm diam; in section stromata in substomatal cavities, hyaline or pale. *Conidiophores* emerging as fascicles through the stomata, hyaline to pale brown, smooth or verruculose, cylindrical. *Conidiogenous cells* holoblastic, basipetal, forming an unconnected chain of conidia delimited by a septum followed by diffuse wall-building below the previous conidium to form the next conidium which is delimited retrogressively, conidia separating by schizolytic secession. *Conidia* formed in simple and branched chains, dry, very variable in shape, ellipsoidal, cylindrical, clavate, Y-shaped, curved or straight, with rounded apices, 1- to multiseptate, pale to dark red-brown or olivaceous-brown, verrucose, (10–)15–17(–23) × (6–)6.5–7(–9) µm.

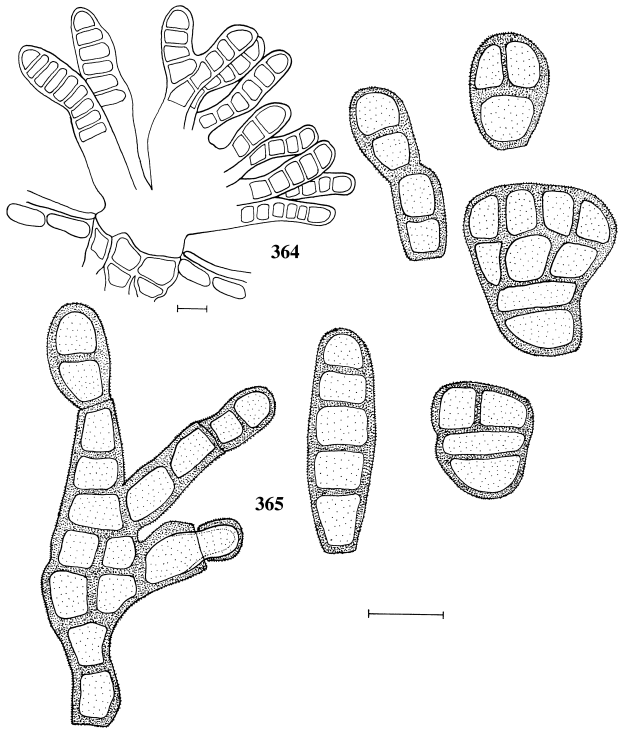
Culture characteristics: *Colonies* black, sooty, sporulating readily; moderately fast-growing, dense with an irregular margin, and with superficial hyphae.

Host range and geographic distribution: *P. petiolaris* (**Malawi**) (Sutton, 1993); *Lsp. conocarpodendron, Leucadendron* sp., *P. nitida, Protea* sp. (**South Africa, EC, NC, WC)** (Saccardo, 1886; Doidge, 1950); *P. angolensis, Protea* sp. (**Zimbabwe**) (Doidge, 1950).

Trimmatostroma protearum Crous & M.E. Palm, *Mycological Research* **103**: 1303 (1999). Figs 364–365.

Symptoms: *Leaf spots* not observed, *sporodochia* amphigenous, black, discrete, evenly distributed.

Causal organism: *Mycelium* internal, consisting of branched, septate, verruculose, medium brown, 4–6



Figs 364–365. *Trimmatostroma protearum*. Fig. 364. Sporodochium on host surface. Fig. 365. Conidiophore with conidia. Scale bars = 10 µm. (From Crous & Palm, 1999).

µm wide hyphae. *Conidiomata* sporodochial pulvinate, punctiform, black, up to 250 µm diam; stromata in substomatal cavities, consisting of thick-walled, smooth, brown cells, 3–6 µm diam. *Conidiophores* fasciculate, emerging through the stomata, dark brown to almost black, thick-walled, verruculose, distoseptate, with horizontal, vertical and oblique septa, 25–120 × 8–20 µm, subcylindrical, straight to curved, frequently branched in the upper region. *Conidiogenous cells* terminal, dark brown, thick-walled, verruculose, integrated, 5–10 × 6–12 µm. *Conidia* simple or in branched chains, variable in shape, multi-celled, frequently constricted at the septa, medium brown, verruculose, with one to several horizontal distosepta, and vertical and oblique distosepta frequently present, (12–)25–35(–45) × (7–)10–15(–25) µm.

Host range and geographic distribution: *P. grandiceps* (**South Africa**).

Disease cycle and epidemiology: Conidia are splash-dispersed.

Control: Unknown.

KEY TO TRIMMATOSTROMA *SPECIES ON* PROTEACEAE

See key to *Mycosphaerella* anamorphs.

Verrucisporota leaf spot

This species is commonly recorded on *Proteaceae* and is widely distributed (Taylor & Crous, 2000).

Verrucisporota proteacearum (D.E. Shaw & Alcorn) D.E. Shaw & Alcorn, *Australian Systematic Botany* **6**: 273 (1993). Figs 366–371.
≡ *Verrucispora proteacearum* D.E. Shaw & Alcorn, *Proceedings of the Linnean Society of New South Wales* **92**: 171 (1967).

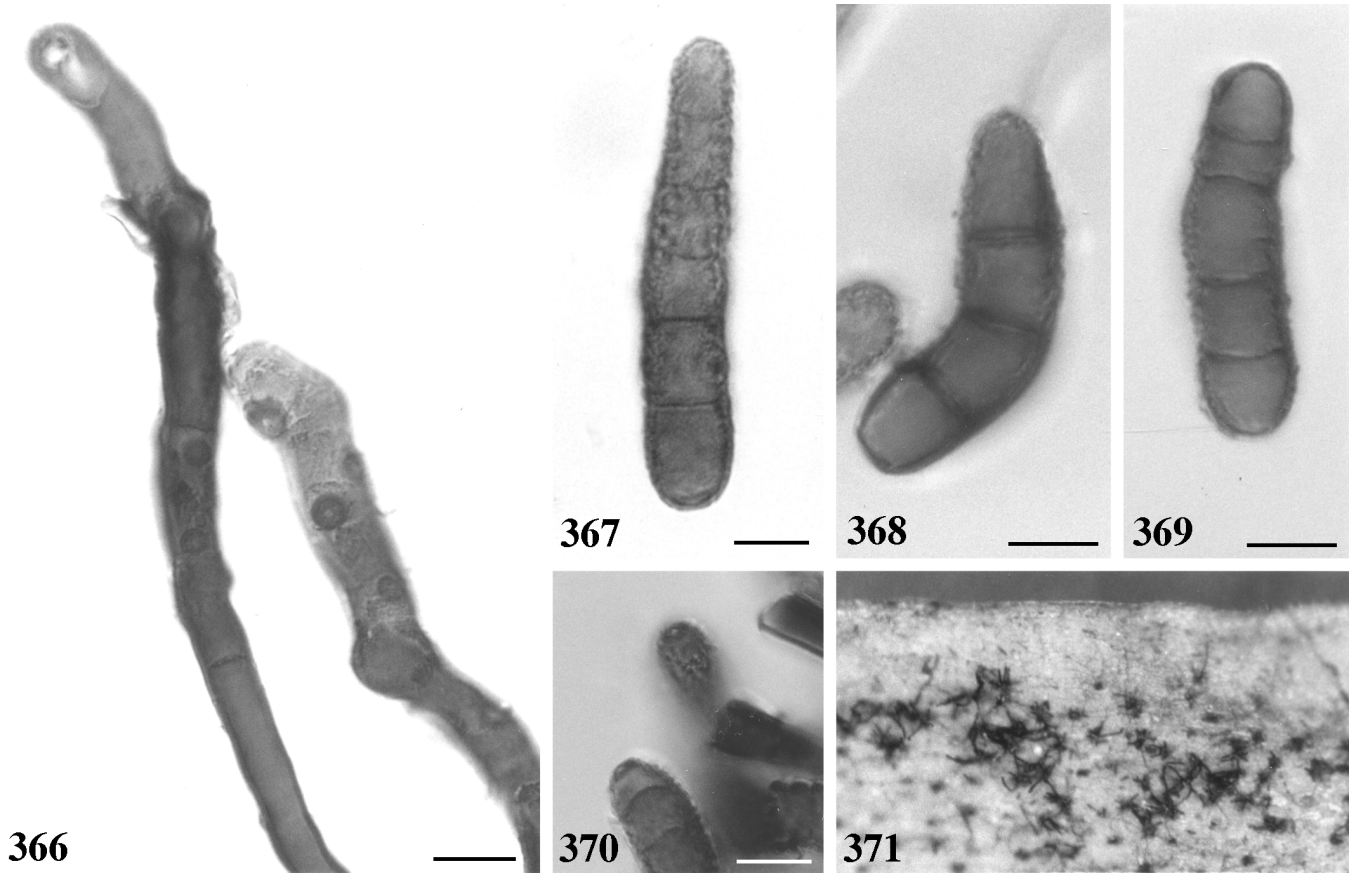
Symptoms: *Leaf spots* not observed, usually on necrotic areas of older, mature leaves, or on leaf litter, colonies round or irregular, hairy, dry, dark brown to black, discrete.

Causal organism: *Mycelium* immersed, hyphae pale brown, septate, verrucose, 3–4 µm wide. *Stroma* forming in substomatal cavities, cells brown-walled, pseudoparenchymatous. *Conidiophores* macronematous, mononematous, caespitose, emerging through the stomata, simple, flexuous, often geniculate, approximately 4-septate, mainly smooth, dark red-brown or slightly olivaceous, from a bulbous base tapering towards the apex, but often becoming more swollen, and also verrucose at the apex, (90–)121–136(–250) × (5–)6–7(–9) µm. *Conidiogenous cells* cylindrical, becoming geniculate, integrated, terminal, becoming intercalary, polyblastic, proliferating sympodially, 15–35 × 7–9 µm, with conspicuous, cicatrised, protuberant, conidiogenous loci, 3–4 µm diam. *Conidia* cylindrical, narrowing slightly to an obtuse apex and with a truncate base with a distinctly thickened hilum, medium red-brown, straight or curved, with 1–6(–7) mainly unconstricted eusepta, thick-walled, verrucose, (20–)31–36(–49) × (7–)8.5–9.5(–12) µm.

Host range and geographic distribution: *Finschia chloroxantha*, *Hakea* sp. (**New Guinea**), *Finschia* sp., *Grevillea* spp., *Hakea* spp., *Protea* spp. (**Australia**); *Protea* spp. (**Malawi, Zambia**); *P. gaguedi*, *Protea* spp. (**South Africa**); *P. madiensis* (**Tanzania**).

Vizella leaf spot

Species of *Vizella* occur in tropical habitats in the Southern Hemisphere. *Vizella interrupta* has been recorded fairly commonly on *Protea, Leucospermum* and *Leucadendron* in South Africa, and on *Banksia, Grevillea, Hakea* and *Isopogon* in Australia, which suggests that this pathogen has had a long association with this host family. Fossil forms of *Vizella*, dating back to the Eocene period and approximately the same age as the *Proteaceae,* have been recorded from Australia, (Van Wyk *et al*., 1976). Although not a serious pathogen, *Vizella* is frequently intercepted as a quaran-



Figs 366–371. *Verrucisporota proteacearum*. Fig. 366. Conidiophores. Figs 367–369. Conidia. Fig. 370. Conidia showing thickened hila. Fig. 371. Conidiophores on host substrate. Scale bars = 10 µm. (From Taylor & Crous, 2000).

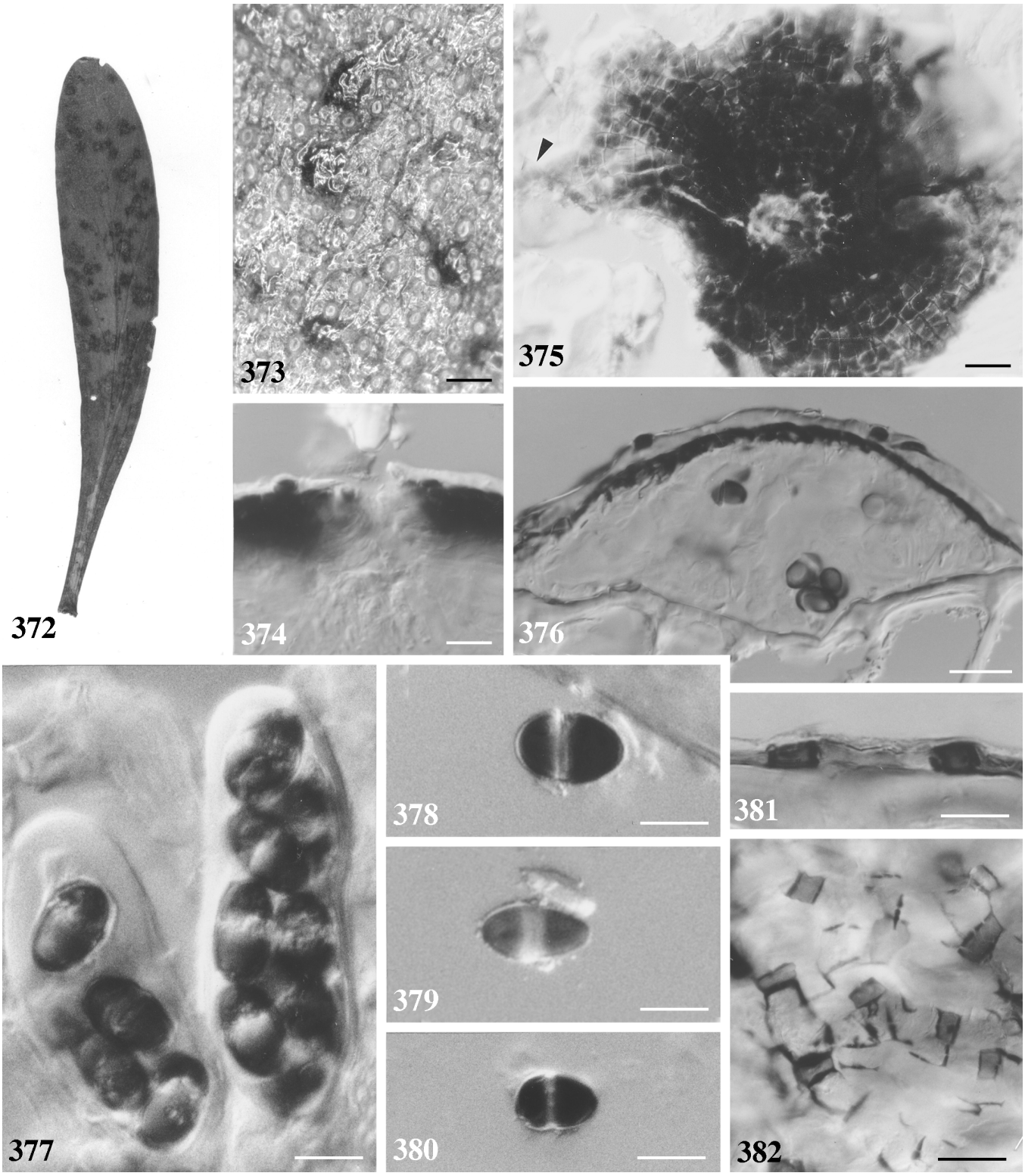
tine organism on South African and Australian *Proteaceae* due to its conspicuous nature.

Vizella interrupta (G. Winter) S. Hughes, *Mycological Papers* **50**: 99 (1953). Figs 372–371, Plate 10.
≡ *Asterina interrupta* G. Winter, *Flora*: 26 (1884).
≡ *Entopeltis interrupta* (G. Winter) Höhn., *S. B. Akademie der Wissenschaften in Wien*, **119**: 420 (1920).
= *Vizella banksiae* H.J. Swart, *Transactions of the British Mycological Society* **57**: 458 (1971).
= *Vizella grevilleae* H.J. Swart, *Transactions of the British Mycological Society* **64**: 303 (1975).

Symptoms: *Leaf spots* circular to irregular, discrete to confluent, pale brown, non-necrotic, with shiny black ascomata clustered towards the centre, 3–7 mm diam. Leaf spots varying in appearance on different hosts, ranging from scattered randomly to concentrically arranged ascomata on the slightly discoloured leaf tissue.

Causal organism: *Mycelium* immersed, intracuticular or of free, flat, ribbon-like hyphae, consisting of alternating, long, hyaline, thin-walled cells, 10–30 µm long and short, dark brown thick-walled cells, 7–17 µm long, or narrower hyphae 4–10 µm wide, composed of long, pale brown, thin-walled cells, 10–20 µm long, alternating with short dark brown thick-walled cells, 2–6 µm long. *Ascomata* amphigenous, circular or with an irregular outline, in surface view cells of the ascomata wall 4–6-angled, often formed in radiating lines, black, singular, gregarious, 130–200 µm diam; in section, flattened conical with a flat base, dimidiate, intracuticular, asci forming on the margin of the ascoma with the apices pointing towards the centre, central column of sterile paraphysoid tissue often present, central false ostiole formed by breaking away of ascomatal wall cells; ascomata (35–)45–59(–68) µm high, (155–)170–202(–215) µm diam. *Peridium* absent at the base but upper wall comprising a stratum of 1–3 layers of compressed, thick-walled, melanised cells with small lumina, 2.5–9 µm. *Pseudoparaphyses* indistinct, trabeculae-like, deliquescing to form a gelatinous mass surrounding the asci. *Asci* multiseriate, saccate to clavate, tapering abruptly to a small pedicel, with a wide, rounded apex, bitunicate, (51.5–)59–64(–72.5) × (17.5–)22.5–25.5(–27) µm. *Ascospores* ellipsoidal, violet-brown with a hyaline, transverse band, aseptate, (11–)15–15.5(–17.5) × (8–)10.4–11(–12.5) µm, narrower in lactophenol, (11–)15–16(–18) × (7–)9–10(–12) µm).

Conidiomata indistinguishable from and interspersed with the ascomata. *Conidiogenous cells* doliiform to ampulliform, hyaline, monoblastic, borne on the upper wall of the conidiomata, 4–5 × 2–3 µm. *Conidia* hyaline, becoming brown after release, ellip-



Figs 372–382. *Vizella interrupta*. Fig. 372. Leaf spot on a *Protea* sp. Fig. 373. Ascomata on host substrate. Fig. 374. Ostiolar region. Fig. 375. Ascoma on host. Fig. 376. Section through an ascoma. Fig. 377. Asci. Figs 378–380. Ascospores. Fig. 381. Characteristic wide hyphae under the leaf cuticle. Fig. 382. Superficial view of hyphae. Scale bars: 373 = 100 µm, 374, 377–382 = 10 µm, 375–376 = 20 µm. (From Taylor & Crous, 1998).

soidal, unicellular, (10–)12.5–16.5 × (5–)5.5–9.5 µm. *Spermogonia* similar in morphology to the conidiomata. *Spermatia* numerous, minute, cylindrical, hyaline.

Host range and geographic distribution: *Lsp. conocarpodendron, Lsp. cordifolium, Lcd. discolor, Lcd. gandogeri, Lcd. laureolum, Leucadendron* spp., *P. cynaroides, P. grandiceps, P. lepidocarpodendron, P. magnifica, P. neriifolia, P. nitida, Protea* spp. (**South Africa, EC, NC, WC**) (Saccardo, 1891; Theissen & Sydow, 1915; Doidge, 1927, 1950; Hughes, 1953; Van Wyk, 1973a; Van Wyk *et al*., 1975a, 1976); *Banksia integrifolia, Banksia* sp., *Grevillea aquifolia, Grevillea*

sp. *Hakea crassifolia, Isopogon anemonifolius* (**Australia**) (Taylor & Crous, 1998j).

Disease cycle and epidemiology: Ascospores are wind-borne.

Control: Unknown.

Notes: This pathogen was first described in 1884 as *Asterina interrupta* from *Leucospermum* and *Leucadendron* collected in the Cape Province of South Africa. Von Höhnel (1910) created the new genus *Entopeltis* with *E. interrupta* as type species. Hughes (1953) treated *Entopeltis* and *Vizella* as synonyms and established the new combination, *Vizella interrupta*. Von Arx & 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 *V. interrupta* (Van Wyk *et al.*, 1976).

Two species of *Vizella* were recorded from *Proteaceae* in Australia, *V. banksiae* and *V. grevilleae*. Based on their similar morphological characteristics, the latter two species were reduced to synonymy under *V. interrupta* (Taylor & Crous, 1998j). The distinguishing features reported for *V. banksiae* were wider hyphae (which have subsequently been observed in *V. interrupta*), and the dimensions of the conidia of the anamorph, which overlap for both species (Van Wyk *et al*., 1976). *Vizella grevilleae* has been reported to have smaller asci, ascospores and conidia than *V. banksiae* and *V. interrupta* (Swart, 1975). However, asci measuring 42–58 × 18–21 µm and ascospores of 13–15 × 7.5–10 µm were recorded for *V. grevilleae* from a cultivated *Grevillea* sp. in Australia (DAR 55653), which overlap with the dimensions of *V. interrupta*. *Vizella banksiae* and *V. grevilleae* are therefore considered to be synonymous with *V. interrupta*. The generic position of the anamorph is uncertain but it is thought to have affinities with *Manginula* (Van Wyk *et al*., 1976). *Vizella interrupta* has not been isolated in culture.

Untreated taxa

Alternaria spp.

Symptoms: Leaf speck and spot.

Host range and geographic distribution: *Leucadendron, Leucospermum, Protea* spp. (**Australia, New Zealand, Portugal [Madeira], South Africa, USA [California, Hawaii], Zimbabwe**). (Anon., 1993; Von Broembsen, 1989; Masuka *et al*., 1998; Moura & Rodrigues, 2001; Taylor *et al*., 2001b).

Notes: Some of these species appear to be pathogenic to *Proteaceae*, and leaf spots associated with *Alternaria* have been regarded as serious in Hawaii, Madeira and Tenerife (Anon., 1993; Moura & Rodrigues, 2001). A serious outbreak of Alternaria leaf spot on *Leucadendron* 'Safari Sunset' was noted from Humansdorp in South Africa. The disease occurred in association with exceptionally warm, humid weather and heavy rain spells. Pathogenicity has, however, not yet been confirmed.

Anthostomella conorum (Fuckel) Sacc., *Sylloge Fungorum* **1**: 283 (1882).
[Reported as *Anthostomella spartii* Berl. & Voglino (Taylor *et al.,* 2001b)]

Symptoms: Associated with leaf spots at leaf margins and tips.

Causal organism: Treated by Lu & Hyde (2000).

Host range and geographic distribution: *P. eximia* (**USA, California**) (Taylor *et al.,* 2001b).

Notes: Pathogenicity unknown.

Ascochyta sp.

Symptoms: Leaf spot.

Host range and geographic distribution: *Lsp. reflexum* (**USA, Hawaii**) (Anon., 1991).

Notes: Pathogenicity unknown.

Bipolaris sp.

Symptoms: Leaf spot.

Host range and geographic distribution: *Lsp. cordifolium*, *Lsp. loliifolium*, *Lsp.* 'Hybrid 24', *Leucospermum* spp., *Leucadendron* 'Safari Sunset' (**USA, Hawaii**) (Anon., 1991).

Notes: Pathogenicity unknown.

Cladosporium spp.

Symptoms: Leaf spot.

Host range and geographic distribution: *Leucadendron, Leucospermum, Protea* spp. (**Madeira, South Africa, USA [California], Zimbabwe**) (Masuka *et al*., 1998; Moura & Rodrigues, 2001; Taylor *et al.,* 2001a).

Notes: Common, but not regarded as serious pathogens.

Coniothyrium spp.

Symptoms: Leaf spots.

Host range and geographic distribution: *Lsp. cordifolium, P. cynaroides* (**USA, California**) (Taylor *et al.,* 2001b).

Notes: Although several *Coniothyrium* spp. have been treated here, many more await description from *Proteaceae*. Generally they are associated with leaf spots.

Kabatiella sp.

Symptoms: Leaf spot.

Host range and geographic distribution: *P. cynaroides* (**USA, California**) (Taylor *et al.,* 2001b).

Notes: Pathogenicity unknown.

Leptosphaeria lonicerae (Fautrey) L. Holm, *Symbolae Botanicae Upsaliensis* **14**: 22 (1957).

Symptoms: Leaf spot.

Host range and geographic distribution: *P. magnifica, P. neriifolia* × *magnifica, Protea* sp. (**USA, California**) (Taylor *et al.,* 2001b).

Notes: Pathogenicity unknown.

Pestalosphaeria leucospermi Samuels, E. Müll. & Petrini, *Mycotaxon* **28**: 475 (1987).
Anamorph: ***Pestalotiopsis* sp.**

Symptoms: On living leaves.

Causal organism: Samuels *et al.* (1987).

Host range and geographic distribution: *Leucospermum* sp. (**New Zealand**) (Samuels *et al.,* 1987).

Notes: Pathogenicity unknown.

Pestalotiopsis guepinii (Desm.) Steyaert (as *guepini*), *Bulletin du Jardin Botanique de l'État Brussels* **19**(3): 312 (1949).
≡ *Pestalotia guepinii* Desm., *Annales des Sciences Naturelles, Botanique, Paris,* Sér. 2, **13**: 181 (1840).

Symptoms: Leaf spot.

Host range and geographic distribution: *Leucadendron, Leucospermum*, *Protea* spp. (**Portugal, Madeira**) (Moura & Rodrigues, 2001).

Notes: Pathogenicity unknown.

Pestalotiopsis sp.

Symptoms: Leaf spot.

Host range and geographic distribution: *Lsp. cordifolium, P.* 'Silver Mink' (**USA, California**) (Taylor *et al.,* 2001b); *Protea* sp. (**Zimbabwe**) (Masuka *et al*., 1998).

Notes: Pathogenicity unknown.

Phoma spp.

Symptoms: Leaf spot.

Host range and geographic distribution: *Lsp.* 'Helderfontein', *P.* 'Pink Mink' (**Portugal, Madeira**) (Moura & Rodrigues, 2001).

Notes: Pathogenicity unknown.

Phomopsis sp.

Symptoms: Leaf spot.

Host range and geographic distribution: *P. cynaroides* (**Portugal [Madeira], USA [California]**) (Moura & Rodrigues, 2001; Taylor *et al*., 2001b).

Notes: Pathogenicity unknown.

Pleospora spp. with ***Stemphylium*** anamorphs

Symptoms: Brown, circular leaf spots and tip blight.

Host range and geographic distribution: *Leucadendron, Leucospermum, Protea* spp. (**South Africa, Portugal [Madeira], Spain [Tenerife], USA [California]**) (Van Wyk, 1973a; Moura & Rodrigues, 2001; Taylor e*t al*., 2001a; unpubl. data).

Notes: Relatively common, and usually associated with tip blight symptoms (especially on *Leucospermum* and *Leucadendron* spp.). Several species are involved, but their taxonomy remains unclear.

Seimatosporium sp.

Symptoms: Leaf spot.

Host range and geographic distribution: *Leucospermum, Leucadendron, Protea* spp. (**South Africa, USA [California]**) (Taylor *et al*., 2001b; unpubl. data).

Notes: Several species occur in the *Seimatosporium*/*Seiridium* complex on *Proteaceae*. Their taxonomy, and status as pathogens, however, remains unresolved.

Seiridium cardinale (W.W. Wagener) B. Sutton & I.A.S. Gibson, *Bulletin of the Fan Memorial Institute of Biology, Botany* **33**: 326 (1972).
≡ *Coryneum cardinale* W.W. Wagener, *Journal of Agricultural Research* **58**: 8 (1939).

Symptoms: Leaf spot.

Causal organism: (Nag Raj, 1993).

Host range and geographic distribution: *Protea* spp. (**South Africa, USA [California]**) (Taylor *et al*., 2001b; unpubl. data).

Notes: Pathogenicity unknown.

Seridium sp.

Symptoms: Leaf spot.

Host range and geographic distribution: *Lsp. cordifolium* (**USA, California**) (Taylor *et al*., 2001b).

Notes: Pathogenicity unknown.

Truncatella angustata (Pers.) S. Hughes, *Canadian Journal of Botany* **36**: 822 (1958).

Symptoms: Leaf spot.

Host range and geographic distribution: *Lsp. cordifolium* (**USA, California**) (Taylor *et al*., 2001b).

Notes: Pathogenicity unknown.

Zetiasplozna cf. ***cordylines*** (Speg.) Nag Raj, in Nag Raj, *Coelomycetous Anamorphs with Appendage-bearing Conidia* (Ontario): 998 (1993).

Symptoms: Leaf spot.

Host range and geographic distribution: *Lsp. cordifolium* (**USA, California**) (Taylor *et al*., 2001b).

Notes: Pathogenicity unknown.

CHAPTER 2

STEM, SHOOT AND FLOWER DISEASES

Cankers and shoot diebacks are common problems on cultivated *Proteaceae*. Stem cankers are typically sunken and darker in colour than the surrounding tissue and a distinct margin generally can be seen at the margin of the healthy and diseased tissue. The underlying woody tissues are discoloured and mycelium of the pathogen can asymptomatically spread some distance beyond the visible lesion. The disease can develop slowly or very rapidly, depending on the physiological condition of the host, and sometimes kills the entire plant. Shoot diebacks are commonly associated with pruning wounds or other damage, and can in severe cases lead to the loss of complete branches or plants. Flower diseases, however, occur mostly under moist, humid conditions, and are encountered in the field, or as post-harvest diseases.

Fungi enter through wounds, leaf scars or lesions caused by other stem pests or pathogens. Wet conditions and wind are favourable for the spread of these fungi, but they also can be spread by contaminated pruning and harvesting tools, in seeds, in soil or plant debris, or by insects (Von Broembsen, 1989; Forsberg, 1993).

Botrytis flower-head blight

Alternative common name: Grey mould

Botrytis is an important pathogen of flowers of *Proteaceae* for several reasons. The inflorescence is susceptible at all stages. Following the death of the inflorescence, the pathogen can move down into the shoot tip and into the leaves. On proteas, *Botrytis* can invade actively-growing tissues and inflorescences that are not yet in the senescent stage (Rohrbach, 1984).

Botrytis is a widespread pathogen that can survive as a saprotroph in debris in and on top of soil. For this reason, *Botrytis* frequently causes seedling damping-off (Forsberg, 1993). It also has been recorded on cuttings showing dieback symptoms, as well as on blighted seedlings of various *Proteaceae* (Benic, 1986; Knox-Davies *et al.*, 1986). *Botrytis* is often associated with decayed rooted cuttings, but it is not known if it is an opportunistic coloniser or true pathogen (Knox-Davies *et al.*, 1987).

Botrytis cinerea Pers. : Fr., *Dispositio Methodica Fungorum. Römers Neues Magazin der Botanik* **1**: 40 (1797). Figs 383–385, Plate 16.

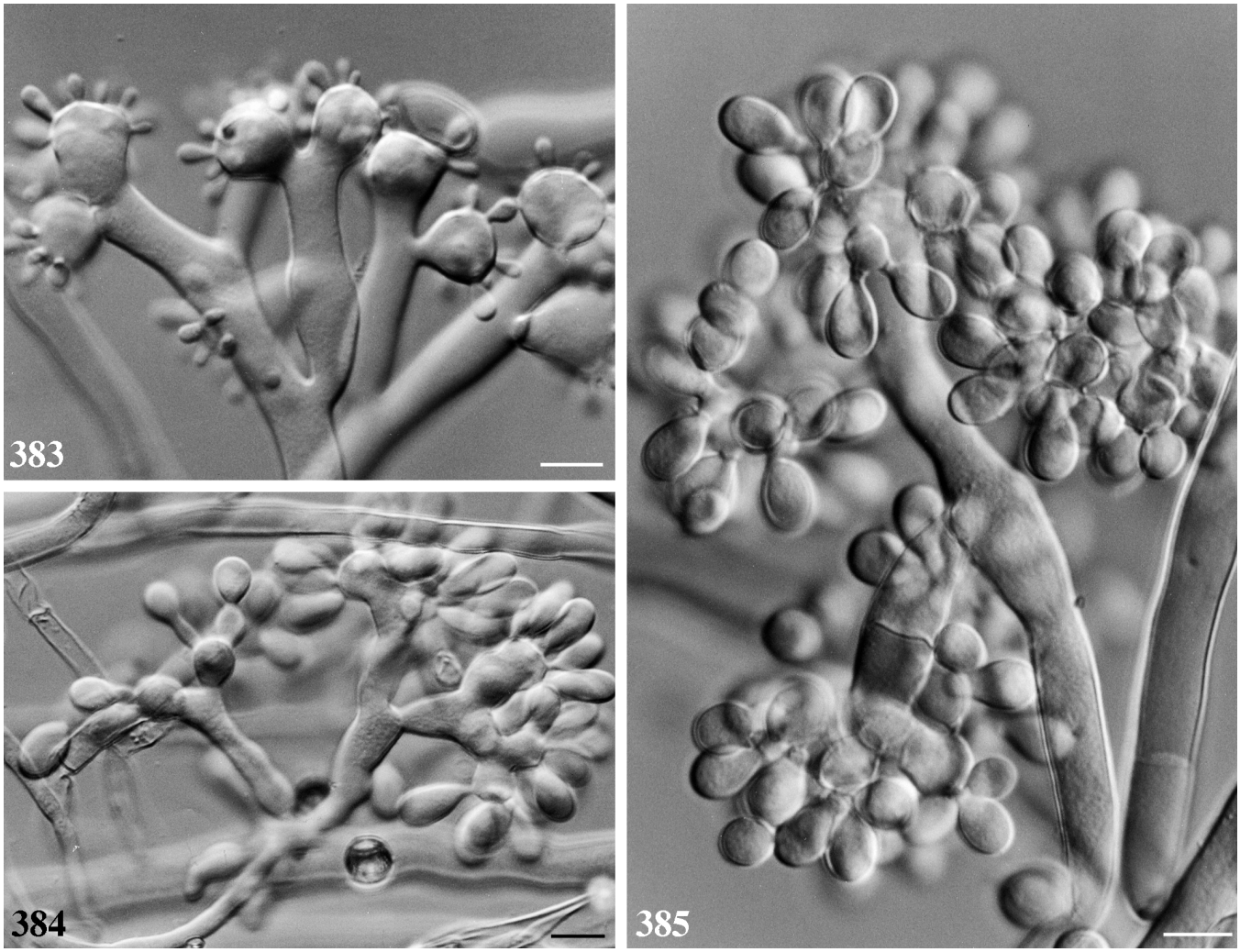
Symptoms: The pathogen causes blight of the flowering branches and flower heads and the symptoms resemble those of Drechslera blight (Forsberg, 1993), with brown spots developing on leaves, flowers and buds. On flowers, symptoms initially occur on the styles and perianths as small, water-soaked lesions, which normally advance into the flower head (Cho, 1977). The lesions expand, often causing the flower heads to become necrotic. The necrosis may extend down the flower head stalks, causing death of affected parts and new shoots (Serfontein & Knox-Davies, 1990b; Forsberg, 1993; Cho, 1977). Infected shoot tips collapse, darken and die. Bending of affected shoots is more typical of Botrytis blight than Drechslera blight. Felty masses of grey-brown spores are seen on affected tissue under wet conditions, thus 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 (Cho, 1977).

Causal organism: *Sclerotia* black, consisting of a white medulla and an outer layer of dark brown to black cortical cells, extremely variable in shape and size. *Conidiophores* frequently up to 2 mm or more in length, 16–30 µm wide, consisting of a stipe with an apical open head of branches, smooth, medium brown below, paler near the apex, with the ends of the branches often hyaline to pale brown. *Conidia* ellipsoidal or obovoid, often with a somewhat protuberant hilum, hyaline to pale brown, smooth, 6–14(–18) × (4–)6–9(–11) µm. *Microconidia* produced on sporodochia (spermodochia), phialides flask-shaped, conidia globose, 2.5–3 µm diam (Ellis, 1971; Samson *et al*., 2000).

Cultural characteristics: Colonies hyaline, becoming grey to grey brown, black sclerotia often forming; 6 cm diam at 20°C after 10 days. Cardinal temperatures for growth are: optimum (21–)22–25(–30)°C, minimum (–2)5–12°C, maximum (28–)33–35°C (Samson *et al*., 2000).

Host range and distribution: *Leucadendron* spp., *Leucospermum* spp., *Serruria florida* (**Australia**) (Forsberg, 1993); *Lcd. discolor, Lcd.* 'Jubilee Crown', *Lsp. cordifolium, Lsp.* 'High Gold', *P. cynaroides, P. repens* (**South Africa, EC, SC, WC**) (Knox-Davies *et al*., 1986; Serfontein & Knox-Davies, 1990b; Denman, 1999); *Lsp. cordifolium, Lsp. cordifolium* 'Pink Star', *Lsp.* 'Hybrid 24', *Leucospermum* sp., *P. cynaroides, P. repens* (**U.S.A., Hawaii**) (Cho, 1977; Greenhalgh, 1981; Anon., 1991); *Lsp.* 'Succession', *Lsp.* 'Luteum' (**Zimbabwe**).

Disease cycle and epidemiology: In Hawaii, the disease is most severe during the winter months, but has been recorded in late spring and early summer (Cho, 1977). Spores spread by wind and water-splash. The disease can rapidly develop under prolonged wet, cool weather (Forsberg, 1993). Infection is favoured by cool, moist conditions, and frequently starts in susceptible tissues of blossoms or else at wound sites. Serfontein & Knox-



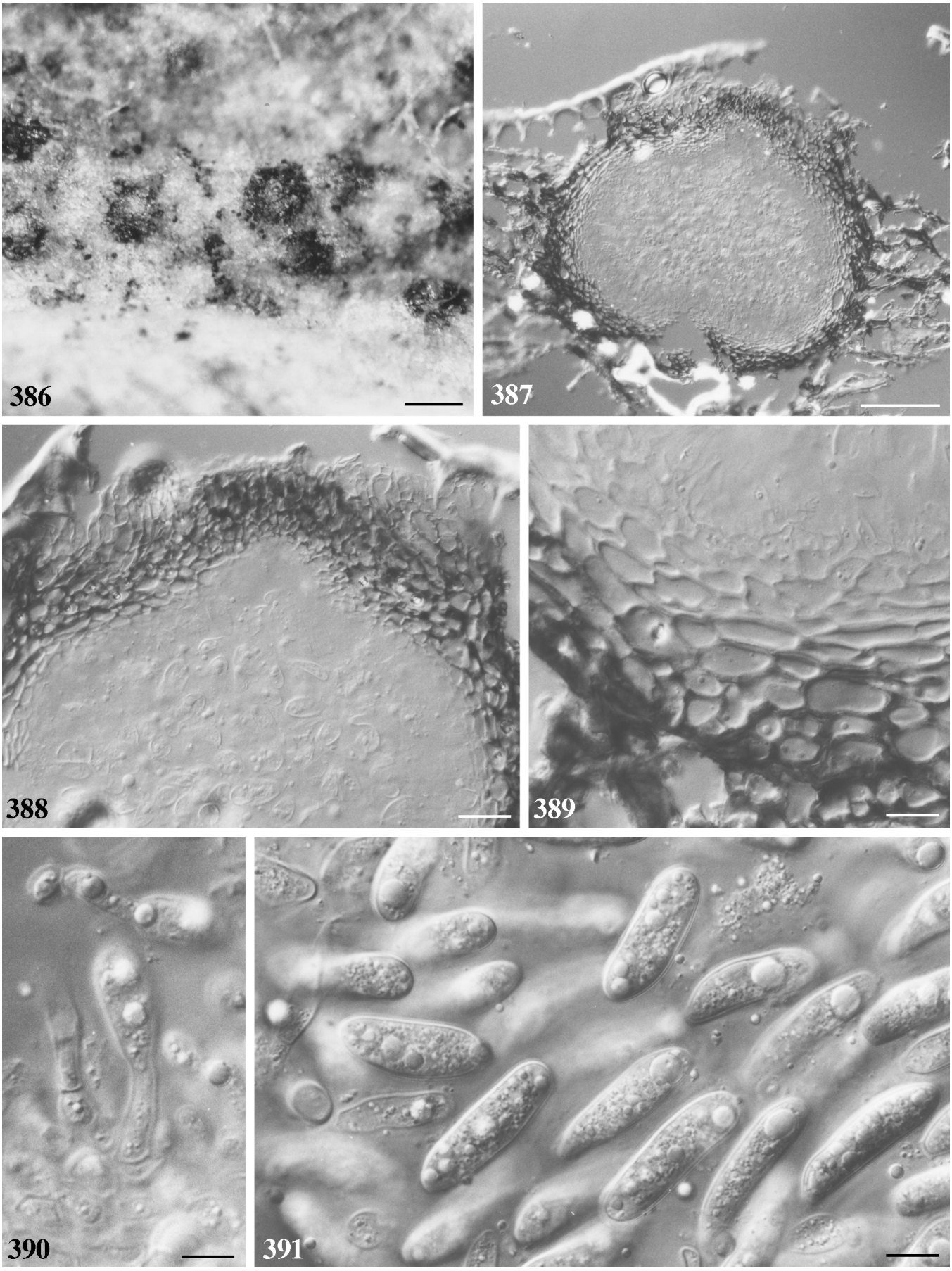
Figs 383–385. Conidiophores, conidiogenous cells and conidia of *Botrytis cinerea*. Scale bars = 10 µm. (From R.A. Samson, unpubl.).

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.

Control: Diseases caused by *Botrytis* can be controlled with benomyl and iprodione sprays (Greenhalgh, 1981). Flowers and young shoots of *Proteaceae* can be protected during moist, cool to moderate weather with two to three weekly sprays of iprodione (Von Broembsen, 1989). A combination of spraying plants at three-week intervals with a mixture of benomyl and iprodione, alternated with procymidone and iprodione, has been found effective (Serfontein & Knox-Davies, 1990b). In all cases blighted flower heads should be removed prior to spraying. When starting new plants, cuttings must be taken only from disease-free mother plants (Benic, 1986). Pre-planting dips of cuttings and spraying with difolatan, captab or benomyl helps to control cutting dieback and blight in mistbeds.

Systemic fungicides are necessary to stop infections once they have already established. There are no systemic formulations specifically registered for *Botrytis* on proteas or ornamentals in South Africa. However, a number of systemic formulations have been registered against *Botrytis* on grapes in South Africa such as benomyl (Benlate), folpet (Folpan or Folpet), iprodione (Rovral) and tebuconazole (Folicur) (Nel *et al*., 1999)]. These fungicides also are registered on ornamentals, although not as post-harvest treatments. It is therefore not known whether there will be a phytotoxic reaction on flowers under conditions of cold storage if either of these fungicides if any of these fungicides are used. Benomyl is destined to be removed from the market in the USA soon, and so growers are advised not to develop a reliance on this chemical. Although iprodione is classified as a contact fungicide it does show a degree of systemic activity. This fungicide has been recommended as a field application by Von Broembsen (1989), Greenhalgh (1981) and Serfontein & Knox Davies (1990b).

Prophylactic applications of contact fungicides to developing buds in the field could help reduce the incidence of bud infection and post-harvest decay. The contact fungicides registered in South Africa on ornamentals include chlorothalonil (Bravo), folpet (Folpan or Folpet) and mancozeb (Dithane, Mancozeb, Sancozeb or Tridex). The only fungicide registered against *Botrytis* on ornamentals in South Africa is chlorothalonil. Folpet is commonly used in the grape industry against *Botrytis*.



Figs 386–391. *Fusicoccum* sp. on *Protea*. Fig. 386. Conidiomata on host substrate. Fig. 387. Vertical section through a pycnidium. Fig. 388. Ostiolar area of a pycnidium. Fig. 389. Pycnidial wall layer. Fig. 390. Conidiogenous cell giving rise to a conidium. Fig. 391. Conidia. Scale bars: 386 = 200 µm, 387 = 50 µm, 388 = 20 µm, 389 = 10 µm, 390–391 = 6 µm.

Botrytis can develop resistance to certain fungicides, particularly to benomyl. If these fungicides are combined or alternated with contact fungicides such as mancozeb or chlorothalonil, the chances of resistant strains developing should be reduced (Cho, 1977).

Sanitation pruning should be implemented to remove diseased plant parts (Von Broembsen, 1989). Crops should be kept well-ventilated to reduce humidity.

When conditions favourable to disease development prevail (wet or high relative humidity and temperatures ranging between 15–25°C), farmers should pick flowers when the blooms are completely dry, or allow blooms to dry before packing. Jacobs (1981) recommended dipping *Lsp. cordifolium* flower heads in benomyl and dichloran (the latter prevents rot from *Rhizopus,* thus blocking an easy entry point for *Botrytis* infections) and drying them thoroughly before packing.

Notes: Botrytis disease occurs on the flower heads and young shoot tips of *Banksia, Leucadendron, Leucospermum, Protea* and *Serruria* 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 also likely to develop Botrytis rot. 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 regarded as a serious problem in Australia, but it could become one in coastal plantings (Forsberg, 1993). Serfontein & Knox-Davies (1990b) reported the pathogen as the cause of flowering branch and flower head blight in the southwestern Cape Province. *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, 1984).

Botryosphaeria stem cankers

Cankers, dieback and blight of mature and seedling *Proteaceae* caused by *Botryosphaeria* spp. are amongst the most important diseases of cultivated *Proteaceae* in South Africa (Figs 386–391, Plates 14, 15).

In earlier papers, Botryosphaeria canker has mainly been attributed to either *B. ribis* or *B. dothidea* (Crous *et al*., 2000a). Recent studies have shown, however, that species such as *B. protearum*, *B. lutea*, *B. obtusa* and several species of *Diplodia* and *Fusicoccum* are also involved (Denman *et al*., 2003). For this reason earlier reports of *B. ribis* or *B. dothidea* should be considered "*Botryosphaeria* sp." and the specimens on which those reports were based should be re-examined.

Botryosphaeria australis Slippers, Crous & M.J. Wingf., *Mycologia*: in press. Figs 392–393.
Anamorph. ***Fusicoccum australe*** Slippers, Crous & M.J. Wingf., *Mycologia*: in press.

Symptoms: Associated with stem cankers and dieback, though its pathogenicity to proteas remains unconfirmed.

Causal organism: *Ascostromata* erumpent through the bark, 1.2 mm diam. *Ascomata* pseudothecial, forming botryose aggregates of 2–10, sometimes solitary; globose with a central ostiole, papillate or not, embedded with only the papilla emerging, black, 100–300 µm diam; pseudothecial wall comprising 5–8 layers of *textura angularis*, outer region of dark brown or brown cells, inner region of 3–6 layers of hyaline cells lining the locules. *Asci* bitunicate, clavate, 60–125 × 16–25 µm, 8-spored, separated by numerous filiform, septate pseudoparaphyses, which are rarely branched towards the tips, and 3–4 µm wide. *Ascospores* fusoid to ovoid, unicellular, hyaline, smooth with granular contents, biseriate in the ascus, 20–23(–25) × 7–8(–9) µm.

Conidiomata (formed on water agar on sterilised twigs) pycnidial, superficial, globose, mostly solitary, covered by mycelium. *Conidiogenous cells* holoblastic, hyaline, subcylindrical, 10–14 × 2–3 µm, phialidic with periclinal thickening, or proliferating percurrently with 1–4 proliferations. *Conidia* fusiform, with subtruncate to bluntly rounded base, (18–)23–26(–30) × 5–6(–7.5) µm, hyaline, unicellular, rarely forming a septum before germination, smooth with granular contents. *Spermatia* not observed.

Cultural characteristics: *Colonies* buff (19''f) to light primrose (23''b), light yellowish pigment most noticeable when grown between 15–20°C in the dark, becoming olivaceous-buff (21'''d) to olivaceous-grey (21'''''i) after 5–6 days, with a sparse to moderately dense, appressed mycelial mat in the centre with sparse tufts of aerial mycelium around the edges, margin smooth; 48 mm diam on PDA after 4 days at 25°C in the dark. Optimum temperature for growth 25°C.

Host range and distribution: *Acacia* spp., *Banksia* spp., *Eucalyptus* spp. (**Australia**); *P. cynaroides* (**South Africa, WC**) (Slippers *et al*., 2004).

Notes: *Botryosphaeria australis* appears to be native to the Southern Hemisphere. The fungus is common on *Acacia* and *Eucalyptus* in Australia, and has also been recorded from *P. cynaroides* in South Africa. The fungus has apparently also been introduced into Europe, as evidenced by an Italian collection on pistachio (Slippers *et al*., 2004). The distribution of *B. australis* on *Proteaceae* in South Africa and elsewhere needs further elucidation, as does its significance as a pathogen.

Botryosphaeria dothidea (Moug. : Fr.) Ces. & De Not., *Commentario della Società Crittogamologica Italiana* **1**: 212 (1863). Fig. 398.
≡ *Sphaeria dothidea* Moug., *Systematic Mycology* **2**: 423 (1823).

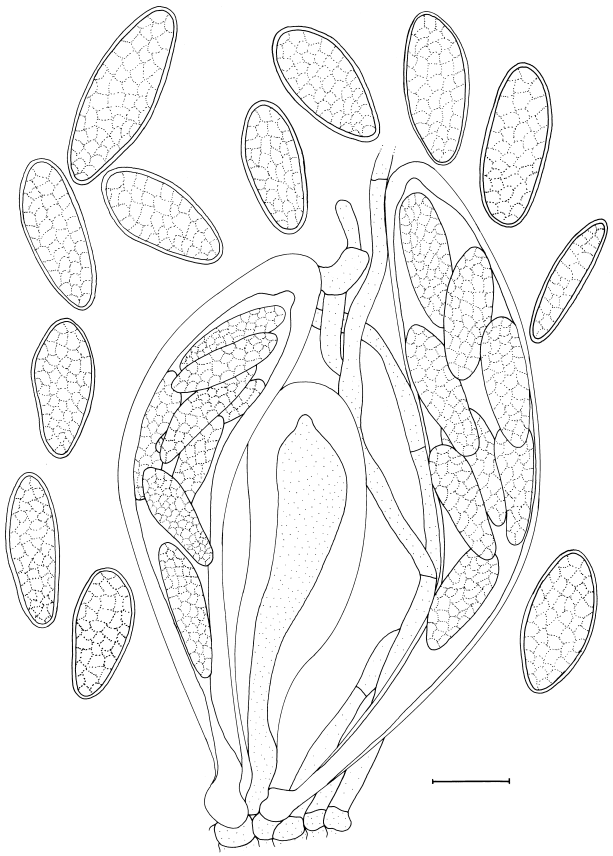


Fig. 392. Asci, ascospores and pseudoparaphyses of *Botryosphaeria australis*. Scale bar = 10 µm. (From Slippers *et al*., 2004).

= *Botryosphaeria berengeriana* De Not., *Sferiacei Italici* (1863).

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

Symptoms: Cankers dark brown-black and sunken (Von Broembsen, 1986). In protea nurseries, Benic (1986) observed disease symptoms to vary from seedling dieback or blight to stem cankers. Basal and tip dieback and leaf necrosis were typical symptoms encountered in mistbeds.

Causal organism: *Ascostromata* erumpent through the bark, 200–500 µm diam. *Ascomata* pseudothecial, botryose aggregates of up to 100, sometimes singular, globose with central ostiole, partly emergent, rarely embedded, papillate or not, brown to black; pseudothecial wall 5–15 layers of *textura angularis*, dark brown or brown outer layers, 2–4 layers of hyaline cells lining the cavity. *Asci* bitunicate, 8-spored, clavate, 63–125 × 16–20 µm, interspersed with numerous filiform, septate, pseudoparaphyses, 2–4 µm wide. *Ascospores* unicellular, hyaline, granular, smooth surface, fusoid to ovoid, often narrower at the ends (17–)19–24(–32) × (6–)7–8(–10) µm.

Conidiomata in vivo pycnidial, morphologically indistinguishable from the ascomata. *Conidiogenous cells* holoblastic, hyaline, subcylindrical, 6–20 × 2–5 µm, proliferating percurrently once or twice. *Conidia* unicellular, rarely forming a septum before germination, narrowly

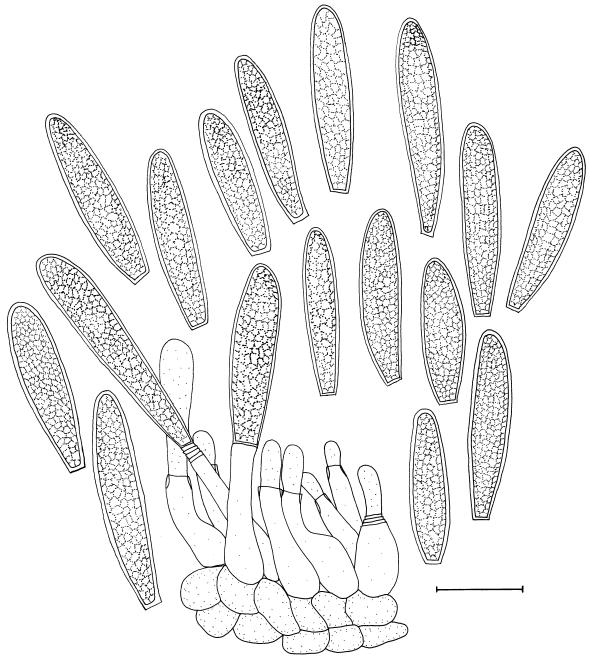


Fig. 393. Conidia and conidiogenous cells of *Fusicoccum australe*, the anamorph of *Botryosphaeria australis*. Scale bar = 10 µm. (From Slippers *et al*., 2004).

fusiform, or irregularly fusiform, with subtruncate to bluntly rounded base, granular, hyaline, smooth surface, (17–)18–20(–22) × 4–5 µm. *Spermatiophores* hyaline, cylindrical to subcylindrical, 4–10 × 1–2 µm. *Spermatia* unicellular, hyaline, allantoid to rod-shaped, 3–6 × 1.5–2 µm (Slippers *et al*., 2004).

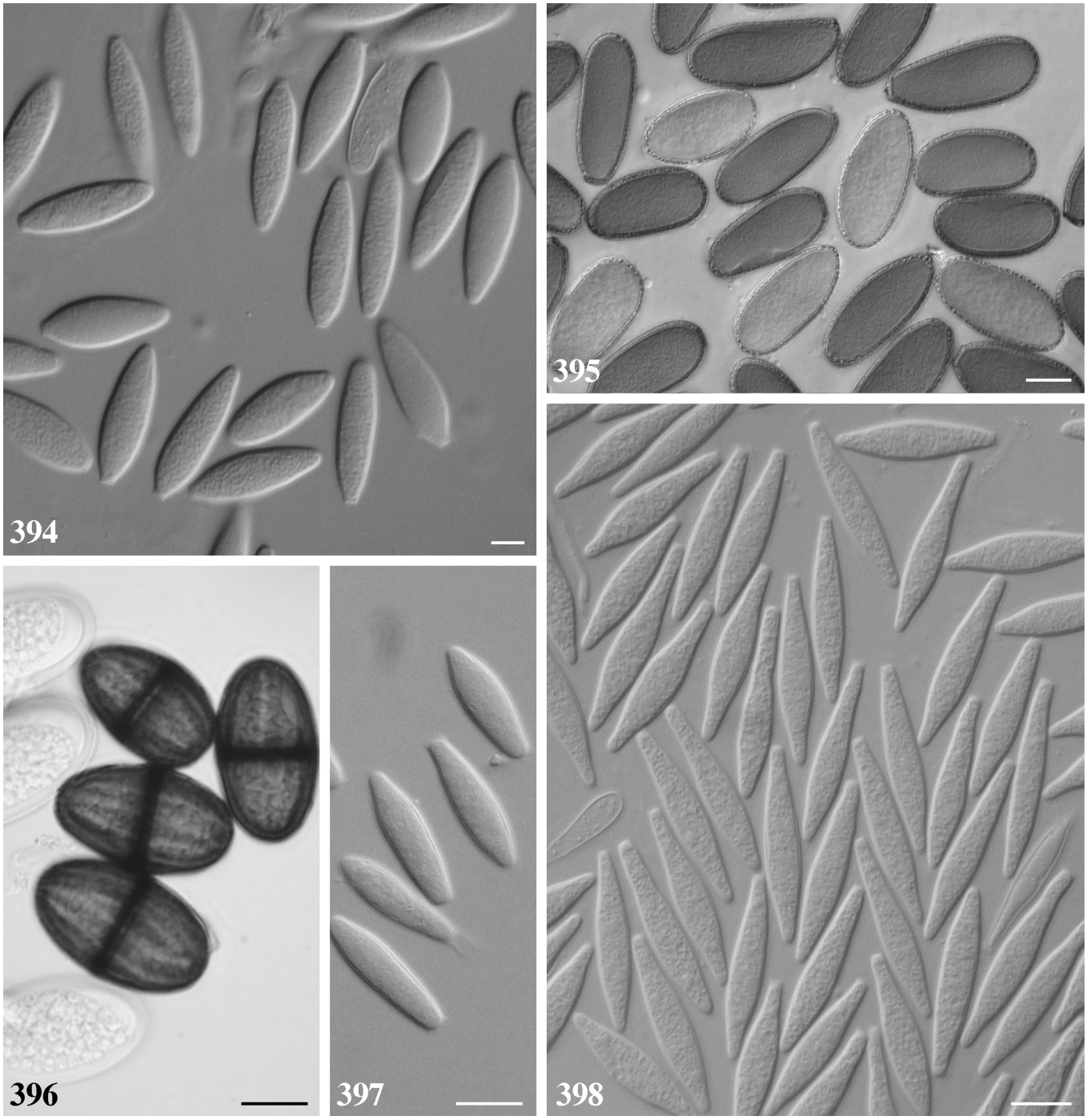
Conidiomata formed *in vitro* on water agar on sterilised twigs of *Malus* sp., *Eucalyptus* sp., *Populus* sp., or needles of *Pinus* sp. within 7–14 days, pycnidial, superficial, globose, mostly solitary and covered in mycelium. *Conidia* longer and more narrowly fusiform than those formed *in vivo*, with particularly narrow ends, (20–)23–27(–30) × 4–5(–6) µm (Slippers *et al*., 2004).

Cultural characteristics: *Colonies* olivaceous-buff (21’’’d), becoming olivaceous-grey (21’’’’’i) to violaceous-black (65’’’’k), with a sparse to moderately dense, appressed mycelial mat, having occasional columns of aerial mycelium reaching the lid, and a smooth margin that appears crenulate as the colonies become darker with age; 12.6 mm/day at 25(–30)°C (Slippers *et al*., 2004).

Host range and distribution: *Lcd. argenteum, Lcd. laureolum, Lcd. spissifolium, Lsp. cordifolium, Lsp.* ‘Flame Spike’, *Lsp.* ‘Succession’, *P. compacta, P. cynaroides, P. eximia, P. grandiceps, P. repens* (**South Africa, EC, SC, WC**) (Oliver, 1951; Van Wyk, 1973a, b; Knox-Davies *et al*., 1981; Benic & Knox-Davies, 1983a; Benic, 1986; Von Broembsen, 1986), *P. longifolia* var. *minor* (as *P. minor*)*, P. repens* (**South Africa, GT**) (Swart, 1999); *Lcd.* ‘Silvan Red’, *Lcd. discolor, P.*

cynaroides, P. 'Silver Mink', *P. eximia, P. laurifolia, P. magnifica, P. obtusifolia*, *Protea* sp. (**U.S.A., California**) (Taylor *et al*., 2001b); *Grevillea wilsonii, Lcd.* 'Safari Sunset', *Lcd. laureolum, Leucadendron* sp., *Lsp.* 'Hybrid 31', *Lsp.* 'Sunset', *Lsp.* 'Yellow Bird', *Leucospermum* sp., *P.* 'Carnival', *P. compacta*, *P.* 'Lady Di', *P.* 'Pink Ice', *P. cynaroides, Telopea* sp. (**U.S.A., Hawaii**) (Anon., 1991; Taylor *et al*., 2001b); *P. repens* (**Zimbabwe**) (Masuka *et al*., 1998).

Disease cycle and epidemiology: On apples, *B. dothidea* predominates in summer and fall in the Northern Hemisphere (Pusey, 1989). Warm, wet weather favours the release and germination of conidia (Weaver, 1979; Pusey, 1989). White spore masses exude from the pycnidia after rainy periods (Crist & Schoeneweiss, 1975). Optimum sporulation occurs at 25–30°C with 100% relative humidity (Eid & Heuberger, 1959). Ascospores are most abundantly produced during the spring (Pusey, 1989) and summer (Sutton, 1981). Rainfall triggers ascospore discharge, which are then air- and waterborne. Rainsplash is the most important mechanism for conidial dispersal. Therefore conidia may be more important in intra-tree spread, while air-



Figs 394–398. Conidia of *Botryosphaeria* anamorphs. Fig. 394. *Fusicoccum ribis* anamorph of *B. ribis*. Fig. 395. *Diplodia* anamorph of *B. obtusa*. Fig. 396. *Lasiodiplodia theobromae* anamorph of *B. rhodina*. Fig. 397. *Fusicoccum luteum* anamorph of *B. lutea*. Fig. 398. *Fusicoccum aesculi* anamorph of *B. dothidea*. Scale bars = 10 µm. (From A.J.L. Phillips & B. Slippers, unpubl.).

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 have been diseased for several years. Because spore release and infection by *B. dothidea* on peaches 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 & Van der Merwe (1990) on *P. grandiceps* in South Africa, indicated that *B. dothidea* sporulates from spring to late summer following rain and with average daily temperatures above 20°C . Sporulation is negligible during the winter months despite abundant rainfall. Wounding is necessary for infection, and infection is greatest at 25–30°C (Von Broembsen & Van der Merwe, 1990).

On almonds, the mycelium of the fungus is found in the cell lumina (largely intracellular) in both xylem and phloem, and grows from cell to cell through pits. Some branching into adjacent parenchyma cells is evident (English *et al.*, 1975; Wene, 1979). On almonds, once the fungus invades the vascular tissue, the mycelium moves rapidly down the stem (English *et al*., 1975). On blueberries, frequently, only one side of an affected branch shows the brown discoloration (Milholland, 1972). Milholland (1972) and Wene (1979) found strong formation of tyloses in the xylem vessels of infected stems, while protrusions were also observed in the xylem, which led to complete occlusion of the vessels.

On other woody hosts, the hyphae in water-stressed plants occur more often in the youngest xylem vessels, but during colonisation of seedlings subjected to freezing stress, hyphae occur mainly in the older xylem (Wene, 1979). 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 the xylem some distance beyond the bark canker margin (English *et al.*, 1975; Wene, 1979). In almonds, Botryosphaeria cankers 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.

Notes: Widespread and destructive necrotic lesions associated with leaf spots caused by *Coleroa senniana* on *P. magnifica* actually are caused by secondary invasion of the Coleroa leaf spots by *Fusicoccum aesculi* (Serfontein & Knox-Davies, 1990a). *F. aesculi* might gain entry when the *C. senniana* pseudothecia burst through the cuticle, or perhaps is present as an endophyte or latent infection and is somehow activated to cause disease.

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. *B. dothidea* was viewed as an opportunistic pathogen that infects trees only under unfavourable conditions. In contrast to these findings, Smith *et al.* (1996) showed that the fungus exists as a symptomless endophyte in healthy eucalypt trees.

Some records of this pathogen on *Proteaceae*, were made prior to 2000. Denman *et al.* (2000b) pointed out the taxonomic uncertainty of *Botryosphaeria* species previously identified on proteas. Thus, these records must be treated with circumspection. The epidemiology data presented here are derived from work carried out on almonds, apples and peaches in the Northern Hemisphere and may not be strictly applicable to *Proteaceae*.

Botryosphaeria lutea A.J.L. Phillips, *Sydowia* **54**: 59 (2002). Fig. 397.

Anamorph: ***Fusicoccum luteum*** Pennycook & Samuels, *Mycotaxon* **24**: 456 (1985).

Symptoms: Associated with black, sunken, elongate stem cankers. Also isolated from leaves with tip blight symptoms.

Causal organism: *Ascostromata* initially immersed, later becoming erumpent through the host tissue, black, < 0.5 mm diam, uni- or multilocular, locules spherical to ovoid, 150–200 µm diam. *Ascomata* with a short neck, opening through a non-periphysate ostiole, wall consisting of 8–12 layers of dark brown to black, thick-walled cells of pseudoparenchymatous *textura angularis*, up to 60 µm thick, with 3–4 layers of thin-walled, hyaline cells lining the cavity. *Asci* bitunicate, cylindrical, to clavate, stipitate, 84–176 × 16–24 µm, 8-spored. *Pseudoparaphyses* hyaline, septate, branched, 2–3.5 µm wide. *Ascospores* irregularly biseriate, hyaline, guttulate, smooth, aseptate, oval to broadly fusiform, widest in the middle or upper third of the ascospore, tapering to the obtuse base and apex 18–22.5(–24) × 7.5–12 µm.

Conidiomata eustromatic, separate or confluent, dark brown to black, uni- or multilocular, immersed in the host, sub-peridermal, locules up to 150 µm diam, walls consisting of a dark brown *textura angularis*, becoming smaller, thinner-walled and hyaline towards the conidiogenous region. *Ostioles* papillate, circular. *Conidiomata* frequently form in the same stromata as the ascomata. *Conidiophores* hyaline, smooth, thin-walled, rarely branched at the base, cylindrical, formed from the cells of the inner locule wall, 8–19 × 3–4 µm. *Conidiogenous cells* discrete, integrated, hyaline, smooth, cylindrical, producing the first conidium holoblastically and subsequent conidia enteroblastically, proliferating percurrently with 2–3 indistinct annellations, or determinate with periclinal thickening, (6–)8–16(–18) × (2.5–)3–4(–4.5) µm. *Conidia* hyaline, thin-walled, aseptate, smooth, fusiform, widest in the middle or upper third of the conidium, with a subobtuse apex, and a

truncate base, (12–)16.5–22.5(–24) × 4.5–6(–7.5) µm, often with a minute basal frill (Phillips *et al*., 2002).

Cultural characteristics: *Colonies* on PDA initially pale to colourless, gradually darkening with age, becoming grey to dark grey; producing a distinctive yellow pigment that diffuses into the agar ahead of the leading edge of the colony, colour most intense after 3 d at 25°C, thereafter it becomes violaceous and by 6–7 d no more yellow colour is visible (Phillips *et al*., 2002). Similar to *B. dothidea* but differing in anamorphic characters formed in culture, of which the formation of a yellow pigment is the most distinctive.

Host range and distribution: *Banksia* sp*., Buckinghamia* sp. (**Australia**); *P. cynaroides* (**South Africa, WC**) (Denman *et al*., 2003).

Botryosphaeria obtusa (Schwein.) Shoemaker, *Canadian Journal of Botany* **42**: 1298 (1964) Fig. 395.
≡ *Sphaeria obtusa* Schwein., *Transactions of the American Philosophical Society, New Series* **4**(2): 220 (1832).
≡ *Physalospora obtusa* (Schwein.) Cooke, *Grevillea* **20**: 86 (1892).
= *Physalospora corni* Ellis & Everh., *Sylloge Fungorum* **11**: xxvii (1878), non Sacc., *nom. illeg*.
= *Physalospora everhartii* Sacc. & P. Syd., *Sylloge Fungorum* **14**: 521 (1899).
Anamorph: ***Diplodia*** sp.

Symptoms: The fungus was isolated as an endophyte, and thus its relative importance as a pathogen of *Proteaceae* remains uncertain. However, this species causes serious stem cankers on fruit trees and grapevines growing in the vicinity of proteas in South Africa, and should be regarded as a potentially serious pathogen.

Causal organism: *Ascomata* subepidermal, up to 3 mm diam, scattered, solitary, stromatic, dark brown to black, with ostioles darker around the neck region. *Asci* 8-spored, bitunicate, clavate, interspersed amongst filiform, septate paraphyses, 90–120 × 17–23 µm. *Ascospores* irregularly biseriate, hyaline, unicellular, sometimes becoming 1-septate with age, 25–33 × 7–12 µm.

Conidiomata pycnidial, immersed, separate or aggregated, dark brown, unilocular, thick- or thin-walled, wall formed of dark brown thick-walled *textura angularis*; ostioles central, single, papillate. *Conidiogenous cells* holoblastic, discrete, determinate or indeterminate, with one or two percurrent proliferations, long lageniform, hyaline, smooth, formed from cells lining the inner wall of the pycnidium. *Conidia* oblong, straight, aseptate, broadly rounded at apex, truncate at the base, wall brown, internally ornamented, (16–)18–26(–27) × (9–)10–15 µm. Both hyaline and dark-walled conidia can be seen in the same pycnidium (Punithalingham & Waller, 1973; Phillips, 2002).

Cultural characteristics: *Colonies* on PDA grey-brown with dense aerial mycelium; conidiomata start to form on OA after 4–5 days at 23°C; conidia exuded in 7–14-day-old cultures.

Host range and distribution: *P. magnifica, P. longifolia, P. punctata* (**South Africa, WC**) (Knox-Davies *et al.,* 1981; Denman *et al*., 2003).

Botryosphaeria protearum Denman & Crous
Anamorph: ***Fusicoccum protearum*** Denman & Crous

See Section 3, Chapter 1, Botryosphaeria leaf blight and stem canker.

Botryosphaeria rhodina (Berk. & M.A. Curtis) Arx, *Genera of Fungi Sporulating in Pure Culture* (Lehr): 143 (1970). Fig. 396.
≡ *Physalospora rhodina* Berk. & M.A. Curtis, *Grevillea* **17**: 92 (1889).
Anamorph: ***Lasiodiplodia theobromae*** (Pat.) Griffiths & Maubl., *Bulletin de la Société Mycologique de France* **25**: 57 (1909).
≡ *Botryodiplodia theobromae* Pat., *Bulletin de la Société Mycologique de France* **8**: 136 (1892).

Symptoms: Commonly associated with dieback and stem cankers of numerous hosts and basal stem cankers of *Leucospermum* spp. (S. Denman, unpubl. data).

Causal organism: *Ascomata* on stems, subepidermal, 250–300 µm diam, scattered to gregarious, stromatic, dark brown to black. *Asci* 8-spored, bitunicate, clavate, interspersed amongst filiform, septate paraphyses, 90–120 × 15–28 µm. *Ascospores* irregularly biseriate, hyaline, unicellular, 24–42 × 7–17 µm.

Conidiomata pycnidial, immersed, separate or compound, dark brown, thick-walled, wall formed of dark brown, thick-walled *textura angularis*; ostioles central, single, papillate. *Conidiogenous cells* holoblastic, discrete, determinate or indeterminate, with one or several percurrent proliferations, cylindrical to obpyriform, hyaline, smooth, formed from cells lining the inner wall of the pycnidium. *Conidia* initially hyaline, 1-celled, granulose, thick-walled, subovoid to ellipsoid-oblong with a truncate base, mature conidia dark brown, 1-septate, longitudinally striate, 18–30 × 10–18 µm. *Paraphyses* hyaline when present, cylindrical, sometimes septate, up to 50 µm long (Sivanesan, 1984).

Host range and distribution: *Protea* spp. (**Australia**) (Forsberg, 1993); *Protea* spp. (**Madeira**) (Moura & Rodrigues, 2001); *Banksia dryandroides*, *Leucospermum* sp., *P. cynaroides*, *Telopea* sp. (**U.S.A., Hawaii**) (Taylor *et al*., 2001b).

Botryosphaeria ribis (Tode : Fr.) Grossenb. & Duggar, *New York Agricultural Experiment Station Technical Bulletin* **18**: 128 (1911). Fig. 394.
Anamorph: ***Fusicoccum ribis*** Slippers, Crous & M.J. Wingf., *Mycologia*: in press.

Symptoms: Associated with cankers that usually originate at pruning wounds and move down the branch, causing a black, sunken lesion, frequently only on one side of the branch, but completely girdling thinner branches; eventually causing branch and plant death.

Causal organism: *Ascostromata* erumpent through the bark, pulvinate, 100–400 µm diam. *Ascomata* pseudothecial, botryose aggregates of 5–50, globose with central ostiole, papillate or not, brown to black, 175–250 µm, pseudothecial wall 5–15 layers of *textura angularis*, with dark brown or brown outer layers, and 2–4 layers of hyaline cell layers lining the cavity. *Asci* bitunicate, 8-spored, clavate, 80–120 × 17–20 µm, between numerous filiform, septate, pseudoparaphyses, rarely branched towards the top, 2–4 µm wide. *Ascospores* unicellular, hyaline, granular, with smooth surface, fusoid to ellipsoid, often rounded at the ends, giving a broadly ellipsoid shape, (14–)18–23(–27) × 6–8(–10) µm.

Conidiomata pycnidial, morphologically indistinguishable from the ascomata, formed in the same stromata, or singular and embedded in young host shoots. *Conidiogenous cells* hyaline, subcylindrical, 6–22 × 2–5 µm, proliferating percurrently with 1–2 annellations. *Conidia* unicellular, fusiform, sometimes irregularly fusiform, with subtruncate to bluntly rounded base, hyaline, granular, smooth-walled, rarely septate with age, (16–)19–23(–24) × 5–6(–7) µm. *Conidiomata in vitro* formed on WA on sterilised twigs of *Malus* sp., *Eucalyptus* sp., *Populus* sp., or needles of *Pinus* sp. within 7–14 days, pycnidial, superficial, globose, mostly solitary or in aggregates of 2–4 and covered in mycelium. *Conidia* similar to those formed *in vivo* but shorter, broadly fusiform to ovoid and more regular in shape, occasionally 1–2-septate and pale brown upon aging after discharge, (15–)16–20 × 5–6(–7) µm (Slippers *et al*., 2003).

Cultural characteristics: *Colonies* white to olivaceous-buff (21’’’d), becoming olivaceous-grey (21’’’’’i) to violaceous-black (65’’’’k), often with sectors that rapidly become darker than the rest of the culture, with very thick, felt-like mycelial mat reaching the lid of the Petri dish, margin smooth, in darker sectors appearing lacinate; 16.3 mm/day at 25°C (Slippers *et al*., 2003)

Host range and distribution: *Banksia coccinea, Buckinghamia* sp. (**Australia**) (Shearer *et al*., 1995; Denman *et al*., 2003); *Lcd. argenteum*, *P. cynaroides* (**South Africa, WC**) (Oliver, 1951); *Grevillea* sp., *Leucospermum* sp., *Lcd.* ‘Safari Sunset’, *P. cynaroides, Telopea* sp. (**U.S.A., Hawaii**), *P. cynaroides*, *Protea* sp. (**Zimbabwe**) (Denman *et al*., 2003).

Disease cycle and epidemiology: Previous records of *B. ribis* on *Proteaceae* were from hosts growing in warm, humid climates such as Australia (Queensland), U.S.A. (Hawaii) and Zimbabwe (Schieber & Zentmyer, 1978; Herbert & Grech, 1985). These climatic conditions may be a prerequisite for infection by *B. ribis.* In South Africa, the cultivation of *Proteaceae* is currently expanding into the warm, humid, summer rainfall regions, and this might lead to the more prominent appearance of *B. ribis*.

Control: For all Botryosphaeria stem canker diseases, practices aimed at maintaining hosts in a vigorous condition must be implemented (Schoeneweiss, 1965). Overwatering of nursery cuttings, wounding, and drought stress are all factors favouring seedling 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 (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 sanitation pruning (Olivier, 1951; Von Broembsen & Van der Merwe, 1990) and this should be carried out during the dry winter months. Sanitation practices reduce the levels of conidia and ascospores, which can serve as a source of infection during the growing periods (Sutton, 1981). Dying bushes should be removed as soon as possible after the first signs of wilting appear because pycnidia soon form on the dead tissue. No stump should be left above ground level. The trunk should be removed and burned, as fruiting-bodies will form on any remaining fragments of the dead tree. These measures will not necessarily control the disease, but they will reduce inoculum and limit 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 & Knox-Davies (1983a) suggested that *Botryosphaeria* inoculum could be reduced by burning whole plantations as they become unproductive. Since *B. ribis* has such a wide host range, other nearby infected nursery plants may serve as sources of inoculum for infection.

Pre-planting dipping of cuttings, followed by regular sprays with fungicides such as difolatan, captab 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, 1983a). 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 insecticides, gave better control than fungicides alone. This supports the view that cankers are often associated with insect wounds (Von Broembsen & Van der Merwe, 1990). 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 & Grech (1985) found *Botryosphaeria* to be sensitive to benomyl.

Notes: Using ITS sequence data, Denman *et al*. (2003) identified numerous isolates associated with cankers and dieback diseases of *Proteaceae* as *B. ribis*. It has been shown, however, that rDNA sequence data and RAPD marker data were insufficient to separate *B. ribis* from *B. parva*, a species to which it is morphologically similar (Smith & Stanosz, 2001; Zhou & Stanosz, 2001). In a recent paper, however, Slippers *et al*. (2003) successfully separated these two taxa by employing sequence data from the β-tubulin and EF1-α genes. Furthermore, that study also showed that *B. ribis* might be chiefly confined to *Ribes* spp. occurring in New York, U.S.A. This in turn, would mean that most of the isolates reported as *B. ribis* from elsewhere probably are *B. parva*. Further molecular studies are required, however, to clarify this issue. For the interim, therefore, we choose to use the name *B. ribis* but note that this may be a complex also involving *B. parva*.

Botryosphaeria ribis is a cosmopolitan fungus with a wide distribution, it is found in many of the temperate and subtropical countries (Punithalingam & Holliday, 1973). It has been reported from a wide range of hosts including at least 34 plant genera and 20 plant families (Smith, 1934). The pathogen causes stem, trunk and branch cankers on proteas and on other *Proteaceae* such as *Grevillea* (Schieber & Zentmyer, 1978) and *Macadamia* (Zentmyer & Storey, 1965; Herbert & Grech, 1985), as well as on other woody hosts such as *Rhododendron* (Schreiber, 1964), almond (English *et al.,* 1975), apple (Latorre & Toledo, 1984), peach (Reilly & Okie, 1982) and *Eucalyptus* (Davison & Tay, 1983; Shearer *et al.*, 1987). The fungus can also cause a serious twig blight of Douglas fir and Arizona cypress (Luttrell *et al.*, 1962; Shanin & Claflin, 1980). *B. ribis* also has been recorded as an apparent saprophyte on the leaves of *Eucalyptus* in South Africa (Crous *et al.*, 1989). It is not normally a soil-inhabiting fungus, and is not capable of surviving in the soil for long periods of time (Olivier, 1951).

THE ROLE OF ENVIRONMENTAL STRESS

Although many isolates of *Botryosphaeria* 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; Neely, 1968; Crist & Schoeneweiss, 1975). 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 and plant metabolism functions normally. Many *Botryosphaeria* spp. are endophytes or saprobes of dead and dying plant tissue, but have the ability to attack weakened or stressed plants (Wene, 1979; Schoeneweiss, 1981).

Certain diseases, particularly stem cankers, crown cankers and diebacks, are most prevalent on plants subjected to environmental stress (Schoeneweiss, 1981). *B. dothidea*, for example, may remain viable for many weeks in wounds on vigorous stems, without extensive colonisation, until plants are stressed (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) suggesting a latent type of infection.

Drought or freezing or both appeared to be the most common stress factors predisposing woody plants to disease (Crist & Schoeneweiss, 1975). Drought and poor nutrition have been implicated as factors favouring *B. ribis* infection of *Macadamia* (Herbert & Grech, 1985), *P. magnifica* (Denman, 2000), and pines (Ivory, 1967; Hodges, 1983). Wene (1979) found that colonisation of stems of woody plants was significant only in stems with plant water potentials lower than -12 bars. Following two dry years in South Africa, *B. ribis* caused a severe branch dieback of macadamias in all major production areas. It was hypothesised that water stress had increased the susceptibility of these trees (Herbert & Grech, 1985). Denman (2002) suggested that high levels of nitrogen may also affect plant metabolism, rendering *P. magnifica* more susceptible to disease.

Predisposition caused by short-term droughts is reversible, as cankers caused by non-aggressive pathogens often heal once water stress is reduced. 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. Furthermore, diseases that affect roots, for example *Phytophthora* spp., impair a plant's ability to take up water and soil nutrients and indirectly cause drought stress to other plant parts. This may explain the sudden appearance of stem cankers on field-grown plants that have not displayed visible signs of water stress (Wene, 1979).

After sudden and severe drops in temperature during fall or winter, outbreaks of stem canker and dieback may 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). In South Africa, such low temperatures are less likely to occur, but in mountainous areas with snow, the alternation of warm day time temperatures with freezing night time temperatures could predispose plants to canker diseases. 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. *B. 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).

WOUND PATHOGENS

Many species of *Botryosphaeria* are primarily considered to be wound pathogens (Smith, 1934; Wiehe, 1952; Witcher & Clayton, 1963), especially of stressed plants (Crist & Schoeneweiss, 1975; Spiers, 1977; Schoeneweiss, 1981). The fungus penetrates through wounds and causes perennial dieback and canker diseases. 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 & Claflin (1980) reported that *B. dothidea* could also invade unwounded elm, blueberry stems and Douglas fir, respectively. Milholland (1972) found that penetration of unwounded blueberry stems by *B. dothidea* occurred through open stomata. Natural infection of almond by *B. dothidea* appeared to be through cortical growth cracks (English *et al*., 1975). Brown & Hendrix (1981) reported that although wounded apple trees inoculated with *B. dothidea* showed disease symptoms more frequently than unwounded ones, wounding was not a prerequisite for infection.

PATHOGENS OF PROTEACEAE

Species of *Botryosphaeria* are commonly isolated from leaves and stems of proteas (Anon., 1991), and also are associated with most woody hosts. The stem cankers and dieback caused by species of *Botryosphaeria* 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 as well (Greenhalgh, 1981). Species of *Botryosphaeria* 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).

Benic & 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 *Leucospermum* spp. and an important secondary pathogen of the stem cankers caused by *Drechslera dematioidea*. Orffer and Knox-Davies (1989) isolated a *Botryosphaeria* sp. together with *Phomopsis sacharata* from about one third of canker and dieback lesions on *P. repens* and *P. obtusifolia*.

Olivier (1951) inoculated *Lcd. argenteum* with *B. ribis* and concluded that *Botryosphaeria* is the primary cause of the silver tree disease. However, Olivier'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 *Phytophthora cinnamomi* and came to the conclusion that *B. ribis* causes branch dieback, whereas *P. cinnamomi* is the primary pathogen leading to tree death. *B. ribis* 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* 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* and *B. baxteri* 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).

***KEY TO* BOTRYOSPHAERIA *SPECIES ON* PROTEACEAE**

1 Conidia pigmented at maturity…….. **2**
1 Conidia hyaline at maturity…… **3**
2 Conidia sienna brown at maturity, ovoid to subcylindrical, 20–26 × 9–12 µm, 0(–1)-septate, with warty or finely roughened, 0.5–1 µm thick walls ……... ***B. obtusa***
2 Conidia medium brown, subcylindrical, 7–14 × 2.5–3.5 µm, aseptate, walls finely verruculose …… ***Saccharata proteae*** (see leaf disease section)
3 Colony slow growing (<40 mm/week on PDA at 25 C); conidia fusiform, 20–30 × 4.5–6 µm, aseptate, smooth walls ……..…… ***Saccharata proteae*** (see leaf disease section)
3 Colonies fast growing (>90 mm/week on PDA at 25°C) ……..…… **4**
4 Colonies producing a distinct, bright yellow pigment after 3 d on MEA or PDA at 25°C, not at 20°C ……..…… ***B. lutea***
4 Colony producing pale yellow pigment after 3 days on MEA or PDA between 15–20°C ……..……..…….. ***B. australis***
4 Colonies not producing yellow pigment in culture ……..…… **5**
5 Conidia on average < 25 µm in length, ovoid, 17–24 × 7–11 µm; mycelium thick, woolly, grey on PDA …….. ***B. ribis***
5 Conidia on average 25 µm in length, narrowly fusiform, 19–30 × 4–6 µm; colonies on MEA or PDA appressed with only occasional tufts of grey to buff aerial mycelium, becoming grey to black with age ……..... ***B. dothidea***
5 Conidia on average > 25 µm in length, irregularly fusoid, 20–40 × 9–13 µm; mycelium on MEA or PDA flattened in the centre, with a rim of loose grey aerial mycelium at the edge of the dish …… ***B. protearum***

Ceratocystis canker

Ceratocystis albifundus was recently described from *Acacia mearnsii* trees in South Africa showing gummosis and dieback symptoms (Wingfield *et al*., 1996). Further investigation revealed that it had earlier been incorrectly treated as *Ceratocystis fimbriata*, and that it had also been collected from diseased *Protea* spp. (Crous *et al*., 2000a).

Ceratocystis albifundus M.J. Wingf., C. de Beer & M. Morris (as *albofundus*), *Systematic and Applied Microbiology* **19**: 197 (1996). Figs 399–401.

Symptoms: Reported to cause a stem canker disease on *Protea* spp. (Crous *et al*., 2000a), but attempts to recollect this fungus from *Proteaceae* have proven unsuccessful, hence its relative importance as a pathogen remains uncertain.

Causal organism: *Perithecia* 100–200 µm diam, yellow-brown, globose, not ornamented; perithecial necks 200–850 µm long, 20–30 µm wide at neck base, 16–24 µm wide below the ostiolar hyphae, black at the base, becoming lighter near the tip, smooth-walled, extending into perithecial base with a basal collar. *Ostiolar hyphae* 40–60 µm, hyaline, divergent. *Asci* evanescent. *Ascospores* 1-celled, hyaline, ellipsoid in face view, hat-shaped in side view, accumulating in a slimy droplet at the tip of the neck, double brim present on opposite ends of ascospores above the continuous brim, without a gelatinous sheath, 4–6 × 3.5–5 µm.

Conidiophores 24–104 × 3–5 µm, mononematous, smooth, septate, hyaline. *Conidia* hyaline, aggregating in slimy masses, of two types: cylindrical and 8–24 × 3–4 µm, or barrel-shaped with truncate ends and 6–10 × 4–8 µm (Wingfield *et al*., 1996).

Host range and distribution: *Protea* spp. (**South Africa, MP**) (Crous *et al*., 2000a).

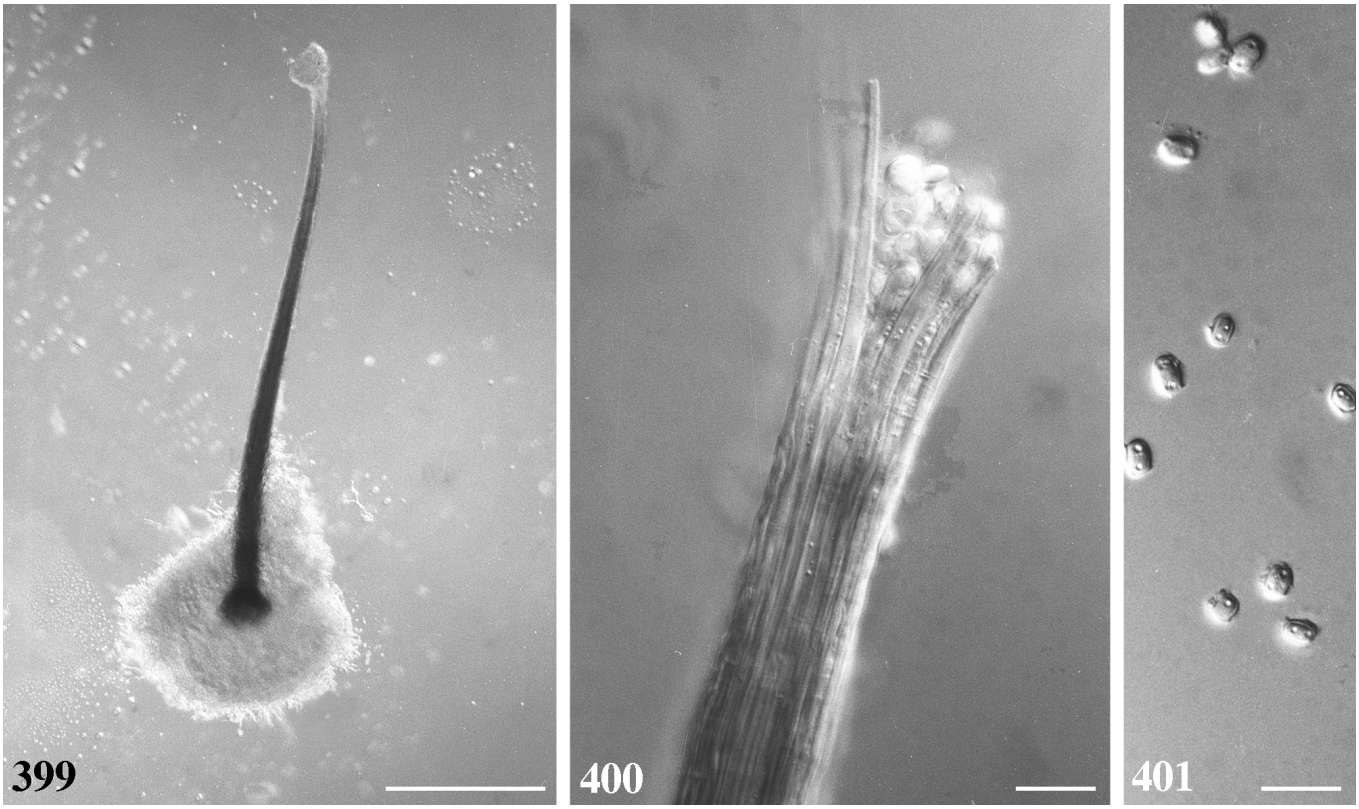
Disease cycle and epidemiology: Probably insect and splash-dispersed.

Control: Unknown.

Colletotrichum anthracnose disease complex

Alternative common name: Shepherd's crook

Colletrotrichum anthracnose is a serious and devastating disease of *Proteaceae*, which occurs wherever proteas are cultivated (Plates 17, 18). To date, the disease has chiefly been ascribed to *Colletotrichum gloeosporioides*. Preliminary molecular data have indicated, however, that as many as four different biological species may be involved (Lubbe *et al*., 2004). Undoubtedly these species will have a different epidemiology, virulence and host range. Although *Glomerella* teleomorphs are known for *C. acutatum* and *C. gloeosporioides*, these states have not been observed on *Proteaceae* and thus only the commonly encountered anamorphs are treated here.



Figs 399–401. *Ceratocystis albifundus.* Fig. 399. Perithecium. Fig. 400. Ostiolar hyphae. Fig. 401. Ascospores. Scale bars: 399 = 200 µm, 400–401 = 10 µm.

Colletotrichum acutatum J.H. Simmonds, *Queensland Journal of Agricultural Science* **22**: 458 (1965). Figs 402–403.

Symptoms: Shoot dieback, necrotic stem and leaf spots, stem rot, stem cankers, seedling damping-off, seedling blight and cutting dieback.

Causal organism: *Conidiomata* acervular, with moist, orange conidial masses. *Setae* developing in a dense layer around the conidiomata, 60–100 µm long, 3–8-septate, medium brown at the base, pale brown at bluntly rounded apex, tapering from a 3–5 µm wide base, to a 1.5–2 µm wide apex. *Conidiophores* branched below, at times pigmented in the lower part, or reduced to hyaline conidiogenous cells. *Conidiogenous cells* subcylindrical, hyaline, smooth, tapering towards a truncate apex with periclinal thickening, 12–20 x 3–4 µm. *Conidia* hyaline, smooth, guttulate, fusoid to naviculate, widest in the upper third, with acutely rounded apex and subtruncate base with abscission scar, (9–)11–13(–16) x (3–)4 µm. *Appressoria* medium brown, ovoid to clavate, 6–13 x 4–5 µm, 0(–1)-septate.

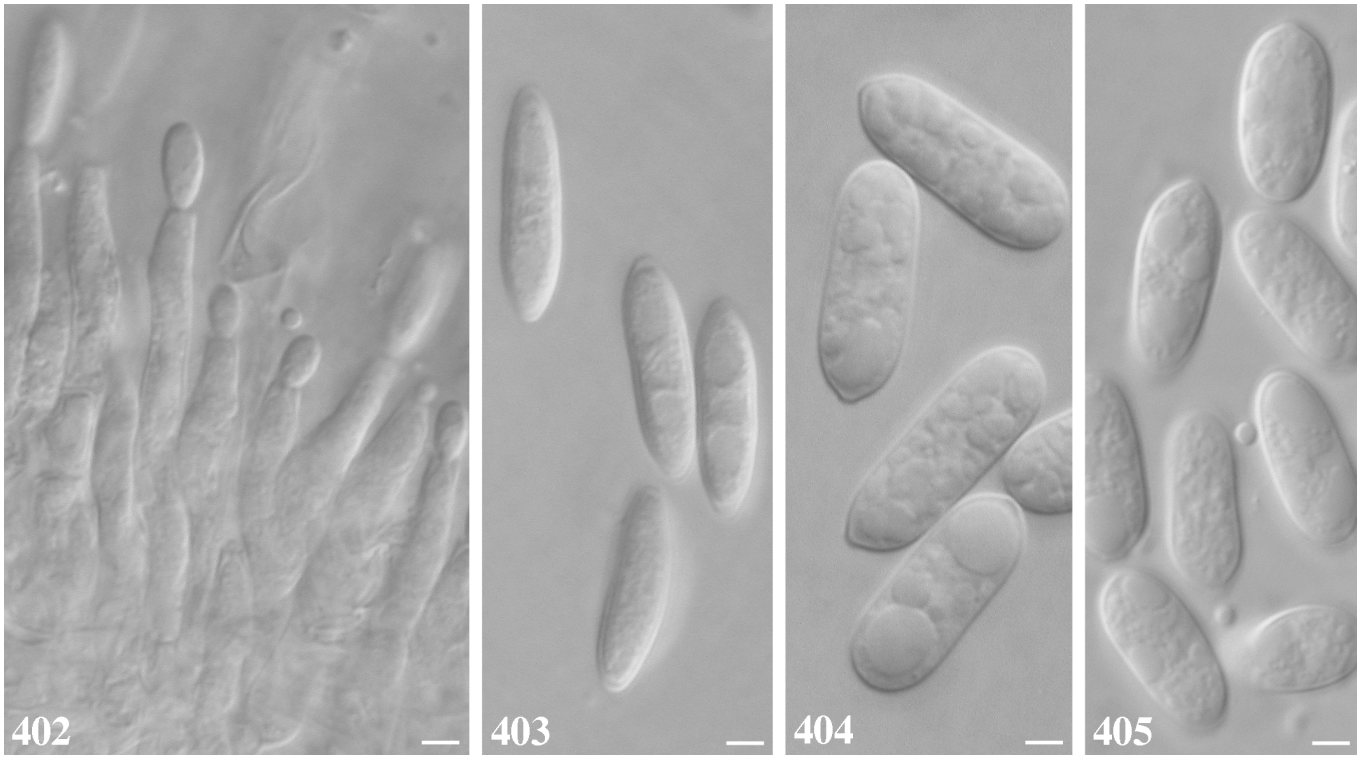
Cultural characteristics: *Colonies* on SNA with moderate, appressed, white aerial mycelium; on PDA with moderate fluffy aerial mycelium and few aerial conidia; rosy buff (13''f) with vinaceous-buff (17'''d) centers on the surface, reverse saffron (15d) with olivaceous-grey (21'''''i) centres; 10.2 mm/day at 25°C. Cardinal temperatures for growth were minimum 5°C, optimum 25°C, maximum 30°C. No growth was recorded at 35°C (Lubbe *et al*., 2004).

Host range and distribution: *Lcd.* 'Safari Sunset', *Leucospermum* sp., *P. cynaroides, P. magnifica, P. repens, Protea* sp. (**South Africa**); *P. cynaroides* (**Zimbabwe**) (Lubbe *et al*., 2004).

Colletotrichum boninense J. Moriwaki, Toy. Sato & T. Tsukiboshi, *Mycoscience* **44**: 48 (2003). Fig. 404.

Symptoms: Shoot dieback and leaf spots.

Causal organism: *Conidiomata* acervular, with masses of orange conidia. *Setae* 75–140 µm long, 3–5-septate, medium brown at the base, pale brown at the bluntly rounded apex, tapering from a 4–6 µm wide base, to a 1.5–2 µm wide apex. *Conidiophores* irregularly branched, frequently pigmented in lower half. *Conidiogenous cells* subcylindrical to obovoid, to fusoid, or irregular, hyaline, smooth, generally tapering from the lower part towards a truncate apex with periclinal thickening, 10–25 x 3–5 µm. *Conidia* hyaline, smooth, guttulate, subcylindrical with bluntly rounded ends and visible abscission scar, at times tapering inconspicuously to a slightly wider apex, or appearing slightly constricted in the middle, (14–)15–16(–18) x 5–6 µm. *Appressoria* medium brown, ovoid to irregularly lobed, 9–11 x 6–8 µm, 0–1-septate.
Cultural characteristics: *Colonies* on SNA with sparse, white aerial mycelium; on PDA with moderate grey



Figs 402–405. *Colletotrichum* spp. Figs 402–403. Conidiogenous cells and conidia of *C. acutatum*. Fig. 404. Conidia of *C. boninense*. Fig. 405. Conidia of *C. gloeosporioides*. Scale bars = 10 µm.

aerial mycelium and few aerial conidia; brown vinaceous (5'''m) with rosy-buff (13''f) centres on the surface, reverse brown vinaceous (5'''m); 8.1 mm/day at 25°C. Cardinal temperatures for growth were minimum 10°C, optimum 25°C, maximum 30°C. No growth was recorded at 35°C (Lubbe *et al*., 2004).

Host range and distribution: *Leucospermum* sp. (**Australia**); *P. cynaroides* (**Zimbabwe**) (Lubbe *et al*., 2004).

Colletotrichum gloeosporioides (Penz.) Penz. & Sacc., *Atti dell' Istituto Veneto di Scienze lettere, ed Arti, Séri* 6 **2**: 670 (1884). Figs 405.
≡ *Vermicularia gloeosporioides* Penz., *Michelia* **2**: 450 (1882).

Symptoms: Shoot dieback, stem and leaf necrosis, 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. Affected plant tissue is usually dark to black and a definite boundary is evident between healthy and diseased tissue. Under 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, resulting in the shepherd'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 *et al.*, 1988).

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

Causal organism: *Conidiomata* with masses of orange conidia. *Setae* 40–120 µm long, 1–5-septate, medium brown at the base, pale brown at the bluntly rounded apex, tapering from a 4–6 µm wide base, to a 2–4 µm wide apex. *Conidiophores* irregularly branched, frequently pigmented in the lower half. *Conidiogenous cells* subcylindrical, straight to curved, hyaline, smooth, tapering towards a truncate apex with visible periclinal thickening, 10–25 x 2.5–4 µm. *Conidia* hyaline, smooth, guttulate, subcylindrical with bluntly rounded ends and slightly flattened base, at times slightly constricted in the middle, (11–)12–15(–21) x (4.5–)5(–6) µm. *Appressoria* brown, ovoid, aseptate, 8–15 x 5–8 µm.

Cultural characteristics: Colonies on SNA with sparse, white aerial mycelium; on PDA with moderate grey aerial mycelium and few aerial conidia. Cardinal temperatures for growth were minimum 5°C, optimum 25°C, maximum 35°C. The mean daily growth rate at 25°C, 26.5 mm/day.

Host range and distribution: *P. compacta, P. coronata*, *P. cynaroides, P. longifolia, P. magnifica, P. neriifolia, P. obtusifolia, P. repens, P. stokoei* (**Australia**) (Greenhalgh, 1981); *P.* 'Susara', *P. cynaroides*, *P. neriifolia*, *Leucadendron* spp., *Leucospermum* 'High Gold', *Lsp*. 'Helderfontein', *Lsp*. 'Vlam', *Lsp*. 'Succession II' (**Madeira**) (Moura & Rodrigues, 2001); *P. compacta, P. compacta* × *neriifolia, P. cynaroides, P. eximia, P. grandiceps, P. lacticolor*, *P. laurifolia, P. lepidocarpodendron, P. longifolia, P. magnifica, P. mundii, P. nana, P. neriifolia, P. obtusifolia, P. pudens*, *P. repens, P. scolymocephala*, *P. speciosa, P. stokoei, P. susannae* (**South Africa**) (Benic & Knox-Davies, 1983b; Benic, 1986; Knox-Davies *et al*., 1986); *P. cynaroides* (**U.S.A., California**) (Taylor *et al*., 2001a); *Banksia dryandroides*, *Lsp. cordifolium* 'Coral', *Leucospermum* sp., *Lcd. laureolum, P. cynaroides, P. neriifolia, Protea* sp., *Telopea* sp. (**U.S.A., Hawaii**) (Anon., 1991; Taylor *et al*., 2001b); *P. cynaroides*, *P. magnifica*, *P. neriifolia*, *P. repens*, *Protea* sp. (**Zimbabwe**) (Masuka *et al*., 1998).

Disease cycle and epidemiology: Prolonged wet periods, moderate to warm temperatures (20–25°C), and high humidity favour the development of Colletotrichum dieback. This disease is particularly important in areas where high rainfall occurs during summer or warm periods, and in humid coastal climates. The fungus spreads rapidly by splashing rain or irrigation water (Von Broembsen, 1989; Forsberg, 1993). Drought stress may induce the stem canker phase, involving other secondary fungi in the disease. *C. gloeosporioides* also is frequently associated with canker fungi such as *Botryosphaeria* spp. Lesions infected by *Botryosphaeria* can develop into extensive stem cankers (Benic & Knox-Davies, 1983b; Coetzee *et al*., 1988).

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). Two to three weekly applications of mancozeb, mixed with benomyl or prochloraz during shoot flushing and wet weather has also been suggested (G. Malan, pers. comm.) to protect 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 of difolatan, captab or benomyl. A combination of difolatan and benomyl can also be used, although no longer available in South Africa. After planting, the cuttings can be sprayed regularly with difolatan 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 these fungicides 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). It is suspected that *Colletotrichum* inoculum would be 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), but it only occurs when environmental conditions are suitable for disease development. The pathogen is the cause of a destructive disease of *P. compacta* in the Betty's Bay, Kleinmond and Bot River area of the Western Cape, both in wild and commercial plantings (Benic & Knox-Davies, 1983b). The fungus also is a very important pathogen of protea nurseries in South Africa (Benic, 1986) and is seedborne (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). *Leucadendron* spp. are reported to be resistant (Benic & Knox-Davies, 1983b) and Benic (1986) suggested that *Leucospermum* spp. are resistant as well. According to Coetzee *et al.* (1988), Colletotrichum dieback is the most important disease of *Protea* spp., but *Leucadendron* and *Serruria* spp. are also susceptible.

Drechslera blight disease complex

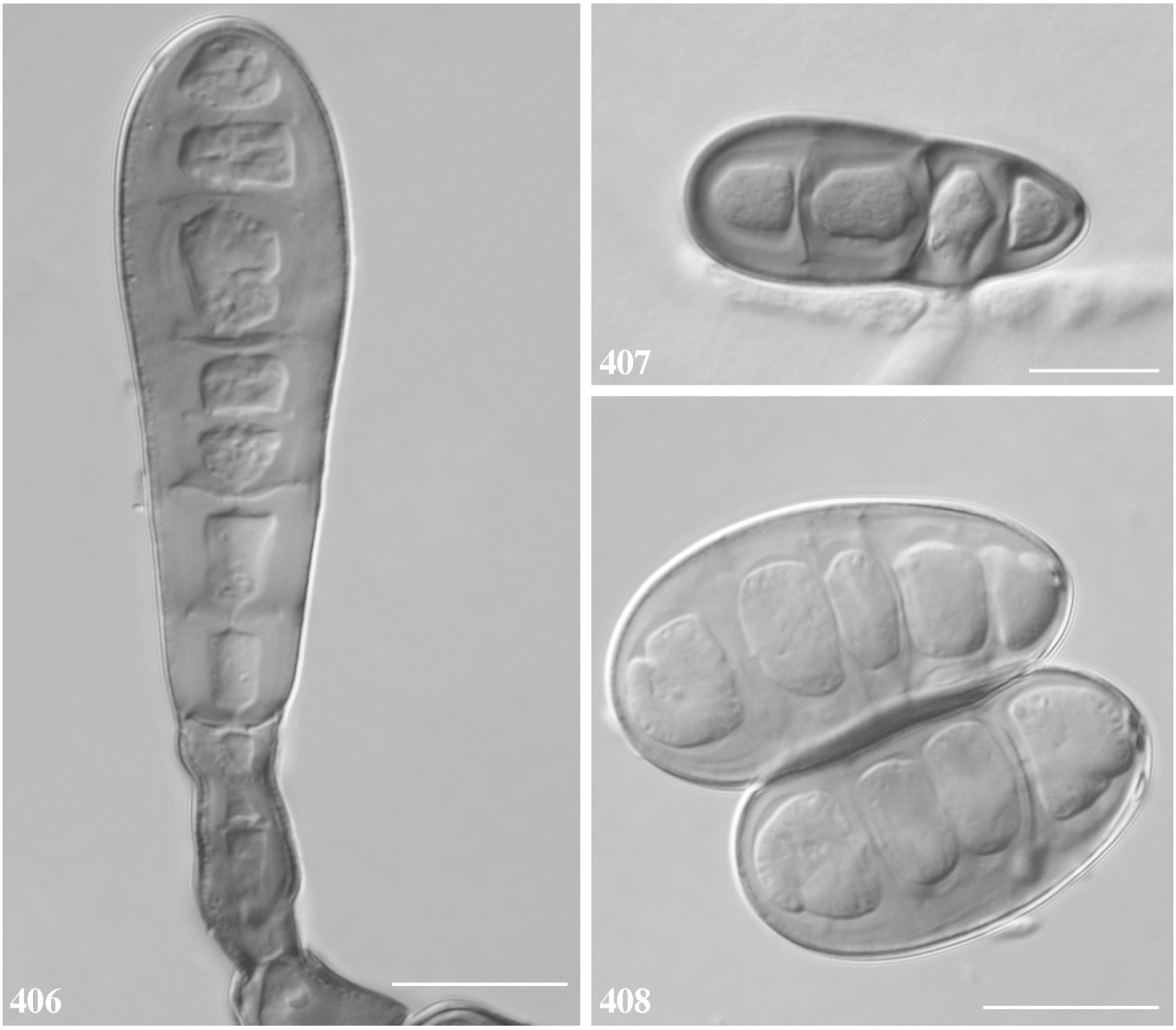
The *Drechslera* complex on *Proteaceae* has never been fully resolved. Two species have been reported from *Proteaceae*, namely *D. biseptata* and *D. dematioidea*. Shoemaker (1998) placed them in a new genus, *Mariellottia*, based on their unusual conidial morphology. Zhang & Berbee (2001) analysed ITS and *gpd* (glyceraldehyde-3-phosphate dehydrogenase) gene se-

quences in a phylogenetic study of *Pyrenophora* and concluded that *Marielliottia* was best retained in *Drechslera*.

Shoemaker (1998) commented that he had in fact studied authentic material of *D. dematioidea* collected from *Leucospermum* spp. in South Africa, but that he also found material of the *Bipolaris* anamorph of *Cochliobolus australiensis* to be present, and concluded that probably more species were involved in this disease complex.

Symptoms: The disease causes a severe blight of current-season leaves, stems, and flower heads, particularly during growth flushes (Plates 19, 20). 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. The lesions are at first pale brown, then darker grey-brown as they become necrotic. Spots can develop with or without purple margins (Von Broembsen, 1986, 1989; Forsberg, 1993).

Leaf lesions can spread to the stems, causing death of new shoots, the stems themselves and the flower heads (Forsberg, 1993). Bright red, superficial cankers develop on the stems where infected leaves are attached. The shoot tips do not collapse and leaves remain aligned with the stem as before infection. Extensive canker development results in shoot death. Young growth of the current season is more severely affected and young plants sometimes die (Von Broembsen, 1989). On developing flower heads, pale 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*, an important secondary pathogen of the stem cankers



Figs 406–408. *Drechslera* spp. Figs 406, 408. Conidiogenous cells and conidia of *D. dematioidea*. Fig. 407. Conidium of *D. biseptata.* Scale bars = 10 µm.

caused by *D. dematioidea*, results in significantly larger lesions than with either alone. Entire branches can die back (Von Broembsen, 1986, 1989).

In Hawaii, *D. biseptata* causes a more severe disease than *D. dematioidea.* Initially the symptoms caused by *D. biseptata* on the leaves are pale brown; these 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 (Anon., 1993). In South Africa, however, that species is highly virulent on *Leucospermum* spp. (Von Broembsen, 1986).

Drechslera biseptata (Sacc. & Roum.) M.J. Richardson & E.M. Fraser, *Transactions of the British Mycological Society* **51**: 148 (1968). Fig. 407.

≡ *Helminthosporium biseptatum* Sacc. & Roum. (*Helmisporium*), *Michelia* **2**: 641 (1881).

≡ *Marielliottia biseptata* (Sacc. & Roum.) Shoemaker, *Canadian Journal of Botany* **76**: 1560 (1999).

≡ *Brachysporium biseptatum* (Sacc. & Roum.) Sacc., *Sylloge Fungorum* **4**: 428 (1886).

= *Helminthosporium biforme* E.W. Mason & S. Hughes (*Helmisporium*), *Transactions of the British Mycological Society* **30**: 114 (1948).

≡ *Drechslera biformis* (E.W. Mason & S. Hughes) Subram. & B.L. Jain, (as *biforme*), *Current Science* **35**: 354 (1966).

Causal organism: *Conidiophores* of two types. Macronematous conidiophores arising from stroma, single to many in a cluster, erect to divergent, long, broad at base, gradually narrowed towards apex, closely septate at intervals of 10–18 µm, dark chocolate-brown; apex truncate, bearing conidia, 100–800 µm long, 10–15 µm wide at base, 9–11 µm in middle, 6–8 µm wide at apex. *Conidiogenous cells* tretic, integrated, proliferating sympodially, cylindrical, cicatrised, sporogenous area gnarled, frequently bent, with numerous dark brown scars. Micronematous conidiophores arising from hyaline to pale yellow hyphae (*in vitro*), slender, sparingly septate, short, erect, densely scarred, dark chocolate brown, rarely branched, 15–105 × 5–8 µm. *Conidia* obovoid, 3-septate, upper septum inconspicuous or lacking in some, rarely constricted at the septa, thick-walled, light to dark olive-brown, except in the basal cell, narrowed to the base, bearing a broad, black, non-protruding 3–4.5 µm wide scar, (21–)32–45(–49) × (9–)11–13(–17) µm. Germination by means of one or three oblique tubes near the scar, rarely one from the apex (Shoemaker, 1998).

Drechslera dematioidea (Bubák & Wróbl.) Subram. & B.L. Jain (as *dematioideum*), *Current Science* **35**: 354 (1966). Figs 406, 408.

≡ *Helminthosporium dematioideum* Bubák & Wróbl., Hedwigia **62**: 337 (1916).

≡ *Marielliottia dematioideum* (Bubák & Wróbl.) Shoemaker, *Canadian Journal of Botany* **76**: 1563 (1999).

Causal organism: *Conidiophores* on host material erect, medium yellow-brown, base slightly enlarged, 7–9(–15) µm, stalk nearly straight, septa approx. 15 µm apart, scars few (1–5), close and dark brown, apex truncate, 22–135 × 4–8 µm. *Conidiogenous cells* tretic, integrated, terminal, proliferating sympodially, cylindrical, cicatrised. *Conidia* mostly obovoid to nearly cylindrical, if 4-septate, widest at the third cell from the base, with hemispherical apex, hemiellipsoidal base, light to medium yellow-brown, but the basal cell lighter, (2–)3–4(–7) septa with a dark central line, slightly constricted at the basal septum, with not-protruding scar, (1.5–)3 µm wide, (31–)36–53(–57) × (9–)11–13(–18) µm.

Host range and distribution: *Leucospermum* spp. (**Australia**) (Greenhalgh, 1981; Forsberg, 1993); *Lsp. cordifolium*, *Lsp*. 'High Gold', *Lsp*. 'Helderfontein', *Lsp*. 'Vlam', *Leucospermum* spp. (**Madeira**) (Moura & Rodrigues, 2001); *Lsp. cordifolium* (**New Zealand**) (Boesewinkel, 1986); *Lsp. conocarpodendron, Lsp. conocarpodendron* × *cuneiforme, Lsp. cordifolium, Lsp.* 'Gold Dust', *Lsp.* 'Yellow Bird', *Lsp.* 'Fire Dance', *Lsp.* 'Vlam', *Lsp.* 'Flame Spike', *Lsp.* 'Red Sunset', *Lsp.* 'Caroline', *Lsp. cuneiforme, Lsp. erubescens, Lsp. glabrum, Lsp.* 'Helderfontein', *Lsp. grandiflorum, Lsp. patersonii, Lsp. pluridens, Lsp. rodolentum*, *Lsp. tottum, Lsp. tottum* × *vestitum*, *Lsp. vestitum* (**South Africa**) (Von Broembsen, 1986); *Lsp. cordifolium*, *Lsp.* 'Pink Star', *Lsp*. 'Red Sunset', *Lsp.* 'Hybrid 24'; *Leucospermum* spp., *Lcd.* 'Safari Sunset', *Leucadendron* spp., *P. cynaroides, P. repens, Protea* spp. (**U.S.A., Hawaii**) (Anon., 1991, 1993).

Disease cycle and epidemiology: Epidemic outbreaks of Drechslera blight on *Leucospermum* spp. occur primarily during the late winter to mid-spring. Warm temperatures of 19–25°C and wet conditions favour spread and development of blight disease (Forsberg, 1993). 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 by the sea, on the shady sides of windbreaks, or in the morning shadows of mountains. Overhead irrigation, crowded stands and weed growth around the base of the plant also favour disease development (Von Broembsen, 1986, 1989; Forsberg, 1993). The fungus can be spread with seed, seedlings, infected cuttings and nursery plants, or from certain grasses and native plants (Von Broembsen, 1989). Conidia are spread by wind and water-splash (Forsberg, 1993).

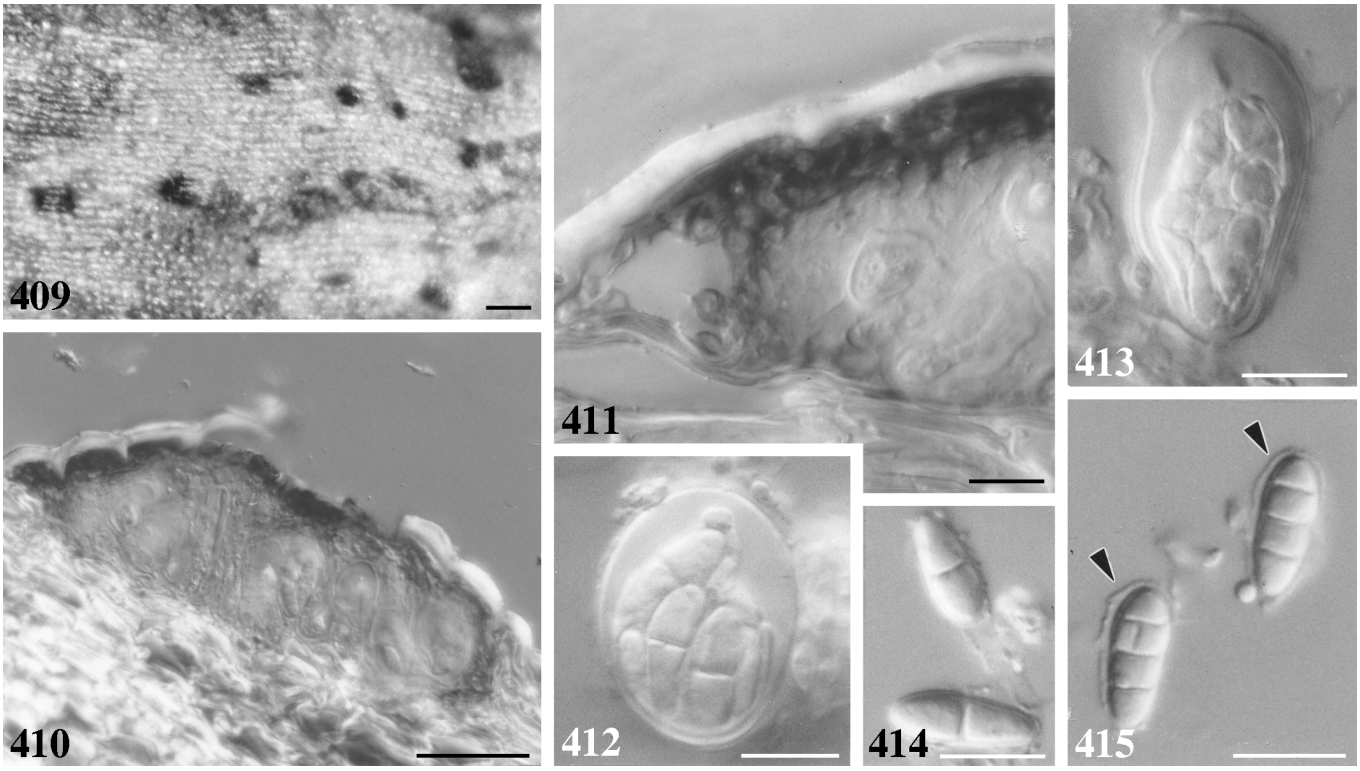
Control: Control of Drechslera blight by fungicides is effective during the early phases of the disease. Iprodione reduces disease severity in the field (Von Broembsen, 1986). Von Broembsen (1989) recommended applications of iprodione or chlorothalonil plus a benzimidazole fungicide every 2–3 weeks during growth periods and flower formation, and under warm, wet conditions. 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 (Anon., 1993). Only propiconazole and prochloraz are effective against the disease in New Zealand. Propiconazole is registered on ornamentals in South Africa (Nel *et al*., 1996). Spraying of the fungicides on a 14-day interval is advised. Seeds can be treated with imazalil or carboxin and thiram (Soteros, 1986), and seed treatment is advisable since *D. dematioidea* can be seed-borne (Von Broembsen, 1986).

In vitro studies showed that a number of fungicides inhibited mycelial growth. They may have potential for controlling Drechslera blight and should be tested in the field. They included chlorothalonil (Bravo), iprodione (Rovral), mancozeb (Dithane or Sancozeb), prochloraz manganese chloride (Octave) and thiram. Less promising, but still worth testing was fenarimol (Rubigan) and poor inhibition of mycelial growth was obtained with kresoxim-methyl (Stroby) and tebuconazole (folicur) (S. Denman unpubl. data).

Pre-plant dipping of the cuttings, followed by an effective spraying programme, can prevent disease development. Cuttings must only be taken from disease-free mother plants (Benic, 1986). Sanitation pruning should be carried out on all shoot cankers that have become dark and sunken (Coetzee *et al*., 1988). Adequate air circulation around and through plants should be employed (Von Broembsen, 1989).

Differences in susceptibility exist among cultivars and control through selection for resistance is possible (Von Broembsen, 1986). *Leucospermum cordifolium* and cultivars derived from *Lsp. cordifolium* ('Gold Dust', 'Yellow Bird' and 'Fire Dance') are most severely affected (Von Broembsen, 1986; Anon., 1993; Forsberg, 1993). According to Von Broembsen (1986), the cultivars 'Luteum', 'Scarlet Ribbon', 'Vlam', 'Caroline', 'Sunrise' and 'Goldie' are highly tolerant to Drechslera blight in South Africa. Benic (1986) stated that hybrid *Leucospermum* cultivars are most susceptible.

Notes: Drechslera blight is the most important disease of *Leucospermum*, particularly the cultivars developed from *Lsp. cordifolium*. Some *Leucadendron* and *Mimetes* spp. are also susceptible to blight (Von Broembsen, 1989). In South Africa and New Zealand, *D. dematioidea* is the most important of the two species. *Drechslera biseptata* is the main cause of disease of *Leucospermum* in the southern states of Australia and Queensland, while both species cause blight on *Leucospermum* in Hawaii (Boesewinkel, 1986; Von Broembsen, 1986, 1989; Anon., 1991, 1993; Forsberg, 1993). A different *Drechslera* sp. that causes leaf spot and blight was reported from the North Island of New Zealand (Soteros, 1986).



Figs 409–415. *Elsinoë fecunda*. Fig. 409. Ascomata on host substrate. Fig. 410. Vertical section through an ascoma. Fig. 411. Ascomatal wall layer. Figs 412–413. Asci. Figs 414–415. Ascospores with sheaths (arrowed). Scale bars: 409 = 100 µm, 410 = 30 µm, 411–415 = 10 µm. (From Taylor *et al*., 2001a).

Commercially cultivated plants seem to be more severely affected by Drechslera blight than those 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 *Leucospermum* spp. to begin shoot growth earlier than would occur in nature (G. Jacobs, Dept. of Horticulture, Univ. of Stellenbosch, South Africa, 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 optimal period for disease development (Von Broembsen, 1986).

Elsinoë scab diseases

Alternative common names: Corky bark or scab

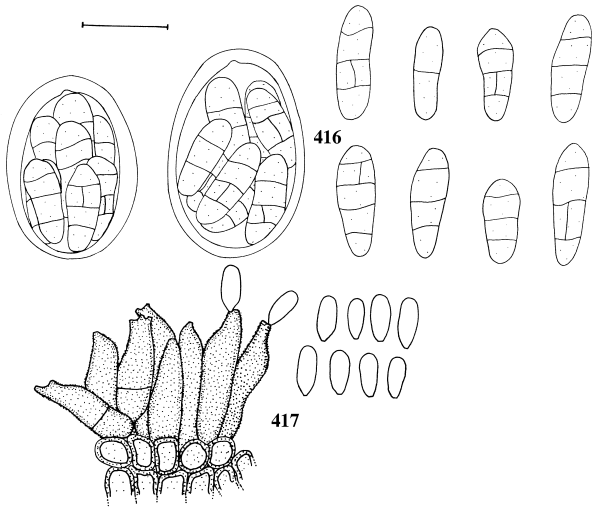
There are over 40 species of *Elsinoë,* which occur mainly in the warmer parts of the world (Kirk *et al*., 2001). Most species have a *Sphaceloma* anamorph and many *Sphaceloma* species are described for which the *Elsinoë* state is not known. Members of this genus are biotrophic or necrotrophic plant parasites, causing scab diseases of fruits, pods, leaves and stems of many cultivated hosts (Von Arx, 1987; Pan, 1994; Phillips, 1994; Gottwald, 1995). Host specificity is unclear at this stage, as many species have been described, but they are nearly indistinguishable from each other by morphological characters (Von Arx, 1987) or symptoms. Recent molecular studies have revealed that several distinct species of *Elsinoë* (or their *Sphaceloma* anamorphs) occur on *Proteaceae* (Swart *et al*., 2001) (Plates 21–23).

Scab disease of *Proteaceae* was first observed in South Africa in 1981, where it was associated with severe losses of commercial *Leucospermum* plantings. Similar disease symptoms were observed on *Leucadendron* spp. in South Africa (Benic & Knox-Davies, 1983a; Von Broembsen, 1989) and *Leucospermum* spp. in Hawaii and Australia (Ziehrl *et al*., 1995). In Australia, scab disease symptoms have been observed on many genera of *Proteaceae*, including *Banksia*, *Leucadendron, Mimetes, Protea* and *Serruria* (Forsberg 1993; Pascoe *et al.*, 1995).

Elsinoë is thought to be distributed via asymptomatic nursery material and this has probably occurred on an international scale. All new disease records are regarded as of great importance to the protea industry.

Elsinoë fecunda Joanne E. Taylor & Crous, *Mycotaxon* **78**: 84 (2001). Figs 409–415.

Symptoms: *Lesions* visible as regularly scattered small black specks on the twigs and some leaves; typical corky and scab-like lesions not observed.



Figs 416–417. *Elsinoë leucospermi* and its *Sphaceloma* anamorph. Fig. 416. Asci and ascospores. Fig. 417. Conidiophores and conidia. Scale bar = 10 µm. (From Swart *et al*., 2001).

Causal organism: *Ascomata* separate and singular, circular to irregular, 70–200 µm diam; in section, pulvinate, intraepidermal, single or multilocular, 125–175 µm diam, 40–65 µm high, with a thin wall of compressed cells between individual locules and each locule possessing an ostiolar opening. *Peridium* composed of medium to dark brown pseudoparenchymatous *textura angularis,* becoming paler and thin-walled inwardly and extending between the asci, 7–18 µm. *Hamathecium* absent. *Asci* attached at the base of the ascoma and distributed irregularly throughout, saccate, broadly clavate and obclavate, thick-walled, 8-spored, hyaline, sessile, with a broad rounded tip and a well-developed ocular chamber, (20–)26–28(–33) × (12–)15–16.5(–20) µm. *Ascospores* multiseriate, hyaline, broadly ellipsoid with rounded to obtuse ends, widest just above the first-formed septum, with 1–4 transverse, and 1–2 vertical septa, oblique septa rare, occasionally constricted at the median septum, (10–)11.5–12.5(–15) × (4–)4.5–5(–5.5) µm.

Host range and distribution: *P. scolymocephala* (**U.S.A., California**).

Elsinoë leucospermi L. Swart & Crous, *Mycologia* **93**: 396 (2001). Figs 416–425.
Anamorph: ***Sphaceloma*** sp.

Symptoms: *Leaf spots* on *Leucospermum* and *Leucadendron* circular to irregular, amphigenous, 1–4 mm diam, extending through the lamina, but more prominent on the initially infected surface; 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 developing 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 pale brown due to the ruptured epidermis; red-brown margins frequently present; lesions becoming roughened and corky at maturity, and often with 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, consisting of small specks to raised, circular spots, pale brown, 1–3 mm diam, surrounded by a distinct, wide, red margin, up to 2 mm wide. On leaves symptoms vary from small necrotic spots with red margins to a blighting of the whole lamina.

Causal organism: *Mycelium* internal, consisting of branched, septate, hyaline to brown, 3.5–4.5 µm wide hyphae. *Ascomata* scattered, separate, pulvinate, subcuticular, composed of medium to dark brown pseudoparenchymatous *textura angularis*, up to 200 µm diam and 60 µm high. *Asci* distributed irregularly throughout the ascoma, ovoid to subglobose, thick-walled, 8-spored, sessile, hyaline, 16–28 × 13–18 µm. *Ascospores* hyaline, broadly ellipsoid with rounded to obtuse ends, with 1–4 transverse, and 1–2 vertical septa; oblique septa rare; constricted at the median septum, (10–)12–14(–19) × 4–5 µm.

Conidiomata acervular, foliicolous but primarily caulicolous, raised, coalescing at maturity, composed of medium brown *textura angularis*, up to 200 µm diam and 1 mm long. *Conidiophores* subcylindrical, pale brown, verruculose, 0–2-septate, 20–30 × 3–6 µm. *Conidiogenous cells* polyphialidic, with 1–2 integrated loci, pale brown, verruculose, ampulliform to doliiform, 10–15 × 3–4 µm. *Conidia* hyaline, granular, aseptate, ellipsoid, with obtuse apex, constricted at the base to a subtruncate hilum, (2–)5–7(–8) × (1–)2.5–3 µm *in vivo*, 5–7 × 2–3 µm *in vitro* in Fries's medium (Swart *et al.*, 2001).

Cultural characteristics: *Colonies* on MEA irregular, erumpent, folded, with smooth, sinuate margins, without aerial mycelium; surface red (71i) to blood-red (71k), sienna (13i) or coral (3'b); 7–12 mm diam on MEA after 1 month in the dark at 25°C. Cardinal temperatures for growth: minimum above 5°C, optimum 20–25°C, maximum above 35°C.

Host range and distribution: *Leucadendron* sp., *Lcd. laureolum, Lcd. thymifolium, Lsp. cordifolium, Leucospermum* spp., *Serruria florida* (**Australia**); *Lcd. pubibracteolatum* × *chamelaea, Leucadendron* spp., *Lsp. cordifolium, Leucospermum* spp., *Serruria florida* (**South Africa**); *Lsp. cordifolium* (**U.S.A., California, Hawaii**); *Lsp. glabrum* × *tottum, Lsp. reflexum* var. *luteum* (**Zimbabwe**).

Elsinoë proteae Crous & L. Swart, *Mycologia* **93**: 374 (2001). Figs 426–429.

Symptoms: *Lesions* on leaves and petioles, circular, separate, becoming confluent, 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.

Causal organism: *Ascomata* scattered, separate, pulvinate, subcuticular, composed of medium to dark brown pseudoparenchymatous *textura angularis*, up to 250 µm diam and 80 µm high. *Asci* distributed irregularly throughout the ascoma, ovoid to subglobose, thick-walled, 8-spored, sessile, hyaline, 28–45 × 19–24 µm. *Ascospores* hyaline to olivaceous, broadly ellipsoid with rounded ends, with 3–5 transverse, and 1–3 vertical septa; oblique septa sometimes present; mostly slightly constricted at the median septum, (14–)16–17(–20) × (5–)6–7 µm.

Sphaceloma state not observed on the host, but induced in Fries's medium. *Conidiogenous cells* polyphialidic, with 1–3 integrated loci, hyaline, smooth, subcylindrical to doliiform, 6–12 × 4–6 µm. *Conidia* hyaline, granular, aseptate, ellipsoid, with obtuse apex, and rounded to subtruncate base, (5–)6–7(–8) × 2–3(–4) µm.

Cultural characteristics: *Colonies* on MEA irregular, erumpent, folded, with sinuate, smooth margins; aerial mycelium whitish, sparse; surface rose to red (71b–71i); older colonies exuding a diffuse red pigment; 12 mm diam on MEA after 1 month in the dark at 20°C. Cardinal temperatures for growth: minimum above 5°C, optimum 15–20°C, maximum below 30°C.

Host range and distribution: *P. cynaroides*, *P. repens* (**South Africa**) (Swart *et al*., 2001).

Sphaceloma protearum L. Swart & Crous, *Mycologia* **93**: 375 (2001). Figs 430–432.

Symptoms: *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.

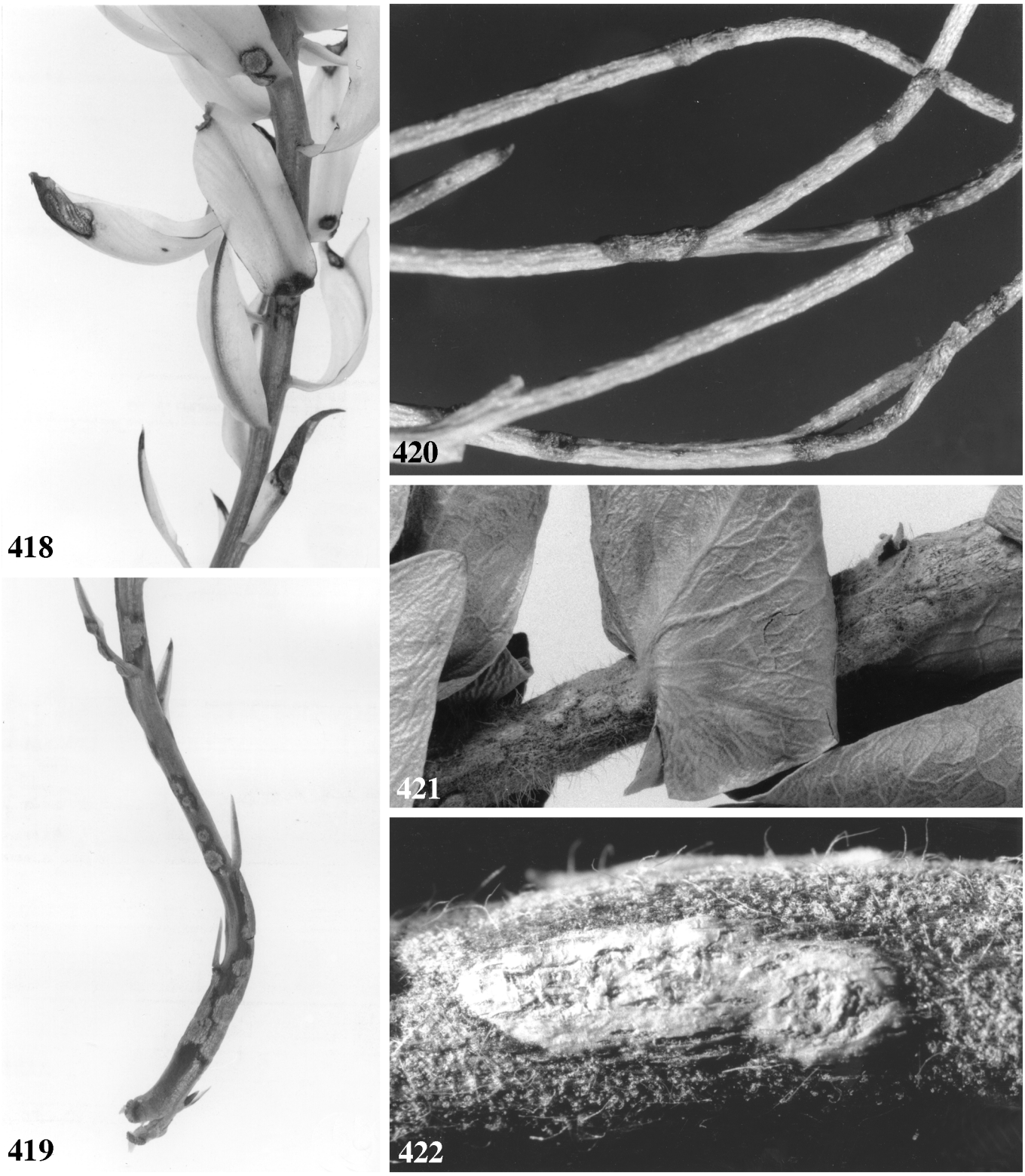
Causal organism: *Mycelium* internal, consisting of branched, septate, hyaline to brown, 3–4 µm wide hyphae. *Conidiomata* acervular on stems, or sporodochial on leaves, composed of medium brown *textura angularis*, up to 100 µm diam and 1 mm long. *Conidiophores* subcylindrical to doliiform, light to medium brown, verruculose, 0–2-septate, 12–20 × 5–6 µm. *Conidiogenous cells* polyphialidic, with 1–2 integrated loci, pale brown, verruculose, doliiform, 5–12 × 4–6 µm. *Conidia* hyaline, aseptate, ellipsoid, with obtuse apex, constricted at the base to a subtruncate locus,

(3.5–)5–6(–7) × (1.5–)2–2.5 µm *in vivo*, not sporulating *in vitro* in Fries' medium.

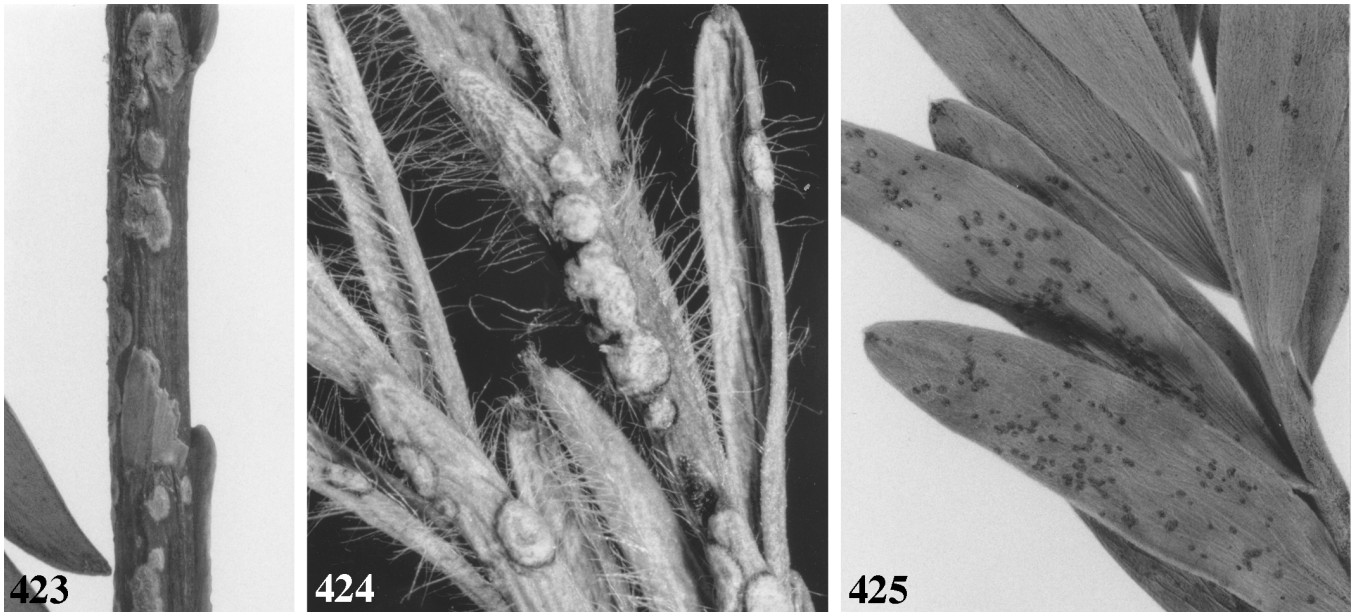
Cultural characteristics: *Colonies* on MEA irregular, erumpent, folded, with sinuate, smooth margins, without aerial mycelium; surface blood-red (71k–71m), a slight red pigment diffusing in the agar with age; 11 mm diam on MEA after 1 month in the dark at 25°C. Cardinal temperatures for growth: minimum above 5°C, optimum 20–25°C, maximum below 30°C.

Host range and distribution: *Protea* spp. (**South Africa, NP**) (Swart *et al*., in prep.), *Protea* 'Sylvia', *P.* 'Pink Ice', *P.* 'Susara' (**Zimbabwe**) (Swart *et al*., 2001).

Disease cycle and epidemiology: Clusters of infection loci, separated by healthy, uninfected tissue, suggest



Figs 418–422. Foliar and stem disease symptoms of *Elsinoë leucospermi*. Figs 418–420. On *Serruria florida*. Figs 421–422. On a *Leucospermum* hybrid. (From Swart *et al*., 2001).



Figs 423–425. Foliar and stem disease symptoms of *Elsinoë leucospermi*. Fig. 423. On *Leucadendron* 'Silvan Red'. Fig. 424. On *Lcd. thymifolium*. Fig. 425. On *Lcd. laureolum.*

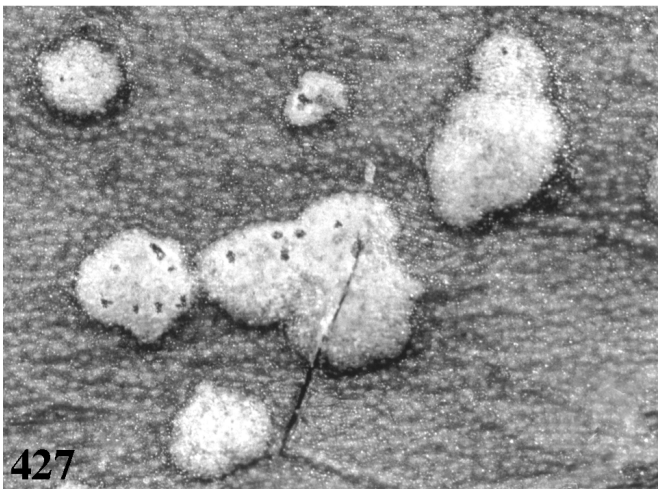
that infection only takes place at certain times in a growing season when spore production, spore dispersal and infection of host tissue are favoured. It seems that only young, actively growing tissue is susceptible. Infection therefore only occurs during periods of active growth (Ziehrl *et al.*, 1995). 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. 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).

Rain or overhead irrigation is a requirement for primary infection, and moist, warm conditions favour disease progression. Although moisture favours infection, optimum conditions of humidity, temperature or the necessary duration of moist conditions are not known (Benic & Knox-Davies, 1983a; Ziehrl *et al.*, 1995). Shaded plants are more susceptible to scab than those in exposed sites. This can be observed on the same plant, where the shaded side is usually more severely affected (Benic & Knox-Davies, 1983a).

Although little is known of the survival strategies of the *Leucospermum* scab fungus, other species of *Elsinoë*/*Sphaceloma* survive as mycelium and spores in old scab lesions (Peltier & Frederich, 1926; Bitancourt & Jenkins, 1937). It is possible that *Elsinoë* species on *Proteaceae* have the same survival strategies. Ziehrl *et al.* (1995) stated that infected plant material often serves as an inoculum source for new infections. It is often the smaller shoots not harvested the previous season that harbour the fungus. Furthermore, Benic & Knox-Davies (1983a) found *E. leucospermi* to form microsclerotia within and on the surface of scab lesions from late summer until the following spring, suggesting that the fungus survives mainly in scab lesions as resting mycelium, stromatic tissue, and as chlamydospores and microsclerotia outside the tissue. Conidia and ascospores likely are less important as survival propagules because of the extremely hot and dry summer climate (Benic & Knox-Davies, 1983a). Plants can be infected without showing visible disease symptoms (Ziehrl *et al*., 2000). Von Broembsen (1989) also noted that some shoot infections were latent and only developed further once plants were stressed.

The disease spreads relatively slowly in the field (Von Broembsen, 1989). Species of *Elsinoë* 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 commonly occur on scab lesions. These insects and the larger chewing insects possibly play a role in dissemination of the fungus (Benic & Knox-Davies, 1983a). In accordance with Jenkins (1930), who isolated *E. fawcetti* from insect faeces, cultures of *E. leucospermi* have also been isolated from insect faeces on *Leucospermum* spp. (L. Benic, Deciduous Fruit Producers Trust, pers. comm.).

Control: Benic & Knox-Davies (1983a) recommended that young plants be sprayed with fungicides while they are actively growing, and when moisture and temperature conditions favour disease development. Prochloraz sprayed during growth flushes gives good protection (Von Broembsen, 1989). Mature plants must be sprayed



Figs 426–427. Leaf symptoms of *Elsinoë proteae* on *Protea cynaroides*. (From Swart *et al*., 2001).

immediately after flowering or after flowers are cut. It may be necessary to spray plants more than once if the flowers are harvested intensively over an extended period. Contact fungicides (e.g. captafol, mancozeb) or systemics 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).

Ziehrl *et al*. (2000) reported contact fungicides such as prochloraz manganese chloride (Octave), captab (Captan) and mancozeb (Dithane) to provide the best control, reducing disease by up to 75%. Since captafol is not longer available in the USA and benomyl is soon to be removed from the USA market there is a need for new fungicides to be tested. Fenarimol (Rubigan), folpet (Folpan or Folpet), and tebuconazole (Folicur) are all registered on ornamentals in South Africa and should be tested against this disease on proteas. Additionally some of the new strobilurin fungicides should be tested.

Benic (1986) reported that difolatan, captab and benomyl can be used to spray cutting material in mistbeds, with difolatan being particularly effective for the control of 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 is required. The Protea Disease Management Group (Anon., 1993) conducted fungicide experiments on potted *Lsp.* '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. However phytotoxicity, indicated by the reddening of the leaves, was observed with chlorothalonil and flusilazole.

Because fungicidal control of Elsinoë scab is poor, sanitation pruning is essential if scab is to be controlled. Lesions are often inconspicuous and difficult to detect in spring and early summer, but typical scab lesions can easily be seen by late summer and sanitation pruning should be carried out at this stage (Coetzee *et al.,* 1988).

Cultivars should be selected for tolerance to 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 used since the fungus can spread in 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). Benic (1986) reported scab lesions on cuttings in a mistbed which results when cuttings are collected from scab-infected mother plants and brought into the mistbed.

Notes: 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 *et al.*, 1995). Symptoms on *Leucospermum* appear on the current season's young shoots. The first symptoms appear as small, elliptical, sunken, whitish lesions, normally accompanied by reddening of the surrounding tissue (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 (Benic & Knox-Davies, 1983a; Ziehrl *et al.*, 1995). Dark fruiting-bodies are sometimes scattered in these lesions.

Older lesions are large, dark, raised, roughened, and often cracked areas with a corky appearance, which may eventually girdle the stem. Stem splitting sometimes occurs and heavy infection may cause abnormal growth and twisting of the stem. Symptoms take be-

tween 3–5 weeks 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 generally less prominent than the extensive stem lesions. Leaf lesions are initially transluscent, then dull, tan-coloured, raised, hard, rough and corky (Benic & Knox-Davies, 1983a).

Scab symptoms vary considerably between the different host genera. Distinctive white, scab-like lesions appear on the older leaves of *P. cynaroides*, while only restricted lesions are observed on the petioles. These symptoms are in direct contrast to those caused by scab on *Leucospermum* spp., which 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 *Leucospermum* spp. However the leaf lesions of the former differ considerably. They are small, circular, red-brown, eventually coalescing and killing the entire leaf. Scab disease on *Protea* spp. in Zimbabwe is much more severe than on *P. cynaroides* or *Leucospermum* spp. in South Africa.

SPECIES SUSCEPTIBILITY

Cultivars and seedlings differ greatly in susceptibility to scab. In Hawaii, *Lsp.* 'Hawaii Gold' appears to be moderately resistant to scab, while *Lsp.* 'Coral' is highly susceptible (Anon., 1991). A selection of *Lsp. saxosum,* identified as highly resistant to scab has produced hybrid seedlings that appear to have inherited its resistance (Leonhardt, 1998). In South Africa, *Lsp.* 'Red Sunset', 'Gold Dust' (Knox-Davies *et al*., 1986) and 'Vlam' are extremely susceptible. The occurrence of scab on *P.* '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. Ziehrl *et al*. (2000) also demonstrated that *Elsinoë* spp. from South African *Proteaceae* could infect Australian proteaceous genera such as *Banksia* and *Dryandra*, but not *Telopea*, *Grevillea* or *Hakea*.

ECONOMIC IMPORTANCE

In a survey conducted on scab disease of *Proteaceae* in Australia, scab 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). The disease has 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 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 AUS $ 602 416 and AUS $ 52 1006 per annum for plantation and nursery growers, respectively (Ziehrl *et al.*, 1995). As no similar surveys have been undertaken in California, Hawaii, South Africa or Zimbabwe, the wider economic impact of this disease complex remains to be determined.

***KEY TO* ELSINOË *SPECIES ON* PROTEACEAE**

1 Occurring on *Banksia* ……..…….…….. **2**
On other *Proteaceae* ……...……...…… **3**
2 Leaf spots on *B. serrata*; colonies grey-olivaceous; optimal growth at 15°C ……..….. ***E. banksiae***
2 Lesions on leaves and veins of *B. prionotes*; colonies blood-red; optimal growth at 20–25°C ……. ***Elsinoë* sp.**
3 Occurring on *Leucadendron*, *Leucospermum* and *Serruria*; ascospores with 1–4 transverse, 1–2 vertical and rarely any oblique septa, (10–)12–14(–19) × 4–5 µm; colonies blood red, optimal growth at 20–25°C; conidia (2–)5–7(–8) × (1–)2.5–3 µm …..…..……..... ***E. leucospermi***
3 Occurring on *Protea* ……. **4**
4 Leaf spots on mature leaves; ascospores with 3–5 transverse, 1–3 vertical and rarely any oblique septa, (14–)16–17(–20) × (5–)6–7 µm; colonies rose to red with diffusing red pigment; optimal growth at 15–20°C; *Sphaceloma* state not observed on host …… ***E. proteae***
4 Shepherd's crook and leaf spot symptoms on juvenile growth flushes, leading to shoot blight; colonies blood-red with slight diffusing red pigment; optimal growth at 20–25°C; *Elsinoë* state not observed on host ……. ***Sphaceloma protearum***
4 Leaf spots small black specks on leaves and twigs; ascospores with 1–4 transverse, 1–2 vertical, and rarely any oblique septa, (10–)11.5–12.5(–15) × (4–)4.5–5(–5.5) µm ……..…… ***E. fecunda***

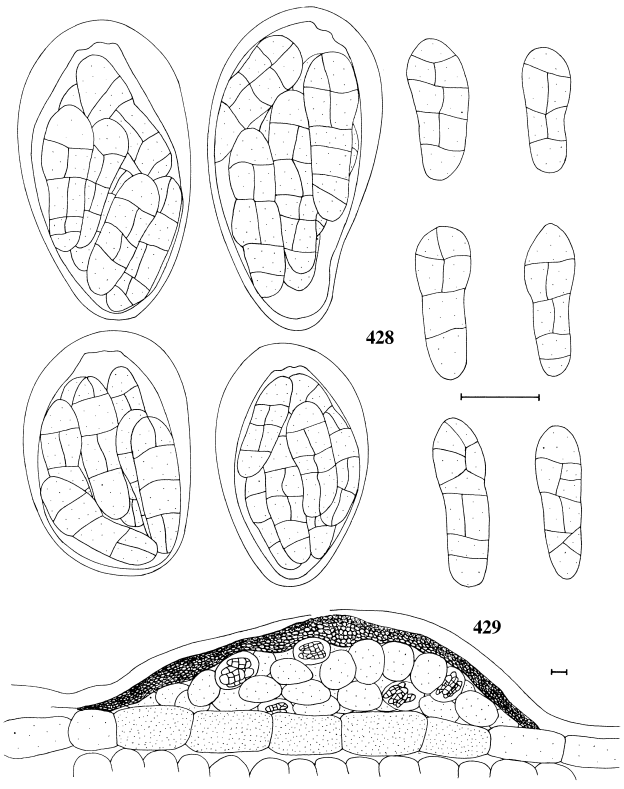
Phloeosporella canker

Species of *Phloeosporella* are commonly associated with leaf spots on a wide range of hosts, but on protea they cause distinctive stem cankers. The genus is characterised by having hyaline structures, sympodially proliferating conidiogenous cells, and acervuli. It is chiefly distinguished from *Pseudocercosporella* in that the latter has superficial to subepidermal sporodochia. *Phloeosporella padi* has been linked to the teleomorph *Blumeriella*. Preliminary molecular data place *P. padi* in the *Mycosphaerella* complex (P.W. Crous, unpubl. data) which suggests that its purported teleomorph, as well as the distinction between *Pseudocercosporella* and *Phloeosporella,* need to be further investigated.

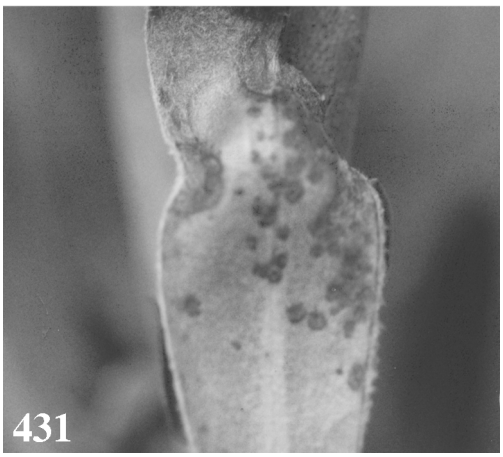
Phloeosporella protearum Joanne E. Taylor & Crous, *Mycological Research* **104**: 627 (2000). Figs 433–439.

Symptoms: *Lesions* associated with stem cankers and characterised by shrunken, pale and parchment-like tissues.

Causal organism: *Conidiomata* acervular, single, in loose groups, immersed, slightly raising the host surface or becoming erumpent, elongate, parallel with veins of stem, pale brown; in section subglobose, subepidermal, raising the host epidermal cells away from the mesophyll cells. *Peridium* a stratum of thin-walled, hyaline, compressed *textura angularis* to *textura intricata,* slightly wider in the corners, with small-celled, dark brown, thin-walled *textura angularis* occurring in places, (15–)21–41(–51) µm thick. *Conidiogenous cells* hyaline, irregularly cylindrical to doliiform or pyriform, with rounded or flattened non protuberant, unthickened apices, conidial ontogeny holoblastic, with sympodial proliferation, (4–)5–6.5(–8) × (2.5–)3–4.5(–6) µm, formed from the upper and lower cells of the conidiomata. *Conidia* narrowly cylindrical, straight, curved or irregular, with a rounded apex and a truncate base, thin-walled, smooth, hyaline, with 1–3 eusepta, (15–)20–22(–26) × (2.5–)3–.5(–6) µm. Germ tubes parallel to the long axis of the conidia, or sometimes perpendicular or irregular, with germination occurring from all cells.



Figs 428–429. *Elsinoë proteae* on *Protea cynaroides*. Fig. 428. Asci and ascospores. Fig. 429. Vertical section through an ascoma on host tissue. Scale bars = 10 µm. (From Swart *et al*., 2001).



Figs 430–431. Leaf symptoms of *Sphaceloma protearum* on a *Protea* sp. (From Swart *et al*., 2001).

Cultural characteristics: Colonies circular, umbonate, with entire margins, broadly concentrically zonate, buff (19”d) to honey (19”b), same in reverse; aerial mycelium, slimy, moderately dense; 21 mm diam after 60 days.

Host range and distibution: *Protea* sp. (**Zimbabwe**) (Taylor & Crous, 2000).

Epidemiology and control: Unknown.

Phoma brown stem canker

Species of *Phoma* are commonly isolated from diseased leaf and stem tissue of *Proteaceae*. Their pathogenicity and relative importance, however, remains unclear. Several species have been recorded to cause canker and wilt diseases on other woody hosts (Holz & Schreuder, 1989) and in some cases this is assumed to be associated with toxin production. Corlett (1991) lists the teleomorph of *P. sorghina* as *Didymella* (*Mycosphaerella*) *holci*, though this connection has never been confirmed.

Phoma sorghina (Sacc.) Boerema, Dorenb. & Kesteren, *Persoonia* **7**: 134 (1973). Figs 440–442.
≡ *Phyllosticta sorghina* Sacc., Michelia **1**(2): 140. 1878.
For additional synonyms see: *Kew Bulletin* **31**: 539–540 (1977).

Symptoms: The disease is characterised by necrotic browning of the stems of young plants from soil level to about 30 cm above the ground. 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 lower 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.

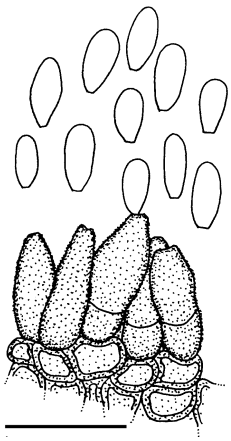


Fig. 432. Conidia and conidiophores of *Sphaceloma protearum*. Scale bar = 10 µm. (From Swart *et al*., 2001).

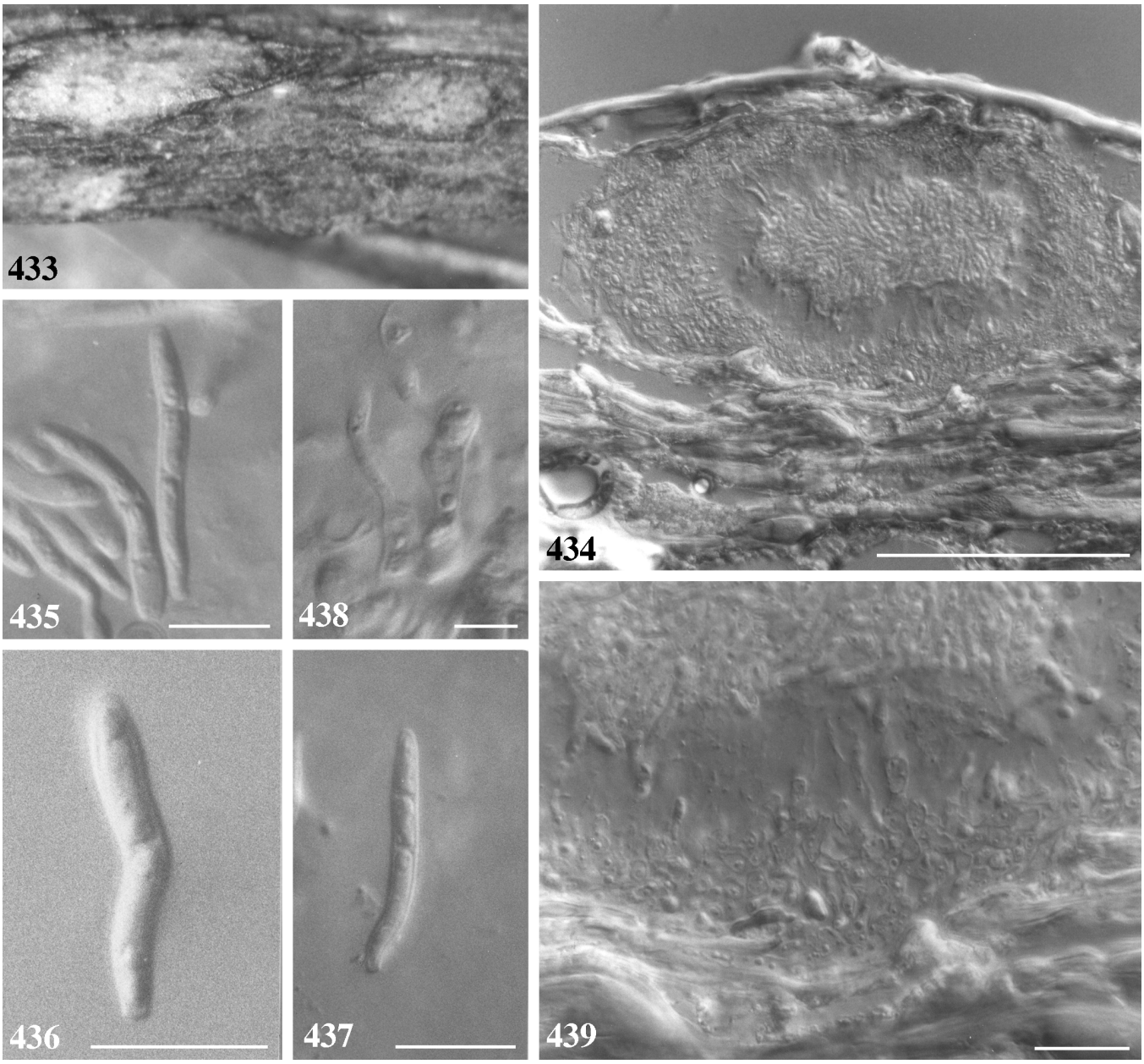
No fructifications have been found in these lesions, but small structures, which look like fruiting bodies to the naked eye, are visible on the surface (Gorter, 1976).

Causal organism: *Conidiomata* on malt agar pycnidial, 50–200 µm diam, subglobose, usually with a straight or somewhat curved, up to 80 µm long neck. Conidial exudate usually salmon. *Conidiogenous cells* hyaline, short, obpyriform, phialidic, lining the inside of the pycnidium. *Conidia* hyaline, non-septate, variable in shape but mostly ovoid-ellipsoidal, (4–)4.5–7(–8) × (1.5–)2–3(–3.5) µm (Boerema, 1993). Conidia sometimes becoming 1-septate at germination (Sivanesan, 1984). Chlamydospores irregular, uni- or multicellular, solitary or in chains, botryoid, smooth, verrucose or tuberculate, subhyaline to brown, 8–35 µm diam (Boerema, 1993).

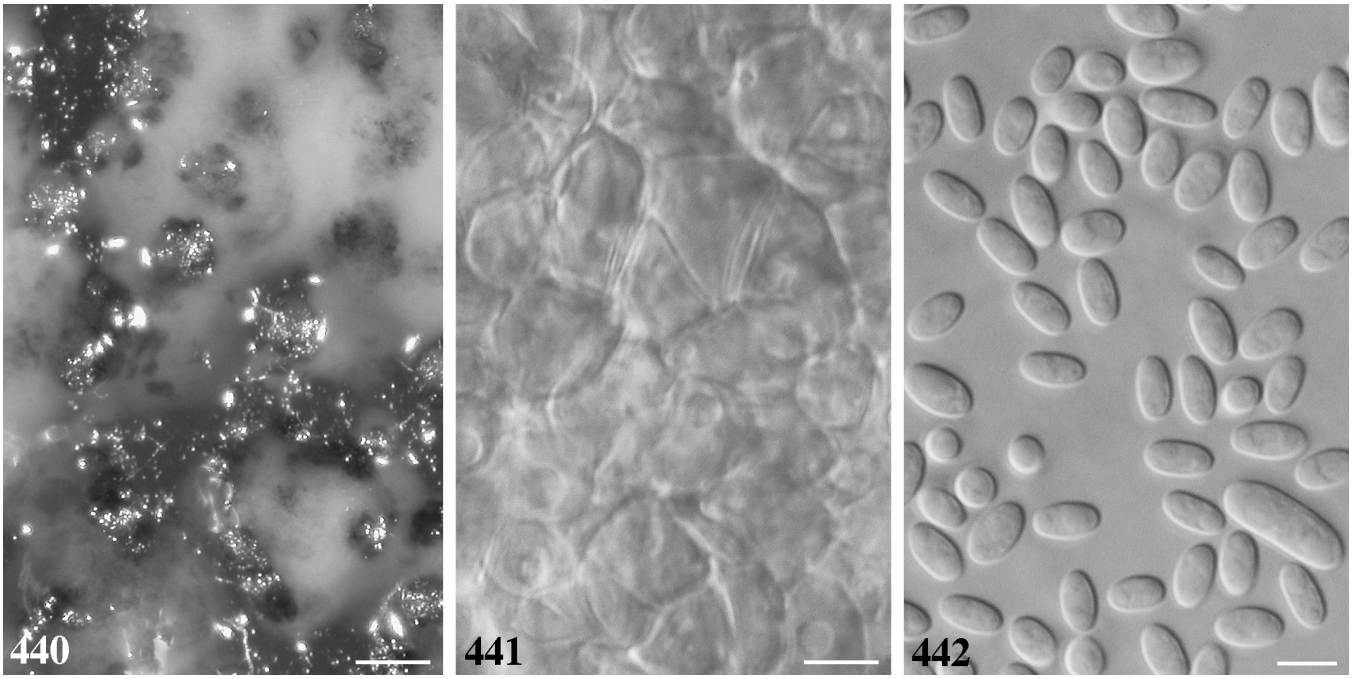
Host range and distribution: *Lsp. cordifolium* (**South Africa, GT**) (Gorter, 1976).

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

In Hawaii, a minor leaf spot disease has been associated with a *Phoma* sp. Symptoms appear when harvesting activities allow the pathogen to enter into the stem, resulting in canker and leaf death. Whether the isolated *Phoma* sp. is the cause of the disease remains



Figs 433–439. *Phloeosporella protearum*. Fig. 433. Lesions on *Protea* stem. Fig. 434. Vertical section through a conidioma. Figs 435–437. Conidia. Fig. 438. Conidiogenous cells. Fig. 439. Conidiomatal wall layer. Scale bars: 434 = 50 µm, 435–437 = 10 µm, 438 = 5 µm, 439 = 20 µm. (From Taylor *et al*., 2000).



Figs 440–442. *Phoma sorghina*. Fig. 440. Pycnidia oozing conidial masses on MEA. Fig. 441. Aggregate chlamydospores. Fig. 442. Conidia. Scale bars: 440 = 200 µm, 441–442 = 10 µm.

uncertain (Rohrbach, 1984). Benic (1986) frequently isolated *Phoma* spp. from seeds of different species of *Proteaceae*, but not from damped-off seedlings. *Phoma* spp. have also been recorded from stems of *Protea* 'Susara', *P. cynaroides*, *P. neriifolia*, *Lsp.* 'High Gold', *Leucospermum* spp., *Lcd.* 'Safari Sunset' and *Leucadendron* spp. in Spain (Moura & Rodrigues, 2001). A *Phoma* sp. has also been reported as an endophyte (Taylor *et al*., 2001c).

Phomopsis stem canker

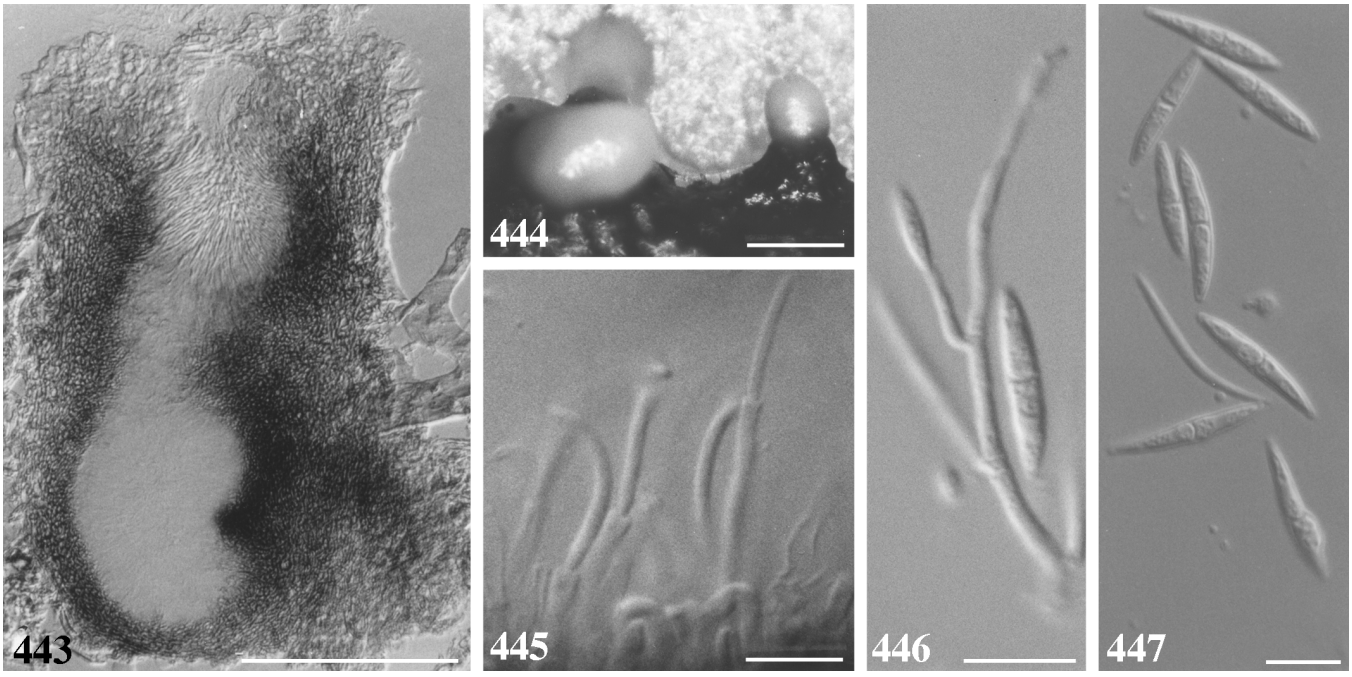
This fungus causes a severe canker and dieback disease on *Protea repens* plants in the Western Cape Province of South Africa. This fungus was found at several localities in the Western Cape Province (Orffer & Knox-Davies, 1989) and has also been observed as the most serious canker disease of *Protea repens* on commercial farms in the Eastern Cape Province. To date this disease is only known from South Africa.

Phomopsis saccharata J.-C. Kang, L. Mostert & Crous, *Sydowia* **53**: 230 (2001). Figs 443–450, Plate 9.

Symptoms: The disease is characterised by dieback of young shoots, diffuse and perennial cankers develop on the older twigs and branches. Young cankers are sunken and maroon-black, while 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 tissue below and above the cankers also becomes discoloured. Pycnidia are often seen on old lesions (Orffer & Knox-Davies, 1989).

Causal organism: *Conidiomata* pycnidial, formed after 33 d on autoclaved *Protea* stems on WA, eustromatic, dark brown to black, almost superficial, covered with hyphae, separate, sparse, ampulliform to barrel-shaped, convoluted, up to 155 µm diam and 170 µm tall; pycnidial wall consisting of two regions: an outer thick-walled region of 10–30 µm, with 3–4 layers of dark brown *textura angularis*, and an inner, pale brown, thinner-walled region of *textura intricata,* 3–10 µm thick. *Conidial masses* hyaline to yellow. *Alpha conidiophores* branched, septate, 7–20 × 2–3 µm. *Conidiogenous cells* filiform, tapering distally, with periclinal thickening, collarette inconspicuous to absent, 13–25 × 1–2 µm. *Conidia* 0–1-septate, fusoid to ellipsoidal with a truncate base and acutely rounded apex, mostly multiguttulate, but also eguttulate, (11–)16–20(–24) × (2.5–)3(–4) µm. *Beta conidiophores* septate, branched, 4–10 × 2–3 µm. *Conidiogenous cells* subcylindrical to reniform, tapering towards the apex, periclinal thickening visible, collarette inconspicuous to absent, 6–26 × 1–2 µm. *Conidia* aseptate, rarely straight, mostly curved to hamate, (15–)18–20(–27) × (1–)1.5(–2) µm; pycnidia with either alpha or beta conidia, or a combination of both conidial types.

Cultural characteristics: *Colonies* flat with smooth margins, zone lines with moderate, fluffy, aerial mycelium after 14 d at 25°C in the dark; predominantly buff (19"d) with white patches, reverse buff (19"d) with greyish sepia (15""i) and some fuscous black (7""k) patches; 5 mm/day at 28°C. Orffer & Knox-Davies (1989) reported that colonies grew from 4–32°C, with optimal growth at 28°C and slow growth below 15°C. The fungus could not be isolated or cultured on media containing streptomycin (0.02%).



Figs 443–447. *Phomopsis saccharata*. Fig. 443. Vertical section through a pycnidium *in vitro*. Fig. 444. Pyncidia oozing conidia. Fig. 445. Conidiogenous cells giving rise to beta conidia. Fig. 446. Conidiogenous cells giving rise to alpha conidia. Fig. 447 Alpha and beta conidia. Scale bars: 443 = 50 µm, 444 = 200 µm, 445–447 = 10 µm. (From Mostert *et al*., 2001).

Host range and distribution: *P. obtusifolia, P. repens* (**South Africa, EC, WC**) (Mostert *et al*., 2001).

Disease cycle and epidemiology: Symptoms generally develop more rapidly during spring than during winter (Orffer & Knox-Davies, 1989).

Control: All unnecessary wounding and damaging of plants should be prevented; other diseases and insects should 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 bigger pruning wounds should be protected with a wound sealant. Avoid planting susceptible species where the disease is known to occur.

Notes: Another *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 seedborne (Orffer & Knox-Davies, 1989). A Phomopsis shoot and stem canker of *Protea* spp. was also recorded in Queensland and that fungus was reported to enter through wounds and cause sunken lesions which result in death of branches and entire plants (Greenhalgh, 1981). Moura & Rodrigues (2001) also recorded a *Phomopsis* sp. on stems of *Protea cynaroides* in Madeira. *Phomopsis* spp. have also been recorded as endophytes (Swart *et al*., 2000; Taylor *et al*., 2001c). Until taxonomic studies are carried out on isolates from these areas it is not possible to know whether they are the same or different from *P. saccharata*.

Poorly understood diseases

Rhizopus mould

Rhizopus sp.

Symptoms: Post-harvest disease, developing on flowers and stems in pack houses, or during export.

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

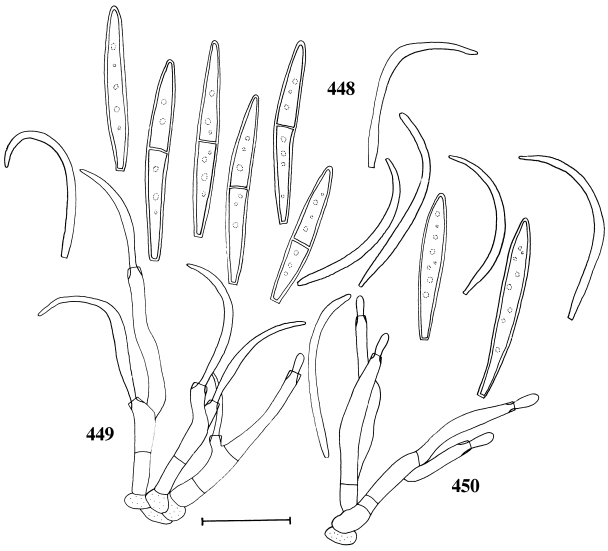
Control: Disease development can be prevented by allowing flowers to dry off and harden either in the field in full sun or if cut, with their stems in the water. Flowers can be sprayed with or dipped in a solution of dichloran and benomyl and dried thoroughly before being packed (Jacobs, 1981; Knox-Davies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

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

Schizophyllum trunk rot

Schizophyllum commune (L. : Fr.) Fr., *Observationes Mycologicae* (Copenhagen) **1**: 330 (1815). Plate 24.

Symptoms: Brown dieback and trunk rot, usually entering the host at pruning wounds, first killing the pruning stub, and sometimes progressing further down the stem.



Figs 448–450. *Phomopsis saccharata*. Fig. 448. Alpha and beta conidia. Fig. 449. Conidiogenous cells giving rise to beta conidia. Fig. 450. Conidiogenous cells giving rise to alpha conidia. Scale bars = 10 µm. (From Mostert *et al*., 2001).

Distinctive small, grey-white frill-like basidiomata are associated with cankered areas. Severely affected plants are often heavily defoliated, have a sparse canopy and support unthrifty growth.

Host range and distribution: *Lcd. argenteum, P. magnifica*, *P. repens* (**South Africa**) (Doidge, 1950; Doidge *et al.*, 1953; Knox-Davies *et al*., 1987; S. Denman, unpubl. data); *Protea* sp. (**U.S.A., Hawaii**) (P.W. Crous & M.E. Palm, unpubl. data).

Control: Possible control measures would include shaping the bushes while they are young so as to avoid cutting out large old branches; limiting the life span of the bushes, i.e. replanting before they are too old in a high-risk area, and finally, but most importantly, applying a fungicide-containing wound sealer immediately after pruning.

Notes: Symptoms are usually only observed on older bushes. The bushes appear in a state of decline and have a thin canopy. Leaf drop is common in *P. repens*. On close inspection of infected branches, a dark canker appears to run the full length of the branch, usually about half the branch in girth, often to the crown. Fruiting structures are frequently seen protruding from the cankered area, which are often initiated from a wound, especially a pruning wound. In commercial situations, *Schizophyllum* infects when old, thick branches are sawed or pruned, leaving a large wound. It seems as though *P. repens* is particularly susceptible.

Sclerotinia dieback

Sclerotinia sp.

Symptoms: Dieback of cuttings and plants in nurseries.

Host range and distribution: *Protea* spp. (**South Africa**) (Benic, 1986).

Control: The disease is associated with wet nursery conditions. By employing cleaner cultivation practices and minimising injury, inoculum and disease levels will be reduced.

Silver leaf disease

Chondrostereum purpureum (Pers. : Fr.) Pouzar, *Česká Mykologic* **13**: 17 (1959).
≡ *Stereum purpureum* Pers., *Neues Magazin für die Botanik* **1**: 110 (1794).

Symptoms: Branches of infected plants develop a silver cast. The fungus eventually sporulates, forming purplish fruiting structures on dead branches (Von Broembsen, 1989).

Hosts range and distribution: *Leucadendron* spp., *Protea* spp. (**New Zealand**) (Von Broembsen, 1989; Forsberg, 1993).

Disease cycle and epidemiology: Fruiting-bodies are flat, later becoming imbricate, 2–8 cm diam, being smooth and purplish underneath, and hairy and tawny on the upper surface. These fruiting structures form on dead wood. Infection occurs via pruning wounds or where mechanical damage has occurred. The fungus apparently spreads from poplars, used as windbreaks, to proteas.

Control: Alternative hosts should not be planted in the vicinity of proteas. Pruning and harvesting tools should be disinfected. Wounds should be protected with a fungicidal sealant (Von Broembsen, 1989).

CHAPTER 3

ROOT DISEASES

Armillaria root rot

Armillaria species are soilborne basidiomycetous fungi that are voracious wood rotting pathogens of trees and shrubs throughout the world (Gregory *et al*., 1991). Species of *Armillaria* have caused losses to *Proteaceae* in Africa (Kenya, Tanzania, South Africa, Zimbabwe) (Doidge, 1950; Gorter, 1977; Masuka *et al*., 1998; Crous *et al*., 2000a; Denman *et al*., 2000a), Australia (Von Broembsen, 1989; Forsberg, 1993; Porter *et al.*, 1996), Madeira (Moura & Rodrigues, 2001), New Zealand (Pennycook, 1989), and the U.S.A. (California, Hawaii) (Laemmlen & Bega, 1974; Farr *et al*., 1989).

Armillaria has only recently been reported from indigenous *Proteaceae* in South Africa (Denman *et al*., 2000a). Presently, species of this genus do not appear to pose a serious threat to the native South African flora or commercial production of these flowers. However, should conditions change that would favour disease development, both the natural proteaceous flora and commercial plantations could be at great risk. In other countries, significant economic losses to indigenous as well as cultivated *Proteaceae* are caused by Armillaria root rot. In Australia, the native *Armillaria luteobubalina* is the main species that causes Armillaria root rot of *Proteaceae* (Kile *et al*., 1983; Pearce *et al*., 1986; Shivas, 1989; Von Broembsen, 1989; Summerell, 1999). Recently, two *Armillaria* species were isolated from proteas in Kirstenbosch gardens in South Africa. Molecular evidence indicated that they are originally northern hemisphere species. This suggests that these fungal pathogens have recently been introduced to South Africa (Coetzee *et al*., 2003). Vigilant quarantine inspections and sensible cultivation practices will thus need to be implemented to avoid a catastrophe in South Africa.

Armillaria gallica Marxm. & Romagn., *Bulletin Trimestriel de la Société Mycologique de France* **103**: 152 (1987).

Symptoms: The first above-ground symptoms include declining, stunted plants with leaf chlorosis which develops from the bottom of the plant upwards (Denman *et al*., 2000a). Some of the branches begin defoliating. When infected plants are removed from the soil, the roots appear blackened and have few feeder roots. White mycelial fans are present in the cambial region under the crown bark.

Causal organism: *Basidiomata* solitary or in small groups arising from roots in the soil. *Pileus* fleshy, ranging in colour from pink to tan, distinctively hairy, 5–9 cm diam, plano-convex. *Lamellae* ivory, becoming dark with age, broad, distant, attached to slightly decurrent; spore print white. *Stipe* centrally attached to the cap, clavate to cylindrical, fibrous, 7–10 cm long, bruising easily. *Annulus* cortinate, white to yellow, with an evanescent, partial veil, leaving white remnants on the stipe. *Rhizomorphs* hard, black, of melanised cortical cells forming cylindrical strands, monopodial, robust, with basidiomata directly attached to them. *Clamp connections* present at the base of the basidia; sub-hymenial tissue binucleate; pigment present in cell walls and vacuoles (Bérube & Dessureault, 1988).

Cultural characteristics: *Colonies* with sparse aerial mycelium; moderately slow-growing, up to 30 mm diam on PDA after 14 days at 25°C. Rhizomorphs developing in culture, long, dark brown to black, cylindrical, fleshy, spidery, extending beyond the colony margins.

Host range and geographic distribution: *Lcd. argenteum, Lcd. gandogeri, P. longifolia, P. scolymocephala* (**South Africa, WC**) (Denman *et al*., 2000a; Coetzee *et al*., 2003).

Disease cycle and epidemiology: Unknown, see section on *A. luteobubalina*.

Control: Since this disease is newly discovered (Denman *et al*., 2000a), experimental work on effective control has not yet been carried out. However, the following guidelines should be used. Avoid establishing *Proteaceae* plantations on sites previously cultivated with trees, particularly if there has been a history of *Armillaria* disease. Ensure that planting material is disease-free and obtained from a reliable pathogen-free source. Remove and burn infected plants on infested sites as soon as possible, taking care to remove as much of the root system as possible. Caution should be exercised if mulching beds with chipped wood and bark since this may be a source of inoculum if derived from diseased, rotted or dead trees or shrubs. Promising developments in biological control with *Trichoderma* species have been reported for control of other *Armillaria* species (Summerell, 1999), and further research should be carried out on control of this potentially serious disease in South Africa.

Armillaria luteobubalina Watling & Kile, *Transactions of the British Mycological Society* **71**: 79 (1978). Plate 25.

Symptoms: Infected plants show poor vigour, leaf yellowing, stunted growth, canopy thinning and limb die-back, and ultimately plants die (Summerell, 1999; Washington, 1999). Shoot tip die-back and plant death is due to infection of the cambial tissues (Porter *et al*., 1996). Resinous gum may exude from the

trunks of infected plants and the surface of infected wood is often pitted in appearance (Summerell, 1999).

Causal organism: *Basidiomata* olive brown to deep yellow, convex becoming broader, dry, covered with small, short, rough scales, pileus 35–90 mm diam. *Lamellae* white but cream or yellowish in age with rusty spots, sinuate, close with both long and short gills. *Stipe* 90–170 mm tall, 10–22 mm wide, tough, with *annulus* near top and small scales below. *Spore print* white. *Rhizomorphs* not readily produced (Summerell, 1999; Washington, 1999).

Host range and geographic distribution: *Proteaceae* (**Australia, Tasmania**) (Shearer, 1994; Summerell, 1999; Washington, 1999).

Disease cycle and epidemiology: The pathogen produces basidiocarps (mushrooms) around the base of infected plants late in autumn (Summerell, 1999; Washington, 1999) or during cool wet weather (Forsberg, 1993). *Armillaria* thrives in moist conditions (Porter *et al*., 1996). In autumn-winter, airborne basidiospores are a source of inoculum (Shearer, 1994), but it is thought that infection only rarely occurs in this way (Porter *et al*., 1996; Summerell, 1999; Washington, 1999). There is debate surrounding the role of rhizomorphs in spread of the pathogen since rhizomorphs are not often produced (Summerell, 1999; Washington, 1999). However, if present, rhizomorphs can grow short distances from the roots of one plant to those of a nearby plant, thus spreading the pathogen (Forsberg, 1993; Washington, 1999). Disease is most likely initiated through introduction of infected plants or contaminated mulch, or via cultivation practices that spread infected pieces of plant tissue, or from existing infection courts including rotting tree stumps and infected root pieces in the soil. Once established in an area, the most common means of disease spread is via root contact, and disease often spreads radially around a central disease centre. Disease spread may be more rapid in close plantings with drip irrigation (Forsberg, 1993). The fungus survives in the soil for extremely long periods, and severe stress (including fertiliser, drought or flood-induced stress) predisposes plants to infection (Summerell, 1999).

Control: Once disease is present in a plantation control is not easily achieved. Von Broembsen (1989) remarked that this disease usually forces producers to abandon the infected land. Prevention is thus better than cure, so it is advisable to choose a site that has no history of Armillaria disease and is free of *Eucalyptus* spp. Washington (1999) suggests a three-year land preparation programme. Trees present on the planned site should be ring-barked 6 months prior to removal and when removed, all material (wood and roots) must be burnt on site. Tree stumps must not be left in or near plantings. Thereafter, the site should be planted with pastures or cereals for 2–3 years prior to establishing the orchard, to give any material remaining in the soil time to decompose (Forsberg, 1993; Washington, 1999). If the site has a history of *Armillaria*, soils should be fumigated before planting (Forsberg, 1993).

Good hygiene is essential to prevent introduction of the pathogen (Summerell, 1999). Ensure plants come from a disease-free source. Soil amendments with organic matter can be expected to retard the activity of the fungus (Summerell, 1999). Prophylactic treatment with appropriate *Trichoderma* biocontrol agents prior to planting may protect roots from colonisation by the pathogen. Plant damage must be avoided (Forsberg, 1993). Exposing the root and crown area of infected plants to air-drying has produced good results (Summerell, 1999). Deep trenching (1 m) and inserting physical barriers between roots may contain the spread of the pathogen in some situations (Summerell, 1999). Chemical control is not effective, although Summerell (1999) claims there is some evidence to suggest that treatment with potassium phosphonates is useful in protecting young trees from infection during establishment, especially where *Armillaria* has been a problem in the past.

Notes: *Armillaria luteobubalina* is widely distributed across the wetter parts of Queensland (eastern Australia) and also in the southern and western reaches of Australia (Shearer, 1994; Summerell, 1999) and in Tasmania. Armillaria root rot is particularly severe on *Proteaceae* in the east near Sydney (Denman *et al*., 2000a). Serious economic losses can be attributed to this pathogen in Australia on both native and South African *Proteaceae* (Summerell, 1999; Washington, 1999).

Armillaria luteobubalina has not been reported from *Proteaceae* in New Zealand, although other species of *Armillaria* have been recorded on *Proteaceae* in that country. Both *Armillaria limonea* and *Armillaria novae-zelandiae* were found on *Knightia excelsa* and the latter was reported on *Grevillea robusta* too (Pennycook, 1989).

Armillaria mellea (Vahl: Fr.) P. Kumm., *Der Führer in die Pilzkunde* (Zerbst): 134 (1871). Plate 26.

Symptoms: The disease symptoms on *Proteaceae* are not well documented but it appears that infection with *A. mellea* ultimately leads to death of plants (Moura & Rodrigues, 2001). Coetzee *et al*. (2003) isolated this pathogen from declining proteas exhibiting stunted growth. The lower leaves of infected plants were chlorotic, and defoliation was evident on branch tips. Main roots were blackened and there was a general lack of feeder roots. White mycelial fans were evident below the bark of infected plants. Black "shoe-string" rhizomorphs may form below the bark and run along the roots into the soil. Clusters of honey-coloured mushrooms (basidiomes) may occur at the base of infected

plants. Neither the mushrooms nor the rhizomorphs have been reported on indigenous *Proteaceae* in South Africa.

Causal organism: *Basidiomata* are characteristically clustered, caespitose, in groups of 8–10, from white fan-like mycelium or from rhizomorphs. *Pileus* fleshy, convex to planoconvex and sometimes plane in shape, 4–9 cm diam, ranging in colour from pale yellow, honey-beige to cigar brown, smooth, without squamules (Bérube & Dessureault, 1988; Volk, 2002). *Lamellae* are ivory-coloured becoming dark with age, close, decurrent, spore print white. *Stipe* centrally attached to the cap, 4.5–14.5 cm long, broad at the apex, cylindrical, tapered at the base. *Annulus* white to yellow, thick, woolly, irregular, decurrent fibrils joined to the stipe. Spores 7–9 × 5.5–6.5 µm (Volk, 2002). *Rhizomorphs* long flattened, black, melanised, belt-like, dichotomously branched, rare. No clamp connections at the base of the basidia. Sub-hymenial cells uninucleate; pigmentation restricted to the vacuoles (Bérube & Dessureault, 1988).

Cultural characteristics: *Colonies* with sparse aerial mycelium, white to creamy beige, woolly at the margin, dark brown and sclerotic in the centre; slow-growing, 20 mm diam after 14 d on MEA at 25°C. Rhizomorphs abundant in culture, long-cylindrical, spidery, extending beyond the colony margin (Coetzee *et al*., 2003).

Host range and geographic distribution: *P. cynaroides* (**Madeira**) **(**Moura & Rodrigues, 2001); *Protea* sp. (**South Africa, WC**) (Coetzee *et al*., 2003).

Disease cycle and epidemiology: Unknown, see section on *A. luteobubalina*.

Control: Since this disease is newly discovered on *Proteaceae* (Denman *et al*., 2000a; Moura & Rodrigues, 2001) experimental work on effective control has not yet been carried out. *A. mellea* is considered to be a very aggressive pathogen, therefore great care should be taken when bringing soil, wood chips or composts made from tree bark into nurseries or plantations. The general guidelines for control of Armillaria diseases should be followed.

Notes: The lack of formation of rhizomorphs and scarcity of basidiomes in the African species makes identification of *Armillaria* from these regions difficult (Swift, 1968). Identification must be based on the morphological features that are present, mating tests between single-spore isolates, and sequencing of various parts of the pathogen genome to reliably identify the fungus to species. The *Armillaria* species reported on South African *Proteaceae* in localities other than South Africa, *viz.* Madeira (Moura & Rodriguez, 2001) and Zimbabwe (Masuka *et al*., 1998), may not be the same species as reported in South Africa; further studies are required to confirm their identity.

Charcoal rot

Macrophomina phaseolina (Tassi) Goid., *Annali della Sperimentazione Agraria* (Rome) **1**: 457 (1947).
≡ *Macrophoma phaseolina* Tassi, *Bolletine del Laboratorio ed Orto botanico Siena* **4**: 9. 1901.

Symptoms: *Macrophomina* has been associated with root and collar diseases of drought-stressed *Proteaceae* in nurseries (Benic, 1986). Infected plants are slightly yellow in colour and lack vigour.

Causal organism: *Conidiomata* solitary or clustered, hard, dark brown, immersed, becoming erumpent, 100–200 µm diam, ostiolate. *Conidiophores* hyaline, short, obpyriform to cylindrical. *Conidia* hyaline, ellipsoid to obovoid, 14–30 × 5–10 µm. *Sclerotia* hard, smooth, black, 100–1000 µm diam.

Cultural characteristics: *Colonies* black, mycelium flattened; moderate growth on PDA at 25°C; sclerotia forming readily in CLA, well-spaced throughout the medium, giving it a spotted look.

Host range and geographic distribution: *Protea* spp. (**Canary Islands, Tenerife**) (Bethencourt Díaz *et al*., 2001); *Protea* spp. (**South Africa, WC**) (Benic, 1986).

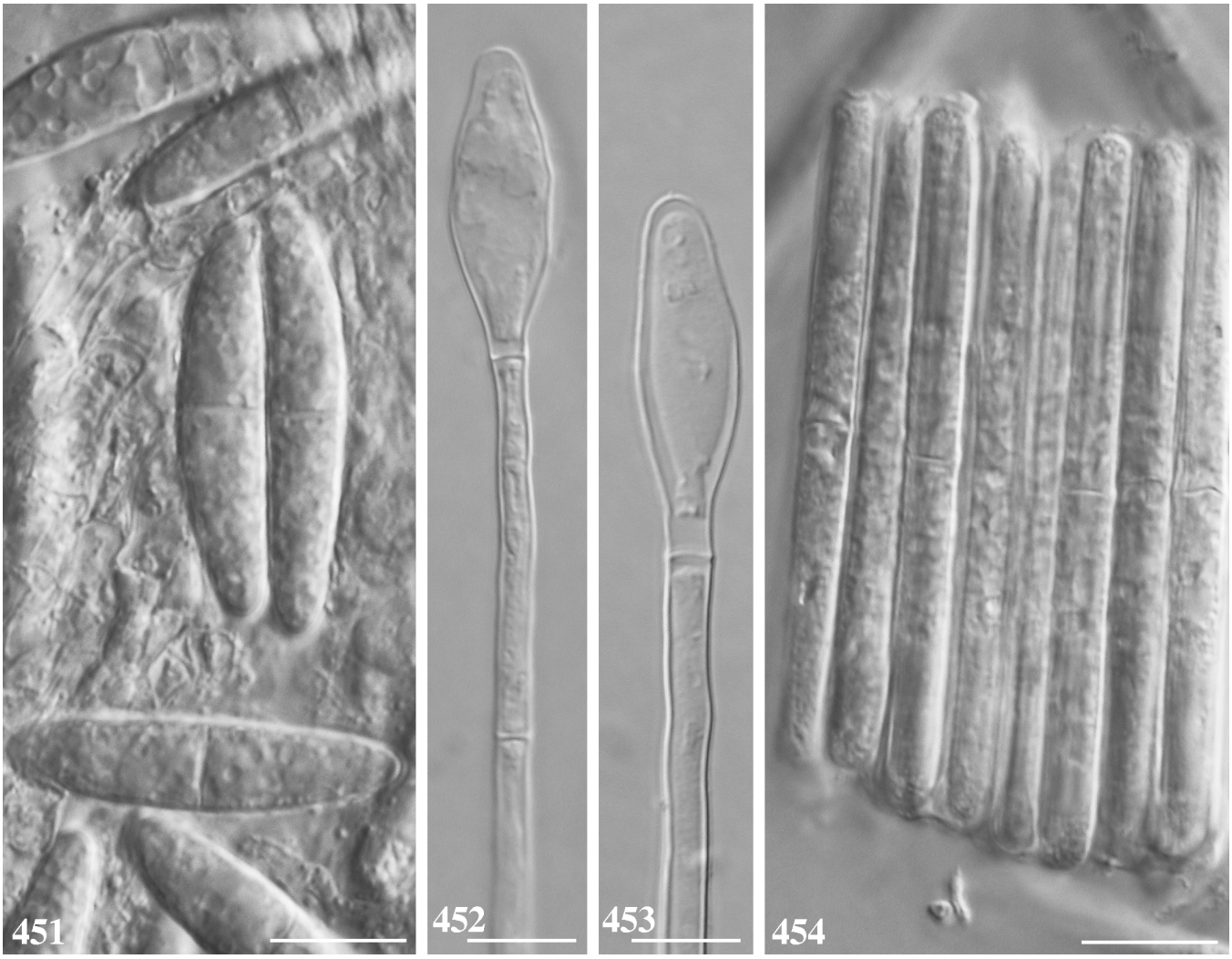
Notes: Von Arx (1981) referred to this fungus as *Tiarosporella phaseolina* (Tassi) Aa, a treatment not accepted by Sutton (1980) or Nag Raj (1993). We use the name to which it is most commonly referred, *M. phaseolina*, but further research is required to clarify the taxonomy of this species.

Cylindrocladium root rot

Cylindrocladium species are reported to be associated with damping-off and cutting end rot of *Proteaceae* in nurseries (Forsberg, 1993; Porter *et al*., 1996) and with root rot of mature *Proteaceae* in Hawaii (Anon., 1991; Crous, unpubl.). Recently Crous (2002) elucidated morphological and molecular differences between the genera *Cylindrocladium* and *Cylindrocladiella*. Most of these records from *Proteaceae* have not been confirmed and the species involved need further study.

Calonectria pauciramosa C.L. Schoch & Crous, *Mycologia* **91**: 289 (1999). Figs 451–454, Plate 18.
Anamorph: ***Cylindrocladium pauciramosum*** C.L. Schoch & Crous, *Mycologia* **91**: 289 (1999).

Symptoms: This fungus causes damping-off, root rot and basal rot. Two types of damping-off symptoms have been observed. In pre-emergence damping-off,



Figs 451–454. *Calonectria pauciramosa* and its anamorph *Cylindrocladium pauciramosum*. Fig. 451. Ascospores. Figs 452–453. Obpyriform to broadly ellipsoid vesicles. Fig. 454. One-septate conidia. Scale bars = 10 µm.

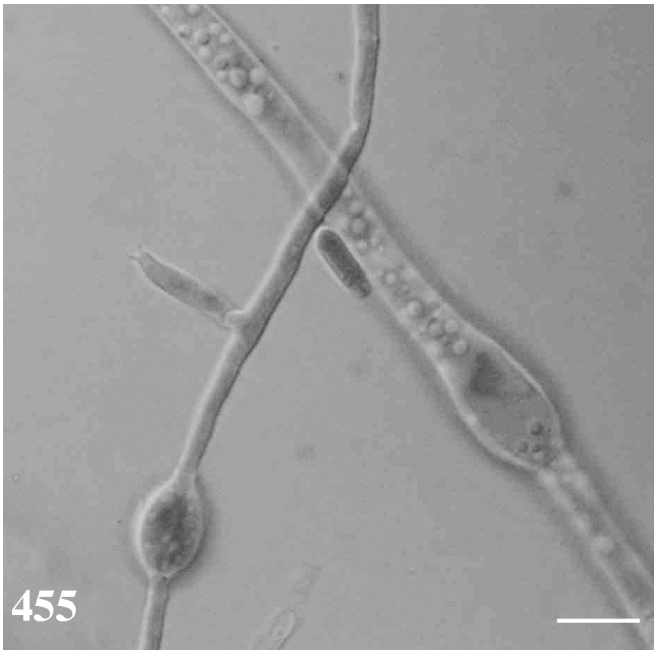
seedlings fail to emerge and rot in the soil (Forsberg, 1993). In post-emergence damping-off seedlings emerge but then decay or rot, often at the soil surface, and the seedling falls over and decays. Typically the root and basal stem tissues become necrotic, water-soaked and collapse.

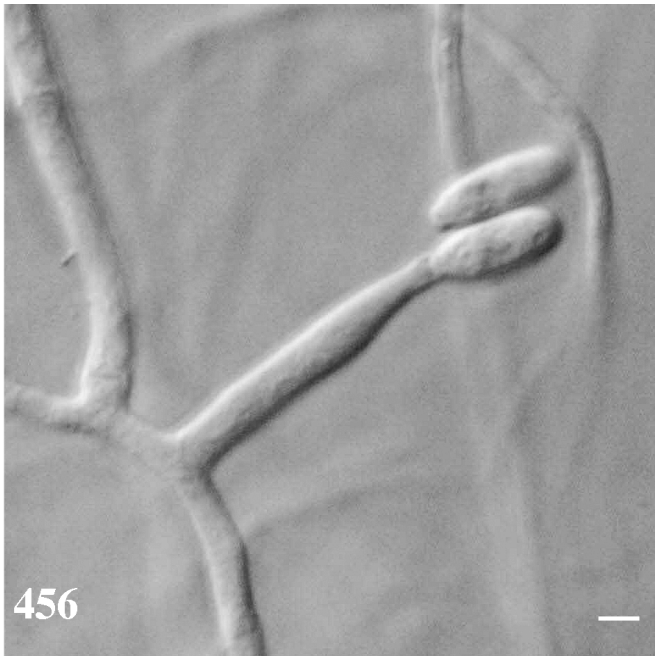
Cutting-end or basal rot is characterised by soft, watery, blackened tissue around the planted end of the cutting. Cankers or blackened tissue extend up the cutting, girdling the stem as the disease progresses (Benic, 1986). Leaves become necrotic and defoliation may occur, and ultimately the plant dies (Benic, 1986).

The symptoms of root rot caused by *Cylindrocladium* sp. on *Proteaceae* in Hawaii include declining plants with a sparse canopy of chlorotic leaves. Stunting and poor vigour is also characteristic. When uprooted, poor root development is evident, roots are blackened and the collar region has soft, necrotic tissue (Crous, unpubl.).

Causal organism: *Ascomata* perithecial, solitary or in groups, orange to red-brown; in section subglobose to ovoid, 250–400 µm high, 170–300 µm diam, apex and body orange to red-brown, base dark red-brown; body turning dark red, and base dark red-brown in KOH+; walls rough, consisting of two thick-walled layers: outside layer of *textura globulosa*, 20–50 µm thick, becoming more compressed towards inner, 5–10 µm thick layer of *textura angularis*, becoming thin-walled and hyaline towards the center, outer cells 40–55 × 15–35 µm, inner cells 5–10 × 3–5 µm; base up to 100 µm thick, consisting of dark red, angular cells, merging with an erumpent stroma, cells of the outer wall layer continuing into the pseudoparenchymatous cells of the erumpent stroma. *Asci* 8-spored, clavate, 70–140 × 8–25 µm, tapering to a long thin stalk. *Ascospores* aggregated in the upper third of the ascus, hyaline, guttulate, fusoid with rounded ends, slightly curved, 1-septate, not or slightly constricted at the septum, (30–)33–38(–40) × 6–7(–8) µm.

Conidiophores consisting of a stipe, a penicillate arrangement of fertile branches, a stipe extension, and a terminal vesicle; stipe septate, hyaline, smooth, 65–100 × 5–6 µm; stipe extension septate, straight to flexuous, 120–230 µm long, 2–3 µm wide at apical septum, terminating in an obpyriform to ellipsoidal vesicle, (5–)7–9(–11) µm diam. *Conidiogenous apparatus* 35–85 µm long, 20–50 µm wide; primary branches aseptate or 1-septate, 12–45 × 5–6 µm; secondary branches aseptate, 15–20 × 5–6 µm, tertiary branches aseptate, 12–15 × 5–6 µm, each terminal branch producing 2–6 phialides;





Figs 455–456. *Fusarium oxysporum*. Fig. 455. Intercalary chlamydospore, and monophialide with microconidium. Fig. 456. Monophialide giving rise to microconidium. Scale bars = 10 µm. (From R.C. Summerbell, unpubl.).

phialides doliiform to reniform, hyaline, aseptate, 10–13 × 2.5–4 µm, apex with minute periclinal thickening and inconspicuous collarette. *Conidia* cylindrical, rounded at both ends, straight, (30–)45–55(–60) × (3.5–)4–5 µm, 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime (Crous, 2002).

Cultural characteristics: Colonies fulvous (13i) in reverse, with abundant white aerial mycelium; chlamydospores extensive, forming microsclerotia; sporulation sparse on aerial mycelia.

Host range and geographic distribution: *Proteaceae* (not specified) (**Australia**) (Forsberg, 1993); *Protea* spp. (**South Africa, WP**) (Crous, 2002); *Banksia prionotes, Leucospermum* spp., *Lsp*. 'Fire Fly', *Lsp.* hybrid '24', *Protea cynaroides*, *P. eximia* and *P. nerifolia* (**USA, Hawaii**) (Anon., 1991).

Disease cycle and epidemiology: Unknown.

Control: A general programme of nursery sanitation must be followed to prevent disease from developing. Sterilise or pasteurise planting media, never re-using pots, planting bags or medium, and do not bring containerised plants from other nurseries into the nursery. Disinfest working surfaces and equipment regularly. A disinfectant footbath and facilities for washing hands must be provided. The nursery area must be fenced off. Pets should not to be allowed within the provided weed-free, bare soil areas in and around the nursery. Elevate planting bags above the ground on free-draining racks/benches and place chipped stone as a floor covering is to be placed under the benches. Remove and burn infected material. Irrigation must be kept to a minimum since wet conditions promote most nursery diseases, including Cylindrocladium rot. Plants must have adequate spacing to allow maximum aeration between the leaves. Lowest leaves on cuttings should be removed to allow air movement between the leaves and the soil surface, thus aiding drying of the soil surface.

Recommended fungicides should be applied preventatively. Benomyl combined with mancozeb applied every fortnight is recommended (Protea Disease Letter, 1991). Mancozeb is a contact fungicide that will only provide preventative protection to the plant if susceptible plant parts are covered prior to contact with infective propagules. This chemical could be applied as a seed treatment as recommended for damping-off caused by *Colletotrichum* (Benic & Knox-Davies, 1983b). Soil drenches with fungicides may also help combat this disease but research needs to confirm this hypothesis and select suitable fungicides. Although benomyl is a highly effective systemic fungicide it is likely to be withdrawn from the market in the future and farmers should try not to develop reliance upon this chemical but should rather attempt disease management through integrated approaches. Iprodione, fenarimaol and tebuconazole are registered on ornamentals in South Africa and should be tested against this disease.

Notes: See section on *Phytophthora* below for extensive guidelines on nursery sanitation.

Fusarium wilt

Fusarium species are widespread, soilborne fungi, many of which are saprobic or non-pathogenic, opportunistic symbionts on *Proteaceae*. Some species are, however, serious pathogens of these hosts. The most serious and destructive of these, which was only recently recognised as such, is Fusarium wilt caused by *F. oxysporum* (Swart *et al*., 1999a). Other *Fusarium* species have been reported to cause damping-off

(Greenhalgh, 1981; Benic, 1986; Forsberg, 1993; Porter *et al*., 1996), blight (Benic, 1986) and root rot of propagation material (Benic, 1986; Anon., 1991). Fusarium wilt has been observed on species of *Protea* (Swart *et al.*, 1999a), *Leucospermum* and *Leucadendron* (unpubl.). Currently, *Fusarium* can be regarded as the second most important root disease of *Proteaceae* in South Africa after *Phytophthora*, and its occurrence is increasing rapidly in commercial plantations, particularly in the summer rainfall regions of southern Africa.

Fusarium oxysporum Schltdl. : Fr. *Flora Berolinensis, 2, Cryptogamia* (Berlin) **2**: 139 (1824).
Figs 455–456, Plates 27, 28.

Symptoms: Infected plants have leaf blackening that begins as irregular patches which coalesce and ultimately cause leaves to die (Swart *et al*., 1999a). During disease development, leaves do not become chlorotic or flaccid, but become stiff and dry after necrosis. Young growth (new flushes) and shoot tips may wilt. A dark, necrotic lesion develops from the roots up the stem, frequently on one side of the stem only. The necrosis extends internally from the bark to the phloem and into the xylem (Swart *et al*., 1999a). Vascular discoloration is clearly evident. The roots are not rotted but remain white and firm, with the exception of the vascular tissue that is red-brown. Disease is recognised on mature (hardened off) plants at a distance in the field by branches or portions of bushes that are dead. In some *Protea* varieties the leaves may become a deep purplish red colour.

Causal organism: *Macroconidia* on CLA abundant on orange sporodochia, 3-septate, straight with a foot-shaped basal cell and attenuated apical cell. *Microconidia* single-celled, comma-, bean- or ellipsoid-shaped, formed in slimy heads on the tips of short monophialides, or individually on mycelial monophialides. *Chlamydospores* thick-walled, single or paired, mostly terminal or lateral, sometimes intercalary, hyaline to refractive yellow-green. *Sclerotia* blue to black, sometimes forming at the base of PDA slants.

Cultural characteristics: *Colonies* circular with smooth margins, ranging in colour from peach to white pink; in reverse peach, white pink to reddish purple on PDA; mycelium fine, felty; approximately 20 mm diam in 4 days at 25°C on PDA.

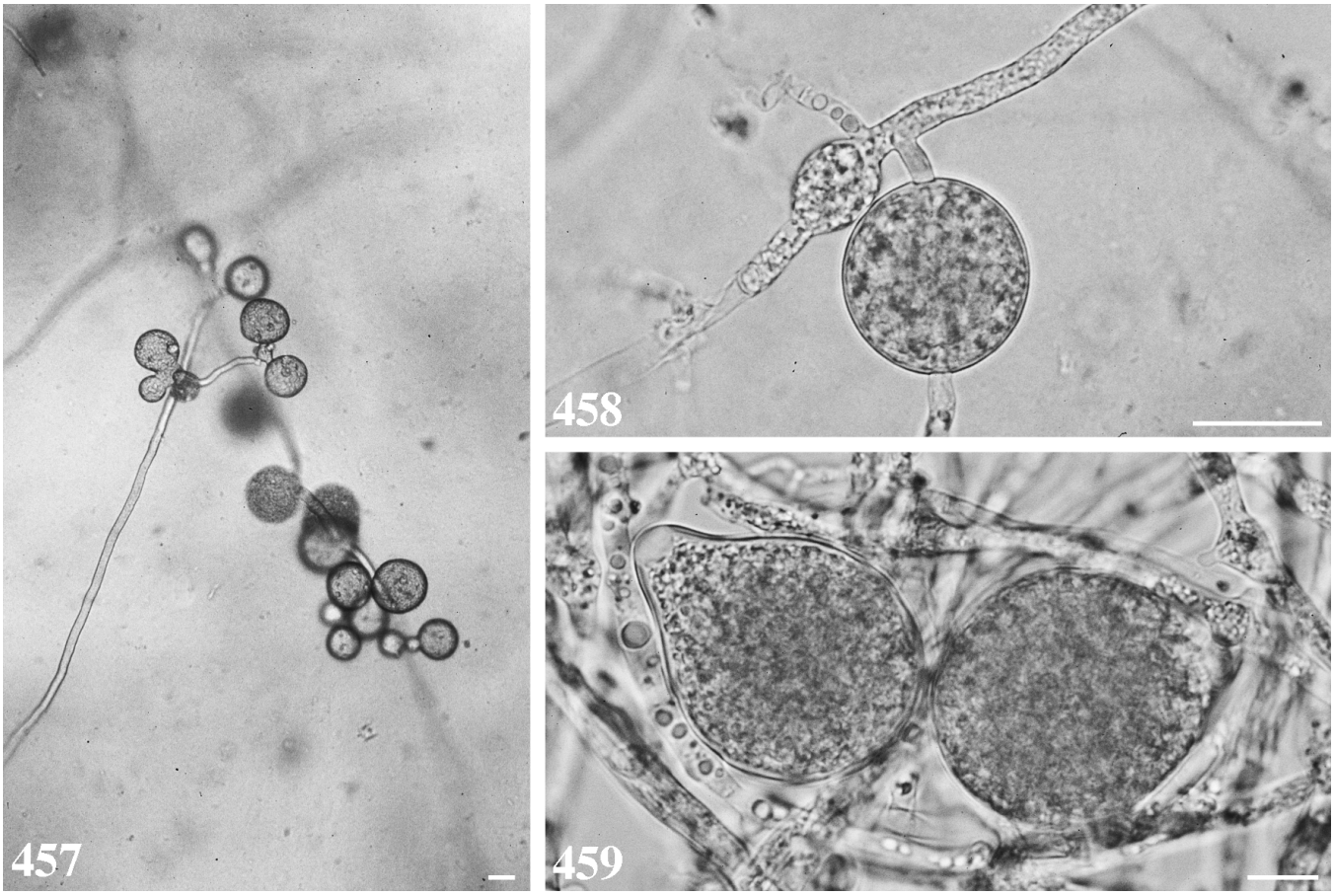
Host range and geographic distribution: *P. repens* and *P.* 'Cardinal', 'Jupiter', 'Pink Ice', 'Susara', 'Sylvia', 'Venus' (**South Africa, NW**) (Swart *et al*., 1999a); *Lsp.* 'Tango' (**South Africa, NW**); *Lcd.* 'Chameleon', 'Pisa', 'Jubilee Crown', *Lsp.* 'Succession' and *P.* 'Venus' (**South Africa, WP**); *Lcd.* 'Blush' and 'Pisa', *Lsp.* 'Succession' (**South Africa, KN**) (*Lsp*. still needs to be confirmed as host via pathogenicity trials); *P. cynaroides*, *P.* 'Pink Ice', 'Sneyd' and 'Sylvia' (**Zimbabwe**) (Swart *et al*., 1999a; Denman, Lubbe & Swart, unpubl. data). Fusarium wilt has also been reported from *Protea* spp. (**Canary Islands, Tenerife**) (Bethencourt Díaz *et al*., 2001).

Notes: In general, Fusarium wilt is a devastating disease, against which there is little control, and management of the disease is difficult (Harveson *et al.*, 2002). Fungicides are generally ineffective. Pre-plant fumigation can reduce inoculum in the soil, but the risks of phytotoxicity of fumigant residues to *Proteaceae* need to be established and costs of fumigation may be prohibitive. Furthermore, one of the most effective fumigants, methyl-bromide, is an ozone-depleting chemical and is in the process of being removed from the market. Other fumigants must thus be considered and the possibility of biofumigation must be explored. Genetic resistance to the pathogen is considered the most stable long-term solution to the problem, although different biological races of the pathogen necessitate multiple resistance genes (Harveson *et al.*, 2002). Since this disease has only recently been recognised on *Proteaceae* (Swart *et al.*, 1999a) much research still needs to be carried out before a screening programme to find inherent resistance can be implemented. No *formae specialis* has yet been named for the fungus on *Proteaceae*.

Suppression of Fusarium wilt on other crops has been recognised in certain soils for more than 70 years. The nature of suppression is fundamentally microbial, but abiotic and soil physico-chemical factors affect the performance of the biological component (Steinberg *et al*., 2002). It would be worthwhile to initiate a search for soils suppressive to Fusarium wilt of *Proteaceae*. The prospects of using antagonistic biocontrol organisms such as *Pseudomonas* and *Trichoderma* spp., or organisms that can induce resistance systemically also need to be investigated, as do the effects of compost amendments. Disease-free nursery material is of the utmost importance in an integrated approach to management of this disease. It is crucial that propagation material selected for cuttings be disease-free. Visual inspection of material is not sufficient to ensure this, and molecular probes may have to be developed to test the material. Once the pathogen has been characterised on a molecular basis, primers should be developed that can be used to test source material and certify it as pathogen-free. Propagation material must be taken from areas where there is no history of Fusarium wilt. Nursery sanitation is also of vital importance, and the use of heat-pasteurised planting medium is recommended.

***Fusarium* spp.**

Symptoms: Benic (1986) associated a *Fusarium* sp. with damping-off and blight of *Protea* seedlings in nurseries in South Africa. Forsberg (1993) reported that cutting rot as well as damping-off diseases caused



Figs 457–459. *Phytophthora* spp. 457. Botryose chlamydospores of *P. cinnamomi.* Fig. 458. Chlamydospore of *P. nicotianae*. Fig. 459. Sporangia of *P. nicotianae*. Scale bars: 457 = 15 µm, 458 = 30 µm, 459 = 10 µm. (From A.W.A.M. De Cock, unpubl.).

by *Fusarium* spp. occurred in Australia. Fusarium root rot has been reported in Hawaii (Anon., 1991) and South Africa (Benic, 1986). Typical damping-off symptoms include both pre- and post-emergence damping-off and necrosis of the cotyledons. Severe blight is characterised by tip die-back of the terminal shoot and leaf necrosis, and in less serious cases mild leaf necrosis will occur (Benic, 1986). In the case of blight, roots remain healthy. In the case of root rot, chlorosis and unthrifty growth of above-ground parts indicate root disease. Upon uprooting, areas on affected roots are blackened and roots break off from rotted parts. In Madeira both *Fusarium equiseti* and *Fusarium solani* have been isolated from diseased roots and crowns of *Proteaceae* (Moura & Rodrigues, 2001).

Host range and geographic distribution: *Proteaceae* (hosts not specified) (**Australia)** (Forsberg, 1993); *Proteaceae* (hosts not specified) (**USA, Hawaii**) (Anon., 1991); *Leucospermum* spp. (*F. equiseti*), *Lcd.* 'Inca Gold', 'Safari Sunset', *Leucospermum* spp. and *Protea* spp. (*F. solani*), *Lcd.* 'Safari Sunset', *Lsp. cordifolium*, *Leucospermum* hybrids, *P.* 'Red Baron', 'Fiery Duchess' (*Fusarium* sp.) **Madeira** (Moura & Rodrigues, 2001); *P. magnifica, Leucadendron* spp. (*F. oxysporum*) **South Africa** (Benic, 1986).

Disease cycle and epidemiology: Unknown.

Control: Largely unknown, although Greenhalgh (1981) reported that a soil drench with benomyl controlled damping-off caused by *Fusarium* spp.

Notes: Diseases caused by *Fusarium* spp. (other than Fusarium wilt) generally are not of major concern to the industry. In nursery situations, damping-off and blight can be avoided by employing good nursery practices including the use of fresh, pasteurised planting medium, applying the recommended seed treatments (Benic & Knox-Davies, 1983; Forsberg, 1993), avoiding over-watering, and spacing plants well to maximise air movement around them. For further information see general guidelines on nursery sanitation in the *Phytophthora* section.

Phytophthora root rot and sudden death disease

A number of *Phytophthora* species are known to affect *Proteaceae*, the most important being *Ph. cinnamomi* and *Ph. nicotianae.* Their extensive distribution and broad host ranges increase the chance of protea plantings becoming infected. Furthermore, *Ph.*

cinnamomi has been isolated from river water and streams (Kliejunas & Ko, 1976; Von Broembsen, 1984a; Von Broembsen & Kruger, 1985), so zoospores of these pathogens can be introduced into areas through irrigation water. Other *Phytophthora* species reported from *Proteaceae* include *Ph. cactorum, Ph. citricola, Ph. cryptogea, Ph. drechsleri,* and *Ph. megasperma* (Hardy & Sivasithamparam, 1988; Tynan *et al*., 1998).

Disease caused by *Phytophthora* species is devastating and usually leads to death of infected plants. Root rot and sudden death are the most commonly encountered diseases caused by *Phytophthora*. Collar rot of *Leucadendron* has frequently been noted in Australia, and this form of the disease has recently been observed in South Africa (WC) as well.

Root rot caused by *P. cinnamomi* is the most important root disease of *Proteaceae* worldwide (Podger, 1972; Van Wyk 1973a; Weste & Law, 1973; Cho, 1981, 1983; Knox-Davies *et al.,* 1986; Von Broembsen, 1989). In South Africa this pathogen causes great losses as it does in indigenous and cultivated *Proteaceae* in Australia (Forsberg, 1993; Shearer, 1994).

Phytophthora cinnamomi Rands, *Mededelingen van het Instituut voor Plantenziekten* (Batavia) **54**: 1 (1922). Fig. 457, Plates 29–32.

Symptoms: A variety of disease symptoms are caused by this pathogen, but the most common one is root rot, which can lead to stem girdling resulting in sudden death. Leaves of plants infected by *Ph. cinnamomi* become a pale dull green to yellow colour. The leaves ultimately dessicate but maintain their upright stance on branches. Young shoot tips may droop or wilt. Plant growth may be stunted. Either part or all of the plant suddenly dies. Generally there is a lack of feeder roots, the root system is reduced, and the main roots are blackened. The internal root tissues are discoloured, dark brown, and in wet areas the tissues may be soft. The dark diseased portion may extend into the crown area (Weste & Law, 1973; Dixon *et al*., 1984).

Collar rot is characterised by a blackening of the stem, beginning below the soil surface, and extending along the stem a short way, ultimately girdling the plant. If the bark is peeled away a striking contrast between pale, healthy and dark brown, diseased tissue can be seen. One or two main roots below the necrotic area on the stem may also be black and the fungus can be isolated from those roots as well. In Australia, root and crown rot are the commonly reported disease symptoms on *Proteaceae* (Marks & Smith, 1992). Radial spread of infection resulting in stem girdling frequently occurs.
Phytophthora cinnamomi also causes damping-off of seedlings in seedbeds, and root and collar rot of young plants in nurseries (Benic, 1986).

Causal organism: *Phytophthora cinnamomi* fits into group VI of the taxonomic classification scheme of Stamps *et al*. (1990). *Hyphae* hyaline, coenocytic, coarse, coralloid, often nodose or inflated, especially at the tips, 5–8 µm wide. (Zentmyer, 1980; Erwin & Ribeiro, 1996). Septa usually develop in the hyphae of old cultures, and cytoplasmic flow may be visible in the hyphae of young cultures. *Chlamydospores* thin-walled, spherical or globose, separated from the mycelium by a basal septum, with an inner thickening of the wall (Waterhouse *et al*., 1983), often pale yellowish in colour, at the terminal ends of hyphae in grape-like clusters, or singly, sessile or laterally attached, in a terminal or intercalary position, 31–50 µm diam (Rands, 1922). *Sporangiophores* simple or sympodially branched. *Sporangia* terminal, non-caducous, non-papillate, ovoid, obpyriform or ellipsoid, with a rounded base, both internal (nested) - or external (sympodial) proliferation possible (Erwin & Ribeiro, 1996), 15–122 µm in length (Zentmyer, 1980), but in isolates from *Proteaceae* usually about 40–75 µm long, diameter varying from 11–63 µm (Erwin & Ribeiro, 1996), but in isolates from *Proteaceae* the diameter ranges from 22–45 µm. The reported average sporangial length : breadth ratio of *Ph. cinnamomi* is 1.54 (Ho & Zentmyer, 1977). Zentmyer (1980) warned that environmental and nutritional factors affect sporangial shape and size markedly and these features are thus not very stable taxonomic characteristics. Sporangia can germinate directly with a germ tube or via the production of motile *zoospores,*. approximately 10–30 zoospores are produced per sporangium (Zentmyer, 1980), ovoid, tapering at the anterior end, with a central groove and an anterior tinsel flagellum and a posterior whiplash flagellum, overall length of zoospores with flagella fully extended is approximately 30 µm (Allen & Newhook, 1973). Kliejunas & Ko (1976) indicated, however, that zoospore diameters were less than 20 µm because water infested with zoospores could be passed through a 20 µm pore sieve and yielded colonies of the pathogen. Zoospores encyst after a few hours of motility. Encysted zoospores usually germinate directly by a germ tube but they can germinate by producing a sporangium sometimes referred to as a *microsporangium* (Blackwell, 1949) or by producing a secondary zoospore (Ho & Zentmyer, 1977). *Ph. cinnamomi* is heterothallic and *oospores* form when an A_1 mating strain is paired with an A_2 strain. Both mating types are found in an approximately even ratio on *Proteaceae* in the Western Cape Province, and in some cases both mating types were isolated from the same plant (Denman & Sadie, 2001). Although it has been reported that heterothallic strains of *Ph. cinnamomi* can be induced into selfing in the presence of certain volatiles (Brasier, 1978) or root extracts (Zentmyer, 1980), this has not yet been reported for *Ph. cinnamomi* on *Proteaceae*. *Oogonia* are round with a tapered base, smooth-walled, hyaline, ranging from 21–50 µm diam, terminal. Antheridia single, amphigynous, cup-shaped, range from 18–22 × 16–17 µm and are devoid of contents after fertilisation has taken

place. A single *oospore* is formed per oogonium after successful fertilisation. Oospores are plerotic and have a thick wall, and when viewed at the base of a Petri dish through a dissecting microscope appear as dark yellow discs ringed with a reddish coloured wall (Denman & Sadie, 2001). Zentmyer (1980) reports that the diameter of oospores is affected by the type of culture medium used, and temperature at which crossing occurred, but in general oospores range between 25–50 µm in diam.

Cultural characteristics: *Colonies* on PDA at 25°C after 4–7 days somewhat variable but distinctive, white, camellia or rose-shaped, mycelium extending through the culture medium in lobes that protrude beyond the aerial mycelium; 40–60 mm diam in 4–7 days on PDA at 25°C.

Host range and geographic distribution: Zentmyer (1980) compiled a comprehensive list of Proteaceous hosts of *Ph. cinnamomi* indigenous to **Australia**; *Lcd. argenteum, Lcd. discolor, Lcd. rubrum, Lsp. reflexum* (as *Lsp. reflexans*), *P. neriifolia, P. obtusifolia* (**U.S.A., California**) (Raabe, 1978); *Lsp. cordifolium, P. cynaroides* (**U.S.A., Hawaii)** (Kliejunas & Ko, 1976; Cho, 1983); *Lcd. argenteum, Lcd. salicifolium, Lcd. tinctum, Lsp. catherinae, Lsp. cordifolium, Lsp. glabrum, Lsp. lineare, Lsp. reflexum, Lsp. tottum, P. magnifica* (**South Africa, WC**) (van Wyk, 1973a, b; Von Broembsen, 1979). However, pot trials revealed *Lcd. salicifolium* (as *Lcd. Strictum), Lsp. conocarpodendron, P. eximia, P. grandiceps, P. longifolia, P. magnifica* (as *P. barbigera*)*, P. longifolia* var. *minor* (as *P. minor*)*, P. mundii, P. punctata* and *P. repens* to be less susceptible than the other hosts listed above (van Wyk, 1973b). Von Broembsen (1984b) added the following South African hosts, *Lcd. album, Lcd. comosum, Lcd. daphnoides, Lcd. discolor, Lcd. galpinii, Lcd. gandogeri, Lcd. laureolum, Lcd. loranthifolium, Lcd. meridianum, Lcd. microcephalum, Lcd. nobile, Lcd. orientale, Lcd. pubescens, Lcd. rubrum, Lcd. salignum, Lcd. spissifolium, Lcd. tradouwense, Lcd. uliginosum, Lsp. conocarpodendron, Lsp. cuneiforme, Lsp. erubescens, Lsp. formosum, Lsp. fulgens, Lsp. grandiflorum, Lsp. muirii, Lsp. mundii, Lsp. patersonii, Lsp. pluridens, Lsp. praecox, Lsp. praemorsum, Lsp. prostratum, Lsp. tormentosum, Lsp. truncatulum, Lsp. utriculosum, Lsp. vestitum, P. aurea, P. cynaroides, P. effusa, P. lanceolata, P. lepidocarpodendron, P. nana, P. neriifolia, P. nitida, P. roupelliae, P. susannae. Lcd. salicifolium* (as *Lcd. strictum*) and *Lcd. xanthoconnus* (? possibly *Lcd. salignum*) were added to the list, respectively, by Von Broembsen & Kruger (1985), and Benic (1986). Many hybrids of *Leucadendron, Leucospermum* and *Protea* are also susceptible to *Ph. cinnamomi*. Von Broembsen (1984b) listed the *Leucospermum* cultivars 'Firefly', 'Gold Dust' and 'Red Sunset' as highly susceptible.

Disease cycle and epidemiology: *Phytophthora cinnamomi* is both a soil- and waterborne pathogen. The waterborne phase is characterised by the production of sporangia and motile zoospores in the presence of sufficient moisture, particularly if soil temperatures are also warm. This process may be repeated many times as long as conditions remain favourable, and this is consequently the stage in which epidemic-producing quantities of inoculum are produced. Thus, wet conditions such as those created by frequent watering or waterlogging alternating with drier conditions would be conducive to the production of vast quantities of zoospores. Zoospores are able to swim small distances (mm) towards their hosts, but they are transported over great distances in free-flowing water, surface run-off water and underground seepage downhill. Zoospores are chemo- and electrotactically attracted to plant roots and encyst before attaching. Zoospore attachment usually occurs in the zone of elongation or on wound sites of roots (Zentmyer, 1980). The pathogen penetrates the host and mycelium rapidly ramifies through the tissues. Although zoospores are considered the main infection propagules, Hill *et al*. (1994) reported that the pathogen can spread via mycelial infection where there is root-to-root contact. Chlamydospores and oospores are formed in infected host tissues within a short time (6–12 days) after root invasion. In South Africa, both mating types of the pathogen occur on *Proteaceae* (Denman & Sadie, 2001) and this suggests a role for the sexual spores (oospores) in the disease, in spite of the conclusion made by Linde *et al*. (1997) that sexual reproduction occurs rarely if at all. Viable oospores would give the pathogen the ability to survive in the soil under adverse conditions and in the absence of the host for many years. Zentmyer (1980) reported that the pathogen could be isolated from soil 6 years after the host had been removed. If sexual reproduction did occur frequently, there would also be a risk of the pathogen developing resistance to some of the systemic fungicides. Podger (1972) also asserts that *Ph. cinnamomi* has appreciable competitive saprotrophic survival ability and the capacity for long saprotrophic survival.

The physiological and biochemical basis of pathogenicity of *Ph. cinnamomi* is little understood (Zentmyer, 1980). The reduced vigour and sudden wilt symptoms associated with infected plants can be attributed to the loss of feeder roots, and malfunction of bigger roots caused by the pathogen. This reduces the plant's ability to absorb and transport soil water and nutrients to the above-ground parts, which places the plant under water and nutrient stress, and finally causes it to die (Von Broembsen, 1989). *Phytophthora*-related deaths are conspicuous in summer in the Southern Hemisphere (January through March) (Von Broembsen & Brits, 1986) but the root damage occurred before that, probably in the spring or previous autumn at the onset of the rainy season.

Control: Prevention of disease is the most effective control measure against *Phytophthora cinnamomi*. Since the pathogen is so extensively distributed in both soil and water, a multifacetted or integrated approach is most likely to yield the best results. When a grower is establishing a plantation, it is absolutely essential to obtain uninfected planting material for establishment. This can be accomplished by purchasing plants from nurseries which utilise strict measures to prevent disease of nursery plants.

To produce disease-free plants, nurseries should be fenced and have limited access. Service vehicles entering the nurseries should drive through a wheel bath since the pathogen can be carried in on soil stuck to tyres. Customer vehicles must be restricted to the (preferably tarred) car park area. Footbaths (dry or wet) must be present at all entrances. Drainage canals must be constructed around the nursery area to lead all surface and run-off water away from the nursery. Pets should not be allowed into the nursery. All pathways should be paved. Plants must be placed on mesh racks that are elevated at least 50 cm off the ground. Wooden slatted benches should not be used because the wood retains moisture and harbours pathogens including the spores of *Phytophthora* that may have been brought into the nursery. The area beneath the plant racks must be covered with a fine chipped stone to reduce the possibility of splash dispersal of the pathogen. Plants must be well-spaced to facilitate air movement and to reduce the risk of splash contamination. There should never be contact between adjacent pots. The water source must be *Phytophthora*-free. Water from rivers or streams must not be used. Municipal or borehole water is usually pathogen-free, but this must be confirmed before use. Water should not be stored in mud or open dams because the pathogen can enter the water source from soil naturally infested around the dam, or can be wind- or animal-dispersed into the water. However, clean, pathogen-free water can be stored in plastic reservoirs that are covered. Hosepipe nozzles must not touch the ground and hosepipes must be hung up above the ground after use and cleaned with a disinfectant before use (Von Broembsen, 1981).

The best way to free soil from pathogens is by steam or heat treatment. Soil treatments with aerated steam (60–62°C) for 30 min is highly desirable because the soil kills pathogens but does not eliminate all of the saprotrophic antagonists (Von Broembsen, 1981). Chemical treatments can also be used but will be less effective if there is a high organic content in the soil, particularly if pieces of roots are present. Von Broembsen (1981) recommends Vapam (sodium N-methyl dithiocarbamate dihydrate) or Basamid (Dazomet) (3,5-dimethyltetrahydro-1,3,5-2H-thiadiazine-2-thione). Both these products break down upon wetting to release (amongst other chemicals) isothiocyanate gas, which plays a major role in killing fungal propagules. The same type of gas can be produced by *Brassica's* or Indian mustard and this is an environmentally more acceptable approach (see below in biocontrol, biofumigation section). Once sterilised, planting media should be stored in disinfested cement bins that are roofed. All media mixing must be carried out on a cement slab that has been disinfested before mixing. If compost is added, it should be broken down into very fine pieces (5 mm) that are first sterilised (essential) and then recolonised with the most appropriate commercial mixtures of composting organisms (see section below on composts). Compost is often a substantial source of pythiaceous fungi in nurseries and thus once it has been sterilised and treated with the correct composting organisms it must be stored in the same way as the sterilised planting mix, i.e. in disinfested, roofed cement bins. Never re-use planting media or planting pots. All footwear and implements must be disinfested before entering the nursery area and before use. A number of disinfectants, such as the quaternary ammonium compounds and peroxide materials are now available. Some of the older recommendations include using a 5% copper oxychloride solution, 4% formalin or 5% sodium hypochlorite (Von Broembsen & Brits, 1986). Containerised plants from other nurseries should not be brought into the nursery. Only select healthy material from the tops of plants for propagation material. Avoid selecting material that has been in contact with the soil or spattered with mud (Von Broembsen, 1981). If seed is used for propagation, it should be heat- and fungicide-treated before planting as recommended by Benic & Knox-Davies (1983) and Benic (1986). All diseased plants in the nursery must be removed and destroyed. Plants must be removed taking care not to drop soil onto other plants: immediate incineration is the best means of destroying material. Remember that, if infected material is disposed into the surrounding area, it will work its way into the soil and irrigation water. Therefore, strong emphasis is placed on immediate destruction of infested planting media and diseased material. The position that the diseased plants occupied on the mesh rack must be disinfested. A certification scheme, in which nurseries are tested for the presence of *Phytophthora* every 4 months, is desirable.

In addition to planting disease-free material, it is important to avoid areas with a history of *Phytophthora* when establishing plantations. *Ph. cinnamomi* is a serious pathogen of avocado trees, eucalypts, grapevines, pineapples and pine trees, among others. Hence caution is to be exercised in planting areas in which these hosts were previously growing. *Proteaceae* should not be established in poorly drained soils. Well-drained, composted soil can be ridged (raised beds) to encourage root growth and facilitate drainage. McLennan (1993) also recommends that any soil or organic material imported to the plantation site must be sterilised. See above for guidelines regarding composts. Never begin establishment of a plantation at the top of a slope, always establish it at the bottom of the slope first and work up to the top of the slope. Ensure that proper drainage canals that lead water away from the plantings are inserted after each planted row, particularly on slop-

ing plantations. Use pathogen-free irrigation water. Municipal or borehole water that has not been stored in dams is usually pathogen-free. Restrict irrigation to a bare minimum but be sure not to drought-stress plants either. Flood irrigation should never be used. Space plants sufficiently bearing in mind their growth and expansion over time. Adequate spacing is essential to ensure air flow through plants which makes conditions unfavourable for foliar pathogens, as well as keeping the lower stem and collar area dry and preventing excess soil moisture build up below the canopy. Once plants are established, Knox-Davies (1981) advises against disturbing them because the risk of injury, particularly root injury, makes plants susceptible to *Phytophthora.* Brits & Von Broembsen (1978) recommend that any cultivation practice that results in soil being disturbed must be avoided, thus ploughing and hoeing should not be practiced. Natural ground cover on the inter-row spaces is recommended (Brits & Von Broembsen, 1978), but care should be taken not to damage plants when mowing the ground cover. The effect of ground cover may reduce sporulation of the fungus (Von Broembsen & Brits, 1986).

Host resistance: The use of resistant rootstocks as part of integrated disease management is highly desirable but to date is not a commercial reality for South African *Proteaceae*. Van Wyk (1973b) provisionally grouped some South African *Proteaceae* in various susceptibility classes. Out of the *Proteaceae* he tested, the most susceptible species were *Lcd. argenteum, Lsp. catherinae, Lsp. cordifolium, Lsp. glabrum, Lsp. reflexum,* and *Lsp. tottum.* Moderately susceptible to moderately resistant species included *Lcd. salicifolium* (as *Lcd. strictum*)*, Lsp. conocarpodendron, P. eximia, P. grandiceps, P. longifolia, P. longifolia* var. *minor* (as *P. minor*)*, P. magnifica* (as *P. barbigera*)*, P. mundii, P. punctata,* and *P. repens*. In the highly resistant species group, *Lcd. uliginosum, P. cynaroides, P. laurifolia, P. neriifolia,* and *P. burchelli* (as *P. pulchra*) were included (Knox-Davies, 1975). Van Wyk & Koeppen (1974) claimed that *Protea cynaroides* was tolerant to attack by *Ph. cinnamomi* due to the presence of *p*-hydroxybenzoylcalleryanin, an antifungal compound found in the bark of the roots. Von Broembsen & Brits (1985) confirmed some of the preliminary resistance ratings made by Van Wyk (1973b): for example, they found *Lcd. uliginosum* to be resistant too. However, their results suggested that *Lsp. reflexum* was not highly susceptible to *Ph. cinnamomi* and therefore they reclassified it as resistant. In addition to the ratings made by Van Wyk (1973b), Von Broembsen & Brits (1985) considered *Lcd. nervosum, Lsp. cuneiforme* and *Lsp. formosum* as having low susceptibiliy to the pathogen. The *Protea* spp. they considered as having resistance or low susceptibility to *Ph. cinnamomi* included *P. caffra, P. compacta, P. lanceolata, P. laurifolia, P. neriifolia, P. nitida* and *P. obtusifolia*. In general, *Protea* species display lower susceptibility to infection by *Phytophthora* than *Leucadendron* or *Leucospermum* species, and *P. compacta, P. cynaroides, P. neriifolia* or *P. repens* can be used to replace *Leucadendron* and *Leucospermum* that have died from *Phytophthora* infections (Von Broembsen & Brits, 1986). Von Broembsen & Brits (1990) reported that various *Leucospermum* breeding lines, particularly T75/11/24 and 153/77/4, developed by the Agricultural Research Council's Fynbos Unit at Elsenburg showed moderate field resistance to infection by *Ph. cinnamomi.* Denman & Sadie (2001) reported that, using a stem inoculation assay on *Leucospermum* hybrids, the shortest lesions were formed on cultivars 'Pink Star' and 'Luteum', whereas in general large lesions were formed on 'High Gold' and 'Helderfontein'. Lesion length on the hybrid 'Spider' was variable. Season and depth of inoculation were important variables affecting results.

Despite many studies, resistance evaluation of *Proteaceae* in South Africa is not yet successfully developed. *In vitro* testing techniques have been variable and problematic (Von Broembsen & Brits, 1985, 1986; Denman & Sadie, 2001), and at best can only be used as a guide to selection of material to be used for further testing. Many factors affect the field performance of *Proteaceae* species and their tolerance to infection. For example, Von Broembsen & Brits (1986) commented that soil conditions and inoculum levels need to be taken into consideration when assessing resistance under field conditions. The seasonal effect on host phenology has also been reported to affect results (Denman & Sadie, 2001). More knowledge on the basic host-pathogen interaction as well as the environmental and ecological factors that affect this interaction is essential before meaningful resistance assessments can be made. During the work carried out to date on evaluating resistance to *Ph. cinnamomi,* it became apparent that even though plants show resistance or tolerance to the pathogen they still become infected. This fact means that resistance must be combined with other disease management practices in order to reduce disease sufficiently to be commercially justifiable. Swart & Denman (2000) demonstrated excellent disease control by using the less susceptible cultivar 'Spider' in combination with chemical control. Natural host resistance to infection will therefore remain an important keystone in integrated disease management, and investment should continue on research into disease resistance.

Assessment of resistance to *Ph. cinnamomi* in Australian *Proteaceae* has received a great deal of attention. A number of studies have demonstrated differences in susceptibility among *Banksia* species (Cho, 1981, 1983). In general, McCredie *et al*. (1985b, c) agreed with results presented by Cho (1981, 1983) but emphasised that susceptibility of *Banksia* was related to the taxonomic group and pattern of rainfall in the area that these plants originated in.

Chemical control: Chemical control of *Phytophthora* is an essential aspect of integrated disease management. During the 1970's, a number of systemic fungicides effective against *Phytophthora* were developed. Although these chemicals do not have good eradicative properties to Phytophthora root and stem

infection of *Proteaceae* once infection has taken place, they have dexcellent prophylactic action against disease (Marks & Smith, 1990, 1992). These systemics have been shown to curtail lesion development and reduce growth of the pathogen. Apart from disease control in plants, the application of certain fungicides as a soil drench also reduces soil populations (inoculum) of the pathogen (Hill *et al*., 1994; Swart & Denman, 2000).

In general, the most effective systemic chemicals are metalaxyl (commonly marketed as Ridomil) and the phosphonates. Metalaxyl worked best when it is applied as a soil drench (Cho, 1983; Swart & Denman, 2000). However, the fungicide is unable to control or reduce lesion length if applied too long (10 d) after infection has taken place (Marks & Smith, 1992). Metalaxyl is also unable to eradicate the pathogen from plant tissues although it does reduce its occurrence and spread (Marks & Smith, 1992; Swart & Denman 2000). Metalaxyl carries a high resistance risk, which means that the pathogen can become resistant to the fungicide and is no longer affected by it. For this reason, only a single application per year is recommended. Furthermore, if repeated applications are applied too soon after the initial soil drench, the chemical is rapidly metabolised in the soil and therefore less available for uptake by the plant. Metalaxyl (soil drenches) can be combined with phosphite (foliar sprays) but metalaxyl applied as a foliar spray is ineffective since it has limited downward translocation in the plant. By combining metalaxyl treatment with the use of resistant rootstocks, significant reduction in disease occurs if disease pressure is not too high (Swart & Denman, 2000).

Von Broembsen & Brits (1986) mentioned that metalaxyl was phytotoxic to *Leucospermum* spp. at rates required to give control under field conditions. However, it appears that the formulation of the chemical is important (S. Denman, unpub. data) and that the granular formulation can be phytotoxic if thrown in a pile on the soil surface. No phytotoxic effects are found when liquid formulations are used or if the granules are thinly distributed over a wide area under the plant canopy. In general, better disease reduction is obtained with metalaxyl compared to fosetyl-Al (Von Broembsen & Brits, 1986, Marks & Smith, 1990; Swart & Denman, 2000). Furalaxyl (marketed as Fongarid), which is closely related to metalaxyl, has also been used with some success (McLennan, 1993). Use of these two fungicides needs to be restricted to one or two applications at the beginning of the season in order to avoid development of resistance in the pathogen.

Due to the resistance risk associated with metalaxyl and furalaxyl, the phosphonate fungicides are indispensible to the protea industry. The latter include fosetyl-Al (an aluminum salt of tris-*o*-ethyl phosponate) (Cohen & Coffey, 1986) and mono- and di-potassium phosphonate (Marks & Smith, 1990, 1992) amongst others. Some of the phosphonate fungicides are now referred to as phosphite, one of the active metabolites of phosphorous acid, which is the simplest of the phosphonate fungicides (Cohen & Coffey, 1986). Fosetyl-Al is effective when applied as a foliar spray because it has excellent basipetal (downward) translocation in plant tissues, but when applied as a root drench was ineffective (Marks & Smith, 1992). Fosetyl-Al and phosphite reduce the rate of disease development (Cohen & Coffey, 1986; Ali *et al*. 1998; Wilkinson *et al*., 2001; Barrett *et al*., 2003) and are thought to trigger host resistance responses (Cohen & Coffey, 1986; Guest & Grant, 1991). These chemicals also have a long residual effect (2 months or more) but when the levels become too low in plant tissues, disease control is overcome (Von Broembsen & Brits, 1986; Marks & Smith, 1992). These chemicals do not kill the pathogen nor reduce its occurrence in tissues (Marks & Smith, 1990; Barrett *et al*., 2003) but they do decrease lesion length and disease development significantly. Barrett *et al*. (2003) suggest that there is an optimum level of phosphite in plants at which maximum disease control is obtained, but they say this is species specific and needs to be investigated for each individual species. However, they recommend phosphite foliar application at 24kg/ha on *Banksias* maintaining that it gives good control with little phytotoxicity. Timing of the fungicide applications is critical and good control of disease can only be achieved if fungicides are applied preventively (Marks & Smith, 1992). Excellent control of disease has also been demonstrated with preventive applications of other phosphonate fungicides (Marks & Smith, 1992; Turnbull & Crees, 1995), providing that disease pressure is not too high. Growers are cautioned not to apply phosphonate fungicides too frequently, as this can lead to leaf burning. There are reports of phosphite toxicity (Komorek *et al*., 1997; Barrett *et al*., 2003). Applying chemicals with the same active ingredients as alternating treatments can cause inadvertent phytotoxicity. For example Fosetyl-Al, phosphorous acid, phosphonic acid (often formulated as potassium phosphonate) and phosphite are all very similar and if applied within 8 weeks of each other can result in a phytotoxic response.

High soil phosphorous that is taken up by plants resulting in high levels in plant tissue may limit phosphite uptake by *Phytophthora* thereby affecting the efficacy of the chemical to control the pathogen (Guest & Grant, 1991; Komorek *et al*., 1997).

Other fungicides have been used to control Phytophthora root rot but with limited success. Dimethomorph (0.6 g a.i./L) applied as a soil drench at 50 mL per L planting medium reduced mortality of *Leucadendron* spp. infected by *Ph. cinnamomi* (Marks & Smith, 1990). Fluazinam applied as a soil drench reduced disease severity on potted *Leucospermum* spp., and decreased soil populations of *Ph. cinnamomi* (Swart & Denman, 2000).

Fungicides are extremely valuable tools in disease control. However, misuses of fungicides, including using the wrong fungicide for the disease, excessive or incorrect applications, poor timing and incorrect dosages, often render them useless or dangerous. All too often protea growers complain that the fungicides are

ineffective, but growers usually apply these chemicals curatively, once they notice disease symptoms. By this time it is too late and too much is expected of the chemicals and the plants. However, by spraying plants two weeks before planting out and then continuing sprays at six weekly intervals, the risk of Phytophthora disease should be reduced significantly, providing that the disease pressure is not too high. Fungicides cannot protect plants against Phytophthora root rot under excessive inoculum loads and highly disease-conducive weather conditions (Turnbull & Crees, 1995; Swart & Denman, 2000). Prophylactic application of phosphonate (phosphite) fungicides is strongly recommended. A single application of metalaxyl or a combination of phosphonate and metalaxyl can be applied if plants become symptomatic. However, growers are reminded that research to date has shown that the curative effects of the fungicides are not satisfactory, so they need to consider the economics of the situation and not bank on total disease control. Fungicide applications must be used in combination with other disease control methods in order to manage the disease.

Biocontrol: Biocontrol in the context discussed here is considered the use of an organism to curtail or stop activity of another organism. In a broader sense, biocontrol would include suppressive soils and the use of composts or organic mulches to reduce disease. Three studies of note have been made on *Proteaceae*. Turnbull *et al*. (1992) discovered that a bacterium, *Pseudomonas cepacia,* showed antagonism to *Phytophthora* and reduced disease in potted protea plants in a nursery environment. However, this protection was not sustainable in the field (Turnbull, 1992). Hardy & Sivasithamparam (1995) investigated the effects of fungi and actinomycetes on the infection capability of *Ph. drechsleri* on *Telopea speciosissima* and concluded that the microorganisms had little effect on the survival of the pathogen. However, infection was reduced and they thought that the mechanism of suppression was complex and could not be attributed to a single taxon. El-Tarabily *et al*. (1996) found that *Micromonospora carbonacea, M. chalcea* and *M. purpureochromogenes* produce enzymes that lyse the hyphae of *Ph. cinnamomi*. *M. carbonacea* in conjunction with *Streptomyces violascens* had a synergistic effect on suppressing root rot caused by *Ph. cinnamomi* on *Banksia grandis*. In general, pathogens are difficult to control using biological methods once infection has taken place, and success with biocontrol will probably only be achieved if the appropriate organisms are applied as preventive treatments. Furthermore, the long-term survival of biocontrol organisms in the rhizosphere under field conditions needs to be addressed in order to ensure a lasting effect.

Suppressive soils: Soils that are suppressive to *Ph. cinnamomi* have been documented (Broadbent & Baker, 1974; Ko & Shiroma, 1989). Broadbent & Baker (1974) found that certain natural soils in the rain forests of Queensland in Australia were suppressive to *Ph. cinnamomi,* especially on avocado pear trees. Suppressiveness could be induced by mulching with green manure and chicken manure (Ashburner system); the mechanism was attributed to high microbial populations, high levels of organic matter, and high exchangable calcium, magnesium and nitrogen. Ko & Shiroma (1989) reported that in Hawaii, 40% of the soils sampled were suppressive to *Ph. cinnamomi* and suppressiveness appeared to correlate with soil pH. At a pH of 4 or less, or 8 and above, soils were suppressive, while between pH 4–8 soils were conducive to the pathogen. This is in contrast to Broadbent & Baker (1974) who recommended that soil pH be maintained above 6 to maintain high populations of antagonistic microorganisms. Soil suppressiveness can be enhanced by the addition of organic mulches. A relatively new concept using decomposing tissues of *Brassica* to suppress pathogen activity, a process called biofumigation, has recently been tested on *Proteaceae in vitro* (Dune, 2002). Dried tissues of *Brassica juncea* completely inhibited the growth of *Ph. cinnamomi* and *Ph. cactorum*, but had no effect on *Ph. cryptogea* and *Ph. megasperma*. The suppressive effect has been attributed to the production of volatile and non-volatile compounds produced in the decomposing process. This suggests that there may be good possibilities for the reduction of *Phytophthora* populations in soil infested with *Ph. cinnamomi* or *Ph. cactorum*.

Composts and organic mulches: The use of organic mulches and composts holds great promise for disease amelioration. Dixon *et al*. (1990) demonstrated a reduction in Phytophthora root rot on *Banksia* spp. when mulches of oat hay and chicken manure, or indigenous vegetation litter were added to the planting medium. However, there appeared to be species-specific effects of the mulches. Oat hay mulched with chicken manure is commonly used in Australian avocado orchards as part of integrated control against root rot caused by *Ph. cinnamomi* (Pegg *et al.,* 1982) and also suppressed disease on *Banksia* in some cases (Dixon *et al*., 1990). However, as a note of caution, some *Proteaceae* are sensitive to the high phosphorous content of chicken manure, thus phytotoxicity could occur if this ingredient is used. In studies carried out in Australia with *Telopea speciosissima* infected by *Ph. citricola, Ph. drechsleri* and *Ph. nicotianae,* the frequently occurring *Ph. drechsleri* was suppressed by using planting media amended with composted eucalyptus bark (Hardy & Sivasithamparam, 1991a, 1995). *Ph. cinnamomi* infection of *Banksia* was also suppressed by planting in the composted eucalyptus bark media (Hardy & Sivasithamparam, 1991a). The nature of suppression was biological because steaming the compost killed the microbes and rendered the compost conducive to disease development. The efficacy of the compost was also dependent upon its age (stage of maturity). The suppressiveness of composts could be enhanced by the addition of certain saprotrophic organisms such as *Penicillium, Streptomyces* and *Trichoderma* species. Compost tea made by infusing 2 L of composted eucalyptus bark in

1 L water for 24 h suppressed sporangial production of *Ph. cinnamomi, Ph. citricola, Ph. drechsleri* and *Ph. nicotianae* (Hardy & Sivasithamparam, 1991b). Suppression of sporangial formation would result in much lower inoculum and therefore in lower disease pressure. Because organic mulches and composts help moderate the effects of temperature and moisture fluctuation (Knox-Davies, 1981), suppress disease and promote microbial activity, their use in integrated disease management is essential. However, the most suitable mulch or compost types for different soil types and proteaceous hosts must be investigated. Protocols for making mulches and composts need to be standardised so that reliable results are achieved. There may be potential to amend composts and organic mulches with organisms that are antagonistic to *Phytophthora.* It is critical to establish the effects of microorganisms on the pathogen because some bacteria and fungi stimulate growth and formation of sporangia and oospores instead of suppressing the pathogen (Malajczuk, 1983). Thus, there is a need for detailed research on the best organic bases to use for composts or mulches, and the appropriate organisms with which to amend these bases. If mulches are used in cultivation, growers must be careful not to overwater plants since mulches reduce water loss from the soil. Unless irrigation schedules are adjusted accordingly, overwatering could occur.

Phytophthora nicotianae Breda de Haan, *Mededeling's Lands Plantentuin* (Batavia) **15**: 57 (1896). Figs 458–459.

Symptoms: Root and collar rot, tree decline and death are symptoms commonly associated with *Ph. nicotianae* infection of *Proteaceae* (Anon., 1991, 1993; Forsberg, 1993). Damping-off of seedlings and cutting-end rot can also be caused by this pathogen, especially in nurseries (Forsberg, 1993).

Causal organism: *Phytophthora nicotianae* fits into group II of the taxonomic classification scheme of Stamps *et al*. (1990). *Hyphae* coenocytic, uniform to slightly irregular, 3–11 µm wide, mostly smooth but often slightly inflated or tuberculate, especially at tips, hyaline to pale yellow. Intercalary hyphal swellings up to 40 µm diam, with four or five radiating hyphae that arise from the walls of the hyphal swellings (Waterhouse *et al*., 1983). *Chlamydospores* mostly abundant (Erwin & Ribeiro, 1996), hyaline to yellow-brown, terminal or intercalary, delimited from mycelium by a septum, spherical, (22–)33(–52) µm, wall 3–4 µm thick (Hall, 1993). *Sporangia* produced abundantly in liquid culture in light or dark (Hall, 1993), especially abundant when incubated under high oxygen concentration, formed singly or in loose sympodia on elongated sporangiophores, mostly spherical or ovate, some ovoid or pyriform, with a prominent papilla and often two or occasionally three papillae, non-caducous (Erwin & Ribeiro, 1996), hyaline to pale yellow or golden-brown, (14–)43(–74) µm long, (12–)36(–60) µm wide, length: width ratio 1.1–1.7, average 1.3. *Zoospores* 7–11 µm long, biflagellate, heterothallic. *Oogonia* delimited from hyphae by a septum, spherical, (16–)26(–36) µm, with a characteristic oogonial stalk that is often eccentric (Waterhouse *et al*., 1983). *Antheridia* amphigynous, cylindrical and unicellular, 10–12 × 9–10 µm (Hall, 1993). *Oospores* aplerotic, thick-walled (2 µm), smooth, spherical, yellow when mature, 12–34 µm diam.

Cultural characteristics: *Colony* morphology is uniform without any specific pattern (Hall, 1993; Waterhouse *et al*., 1983), although Erwin & Ribeiro (1996) report that on PDA colony growth is typically arachnoid. On PDA and cornmeal agar the colony margin is uneven with tufts of mycelium clumping around emerging edges of the culture. The uneven mycelium clumping gives cultures an almost spotty, slightly moth-eaten appearance. On V8 agar the cultures develop more abundant aerial mycelium and are not so distinctive. Growth rate on V8 agar at 25°C ranges from (3–)6(–9) mm/day (Hall, 1993). Cardinal temperatures: minimum 10–12°C (Hall (1993) reports stasis only at 5°C), optimum 24–30°C, and maximum 37°C, although some isolates can grow at 40°C (Hall, 1993). *Ph. nicotianae* is generally a warm-climate species and is reported to grow well at 35°C (Hall, 1993; Waterhouse *et al*., 1983).

Host range and geographic distribution: *Proteaceae* (**Western Australia**) (Hardy & Sivasithamparam, 1988); *Lcd.* 'Safari Sunset', a *Lsp.* hybrid (**U.S.A., Hawaii)** (Anon., 1991, 1993)

Disease cycle and epidemiology: Unknown.

Control: See section on *Phytophthora cinnamomi.*

Pythium root rot

Pythium vexans de Bary, *Journal of the Royal Agricultural Society of England* (London) **12**: 255 (1876). Fig. 460.

Symptoms: Root rot.

Causal organism: *Hyphae* coenocytic, hyaline, fine, silky, up to 5 µm wide. *Sporangia* terminal or intercalary, globose to ovoid or pyriform, occasionally proliferating (Van der Plaats-Niterink, 1981). *Zoospores* produced in an ephemeral, spherical vesicle formed at the end of a short evacuation tube. *Oogonia* thin-walled, mostly terminal on short lateral branches but sometimes intercalary (Van der Plaats-Niterink, 1981), (16–)20(–24) µm diam. *Antheridia* usually single, occasionally two per oogonium, mostly monoclinous, occasionally diclinous, stalked, arising at some distance from the oogonium; antheridial cells

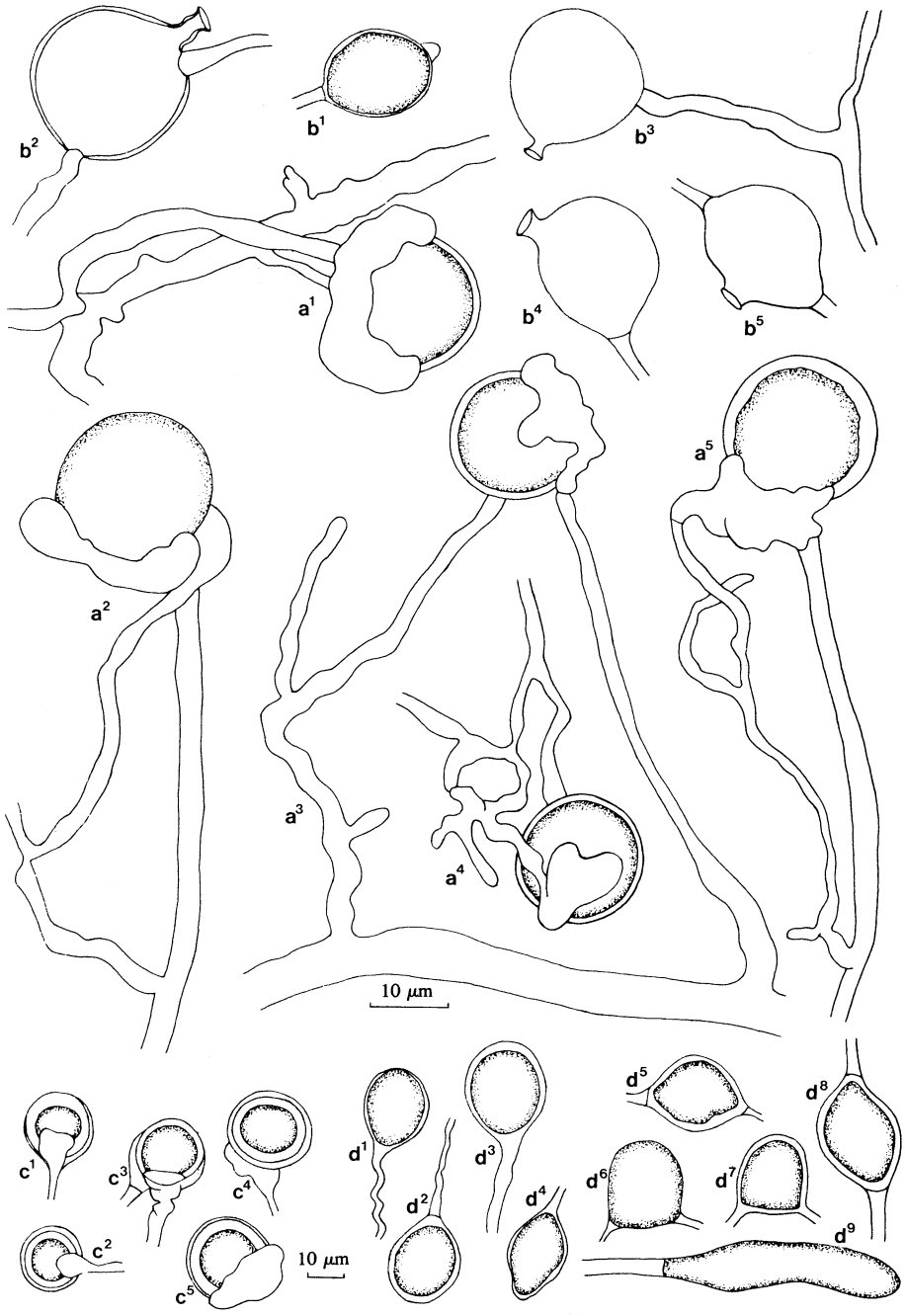


Fig 460a–d. *Pythium vexans*. $a^{1–5}$, $c^{1–5}$. Oogonia and antheridia, $b^{1–5}$, $d^{1–5}$. Sporangia with papilla and discharge tubes, and hyphal swellings. Scale bars = 10 µm. (From Van der Plaats-Niterink, 1981).

large and bell-shaped (Van der Plaats-Niterink, 1981). *Oospores* aplerotic, (14–)17.5(–20) µm, wall 1.5 µm thick.

Cultural characteristics: *Colonies* with no distinctive pattern, aerial mycelium cottony; growth rate 18 mm/day on potato-carrot agar at 25°C. Cardinal temperatures: minimum 5°C, maximum 35°C and optimum 30°C (Van der Plaats-Niterink, 1981).

Host range and geographic distribution: In pot trials *Pythium vexans* was able to infect *Protea eximia* and *P. neriifolia* but did not kill the plants (**U.S.A., California**) (Raabe, 1978). *Pythium vexans* together with *Ph. cinnamomi* were also able to infect *Banksia menziesii, B. speciosa, Lcd. argenteum, Lcd. discolor, Lsp. reflexum* and *P. obtusifolia* (Raabe, 1978).

Disease cycle and epidemiology: The life cycle and spore types of *P. vexans* are similar to those of the *Phytophthora* species.

Notes: Although *Pythium* species do not cause major problems in cultivation of *Proteaceae*, they can be troublesome on young plants or the softer-wooded *Protea* species such as *P. cynaroides* and *P.*

grandiceps. *Pythium* problems develop if plants are cultivated in a warm environment where there is abundant moisture and a high level of inoculum. Nursery environments are often conducive to the development of Pythium diseases and Pythium damping-off has been reported in Australia (Porter *et al*., 1996) and South Africa (Benic, 1986). In Hawaii tissue-cultured plantlets of *Telopea* died from stem rot caused by an unknown *Pythium* species when transplanted in nurseries (Anon., 1993). In Madeira, *P. cynaroides* and a hybrid *Protea* 'Red Baron' were susceptible to root and crown rot caused by a *Pythium* sp. (Moura & Rodrigues, 2001). Contaminated soil or composts are likely to be the most important source of inoculum of this fungus.

Rhizoctonia root rot

Rhizoctonia is a common, serious and usually underestimated pathogen of *Proteaceae*, particularly in the nursery environment (Rohrbach, 1984; Benic, 1986). Seedlings are highly susceptible but this pathogen can also damage rooting cuttings. Little information is available on which species of *Rhizoctonia* attack *Proteaceae* and none of the AG types involved have been identified. There is much need for further work on this pathogen. Elucidation of the etiology and epidemiology will lead to the development of sustainable control measures.

Rhizoctonia solani J.G. Kühn, *Die Krankheiten der Culturgewächse, ihre Ursachen und Verhütung* (Berlin): 224 (1858).

Symptoms: Damping-off typically including seedling rot, water-soaked lesions at the stem base, plant and seedling collapse. Cotyledon necrosis is a damping-off symptom specifically caused by *R. solani* (Benic, 1986).

Causal organism: *Hyphae in vivo* >7 µm wide, brown, branched often at a 90° angle near the distal septum of young vegetative cells, often slightly constricted above the point of distal branching; hyphal cells multinucleate, dolipore septa and clamp connections absent (Sneh *et al.,* 1991). *Conidia* not formed. *Sclerotia* of monilioid cells and not differentiated into medulla and rind (Sneh *et al.,* 1991).

Cultural characteristics: Aerial mycelium abundant at first, later subsiding and colonies becoming smooth textured and brownish; mycelium coarse, hyaline to white at first, later becoming tawny brown; sclerotia on the surface of the colony, dark brown to black, hard, flattened, melanised, irregularly shaped; 40–60 mm diam in 4 d on PDA at 25°C.

Host range and geographic distribution: *Proteaceae* (**Australia**) (Porter *et al*., 1996); *Lcd.* 'Safari Sunset' (**Madeira**) (Moura & Rodrigues, 2001); *Proteaceae* (**South Africa, WC**) (Benic, 1986; Knox-Davies *et al.*, 1986); *Proteaceae* (**U.S.A., Hawaii**) (Rohrbach, 1984); *Lsp.* hybrid, *P. cynaroides, P. eximia* (**U.S.A., Hawaii)** (Anon., 1991).

Disease cycle and epidemiology: Unknown.

Control: Largely unknown, but Greenhalgh (1981) reported that drenching the soil with benomyl controlled *Rhizoctonia*. To control basal-end rot, cuttings should be stripped of their lower leaves, dipped in 0.05–0.15% (m/v) captafol in water and dried briefly, before treating with rooting compounds (Knox-Davies *et al*., 1986).

Since captafol is no longer sold in the USA and Benomyl will likely be removed from the market, there is a need to find other effective treatments. Carboxin, fludioxonil and tolclofos-methyl are fungicides with efficacy again Basidiomycetes and they need to be tested as soil and cutting treatments for control of *Rhizoctonia* in protea nurseries. Oxycarboxin is already registered on ornamental plants in South Africa (Krause *et al*., 1996).

Poorly understood diseases

Rosellinia root rot

Rosellinia spp. Plate 32.

Symptoms: Infected plants show unthrifty growth and general leaf chlorosis, often beginning from the base of the plant. Soil, roots and crowns of plants are covered in a white to olive-green mycelium. Roots are necrotic and in severe cases break off when plants are pulled from the soil. Bethencourt Díaz *et al*. (2001) reported that young shoots of infected plants had dry leaf tips. The leaf margins were also blighted and necrotic circular spots ringed by reddish tissue could also occur along the leaf margin.

Host range and geographic distribution: *Rosellinia necatrix* was isolated from *Lcd.* 'Long Tom', 'Safari Sunset', *Lsp.* 'High Gold', 'Succession II', *P. cynaroides* (**Madeira**) (Moura & Rodrigues, 2001); *Proteaceae* (**Canary Islands, Tenerife**) (Bethencourt Díaz *et al*., 2001).

Disease cycle and epidemiology: *Rosellinia necatrix* is the most frequently encountered root pathogen of *Proteaceae* in Madeira (Moura & Rodrigues, 2001) where it is devastating to *Proteaceae,* especially *Leucadendron*. Wet conditions seem to favour disease development.

Control: Unknown, but Moura & Rodrigues (2001) report that chemical treatments do not control this fungus.

Notes: This pathogen has not been reported on *Proteaceae* in Australia or South Africa where *Proteaceae* are native. Furthermore, *R. necatrix* has not been reported from any other areas such as California, Chile, Hawaii or Zimbabwe, which cultivate *Proteaceae*. *Rosellinia* is a serious pathogen and measures should be taken to avoid introducing it into areas where it has not yet caused disease.

Verticillium wilt

Verticillium spp.

Verticillium is a well-known vascular wilt pathogen of a wide range of plants, but it has not been reported frequently on *Proteaceae*. Koike *et al*. (1991) were the first to report Verticillium wilt on *Leucospermum*.

Symptoms: Infected plants develop terminal shoot wilting, the foliage fades to a light yellowish-green colour and the plants soon die. Dark brown spots (tyloses) were evident in the vascular system (Koike, 1991).

Host range and geographic distribution: *Verticillium dahliae* from *Lsp. cordifolium* (**U.S.A., California**) (Koike *et al*., 1991); *Lsp.* species, *P. neriifolia*, *P.* 'Red Baron' (**Madeira**) (Moura & Rodrigues, 2001).

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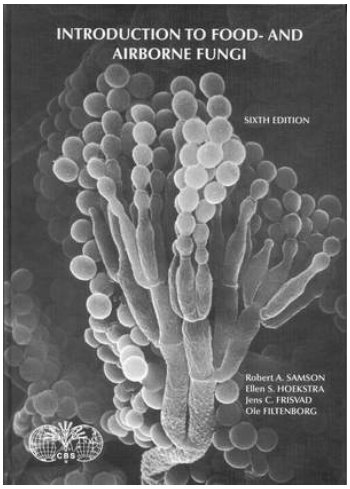

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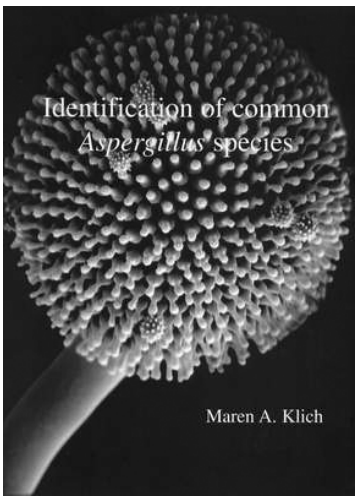

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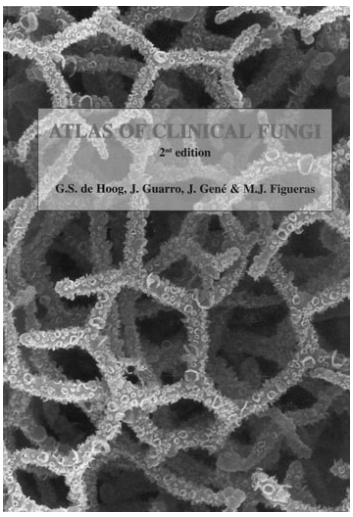

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CHAPTER 4

DISEASES CAUSED BY BACTERIA AND PHYTOPLASMS

Pseudomonas leaf spot

Pseudomonas leaf spot is caused by a pathovar of *Pseudomonas syringae* (Wimalajeewa *et al.*, 1983; Forsberg, 1993). The disease, which was first observed on plants in England, occurs on leaves of older plants as well as seedlings (Paine & Stansfield, 1919). The spotting retards seedling growth and lowers the quality of stem foliage of cut-flowers (Wimalajeewa *et al.,* 1983).

Pseudomonas syringae pv. ***proteae*** Moffatt, Fahy & Persley, *Plant Bacterial Diseases – A Diagnostic Guide*: 327 (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). Each spot is surrounded by a green band, 1–2 mm wide, which separates the sunken spot from the halo (Paine & Stansfield, 1919; Forsberg, 1993). The spot slowly enlarges and, after several weeks, the entire leaf surface usually is affected. In severe cases where several spots enlarge and coalesce, large portions of the leaves wither. Paine & Stansfield (1919) observed numerous dome-shaped blisters of a reddish brown colour scattered over the upper surface of the leaf. Blisters varied in size (1–3 mm diam), and had a raised surface (0.5–1 mm). Instead of the brown blisters, larger blotches occurred on younger leaves, with surfaces frequently being depressed by shrinkage of the underlying cells. It isn't known whether one type of spot changed into the other or whether they were the result of a difference in age of the leaf at the time of infection.

Causal organism: Gram-negative, rod-shaped bacterium, 2.7–3.4 × 0.7–0.9 µm, with 2–7 polar flagella (Wimalajeewa *et al*., 1983). Paine & Stansfield (1919) described the bacteria in culture as small, oval rods, 0.8–1.6 × 0.6–0.8 µm.

Cultural characteristics: *Colonies* with green centres on glucose-yeast extract-chalk agar and yeast-dextrose-carbonate agar, but the colour disappears after a few days; of two types on King's B Medium: one type raised, convex, mucoid and coalescing, while the other is low, convex, translucent and spreading, both produce a diffusible green-yellow pigment that fluoresces blue-green under uv-light (Wimalajeewa *et al.*, 1983).

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

Disease cycle and epidemiology: Spread occurs by rain splash and movement of infected nursery stock. Prolonged wet, humid and cool conditions favour disease spread and development (Forsberg, 1993). Bacteria enter leaf tissue through the stomata. The host tries to prevent the spread and development of the pathogen by the formation and production of wound cork and gum (Paine & Stansfield, 1919; Paine & Berridge, 1922).

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

Witches' brooms

Witches' brooms are commonly observed in the *Proteaceae* and are the result of a proliferation of malformed leaves and dense clusters of slender shoots with long internodes (Plate 13) (Knox-Davies, 2001). This renders the stems unmarketable as cut-flowers.

Causal organism: The disease is caused by a phytoplasma, which is vectored by several mites, namely *Aceria proteae, Proctolaelaps* sp. and *Oxycarenus maculatus*. Although the disease also occurs in species of *Leucadendron*, it seems that the mite(s) involved may differ from the ones on *Protea* (Wieczorek & Wright, 2003).

Host range and geographic distribution: *Aulax umbellate, H. sericea, Lcd. gandogeri, Lcd. procerum, Lcd. salignum, Lcd. stellare, Lcd. teretifolium, Lsp. arenarium, Lcd.* 'Chameleon', 'Inca Gold', 'Jubilee Crown', 'Pisa', *Leucadendron* spp., *Lsp. conocarpodendron, Lsp. hypophyllocarpodendron, Lsp. parile, Lsp. praemorsum, P. acaulos, P. amplexicaulis, P. angolensis, P. aspera, P. burchellii, P. caffra, P. coronata, P. cynaroides, P. denticulata, P. eximia, P. glabra, P. humiflora, P. laevis, P. lanceolata, P. laurifolia, P. lepidocarpodendron, P. longifolia, P. lorifolia, P. magnifica, P. mundii, P. neriifolia, P. nitida, P. obtusifolia, P. punctata*, *P. repens, P. scabra, P. speciosa, P. scolymocephala, P. scolopendriifolia, P. stokoei, P. sulphurea, P. tenax, Serruria furcellata, S. phylicoides, Vexatorella obtusata* (**South Africa**) (<http://protea.worldonline.co.za/-witches.htm>; R. Newton, unpubl. data).

Disease cycle and epidemiology: The phytoplasma is vectored by a mite, and disease control is therefore dependent on controlling the mite vector. There is some question about whether the disease can be spread via pruning shears.

Control: Sanitation in plantations is of the utmost importance. Although spread via pruning shears has not

yet been confirmed, care should be taken to sterilise tools between plants. Infected branches and severely infected plants must be removed. Pesticides may represent an important way of controlling the disease, though this needs to be confirmed.

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Saccharata proteae (Wakef.) Denman & Crous, comb. nov. 104

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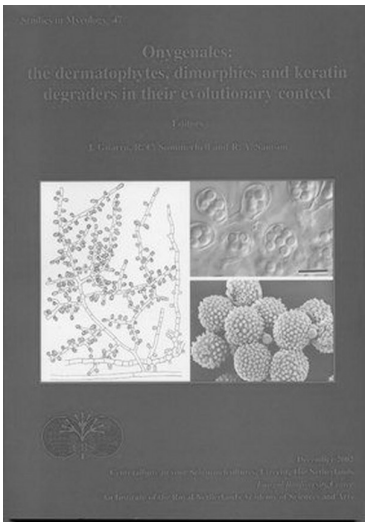
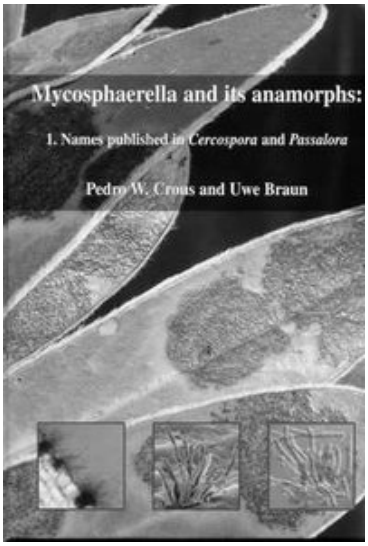


Onygenales: the dermatophytes, dimorphics and keratin degraders in their evolutionary context

J. Guarro, R. C. Summerbell & R. A. Samson (eds)

This volume contains 21 articles on the phylogeny and population genetics of the class of fungi containing many agents of human mycotic disease, including dermatophytes and dimorphic systemic pathogens such as *Histoplasma*. Many common soil-borne and biomedical- contaminant keratinophilic species are also treated. Keys to major keratinophilic and/or onychomycosis-inducing groups such as *Aphanoascus*, *Auxarthron* and *Arachnomyces* (anamorph *Onychocola*) are given, and new molecular characters are presented for dermatophyte identification. There are 17 new species described, four new genera, one new family and one new order.

220 pp, (A 4 format) paperback, 2002. € 49,--

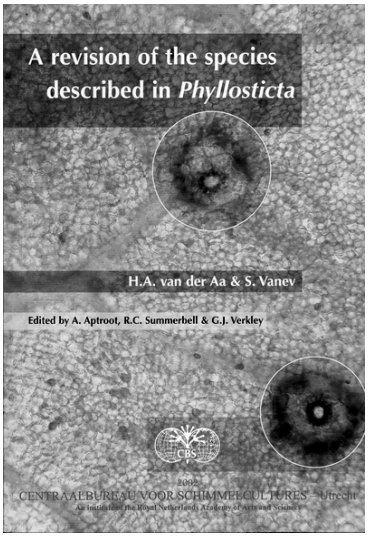


Mycosphaerella* and its anamorphs: 1. Names published in *Cercospora* and *Passalora

P. W. Crous & U. Braun

This book contains a compilation of more than 3000 names that have been published or proposed in *Cercospora,* of which 659 are presently recognised in this genus, with a further 281 being referred to *C. apii s.lat*. Approximately 550 names of *Passalora* emend. (incl. *Mycovellosiella*, *Phaeoramularia*, *Tandonella* and *Phaeoisariopsis p.p*.) are treated in a second list. In total 5720 names are treated. Four hundred and fifty three taxonomic novelties are proposed.

571 pp, 31 figures (17 x 25 cm) hard cover, 2003. € 75,--



A revision of the species described in *Phyllosticta*

H. A. van der Aa & S. Vanev
(A. Aptroot, R. C. Summerbell & G. J. Verkley, eds)

2936 taxa are enumerated, based on the original literature and on examination of numerous herbarium (mostly type) specimens and isolates. 203 names belong to the genus *Phyllosticta s.str*., and are classified in 143 accepted species. For seven of them new combinations are made and for six new names are proposed. The great majority, 2733 taxa, were redisposed to a number of other genera. A complete list of these novelties, as included in the book's abstract, can also be consulted on the web-site of CBS (http://www.cbs.knaw.nl).

510 pp, (17 x 25 cm) paperback, 2002. € 50,--

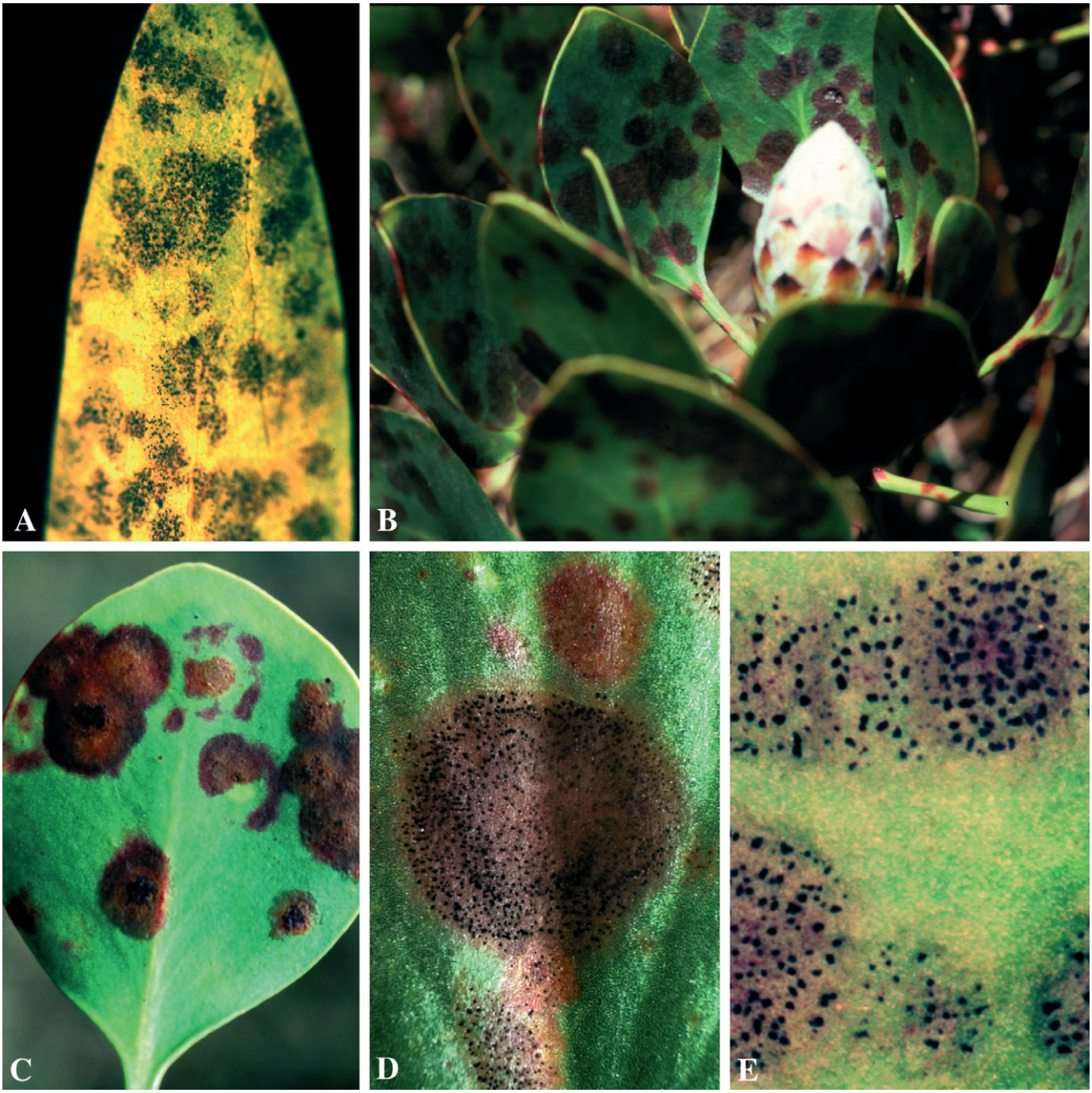


Plate 1A–E. Batcheloromyces leaf spot. A. *Batcheloromyces leucadendri* on *Leucadendron* sp. B–E. *Batcheloromyces proteae* on *Protea cynaroides*.

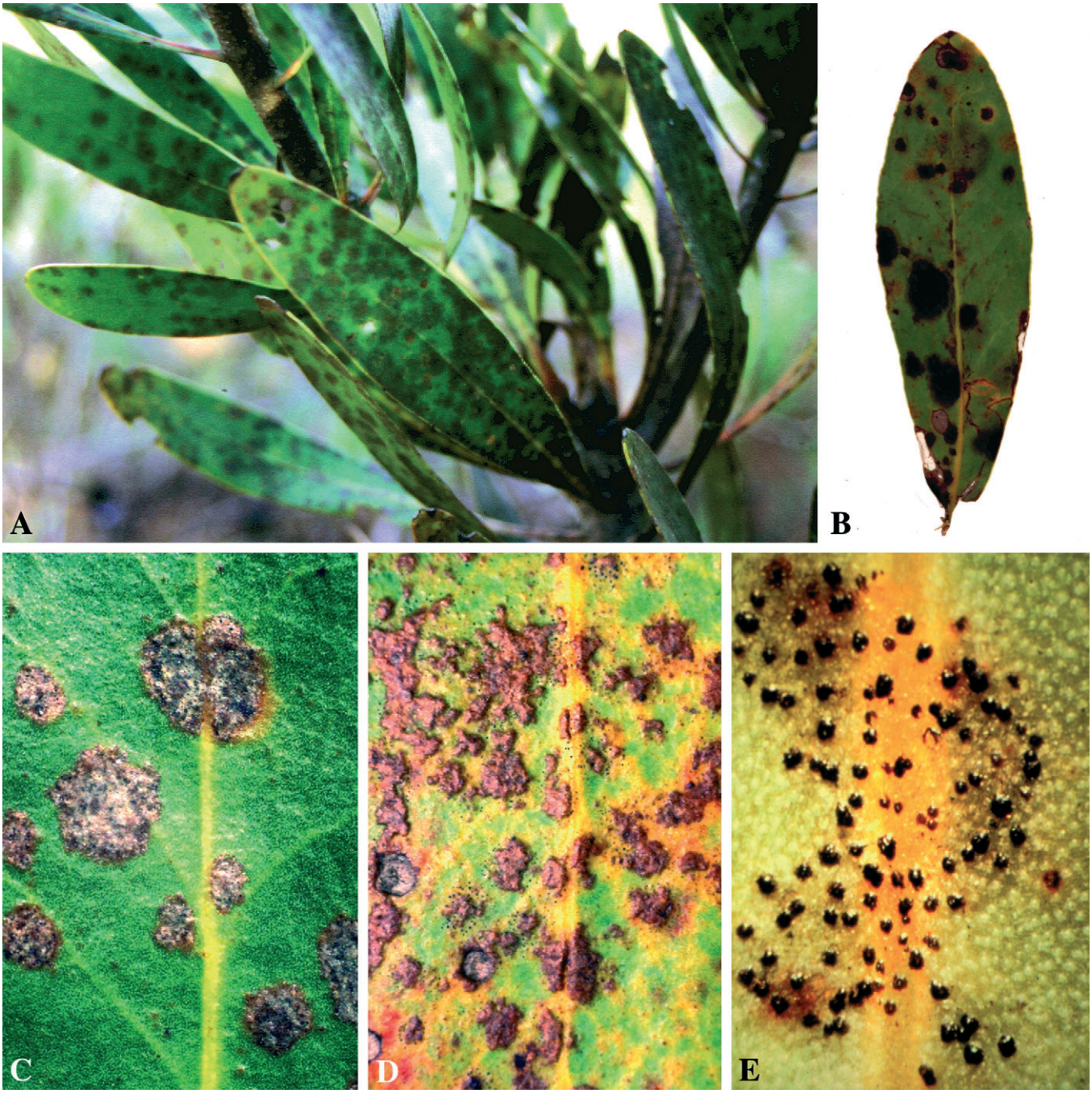


Plate 2A–E. Lembosia "Clasterosporium" and Coleroa leaf spot. A–B. *Lembosia proteae*. C–E. Leaf spots and fungal fruiting bodies of *Coleroa senniana*.

Plate 3A–E. Coleroa leaf spot. A–E. Leaf spots and fungal fruiting bodies of *Coleroa senniana*.

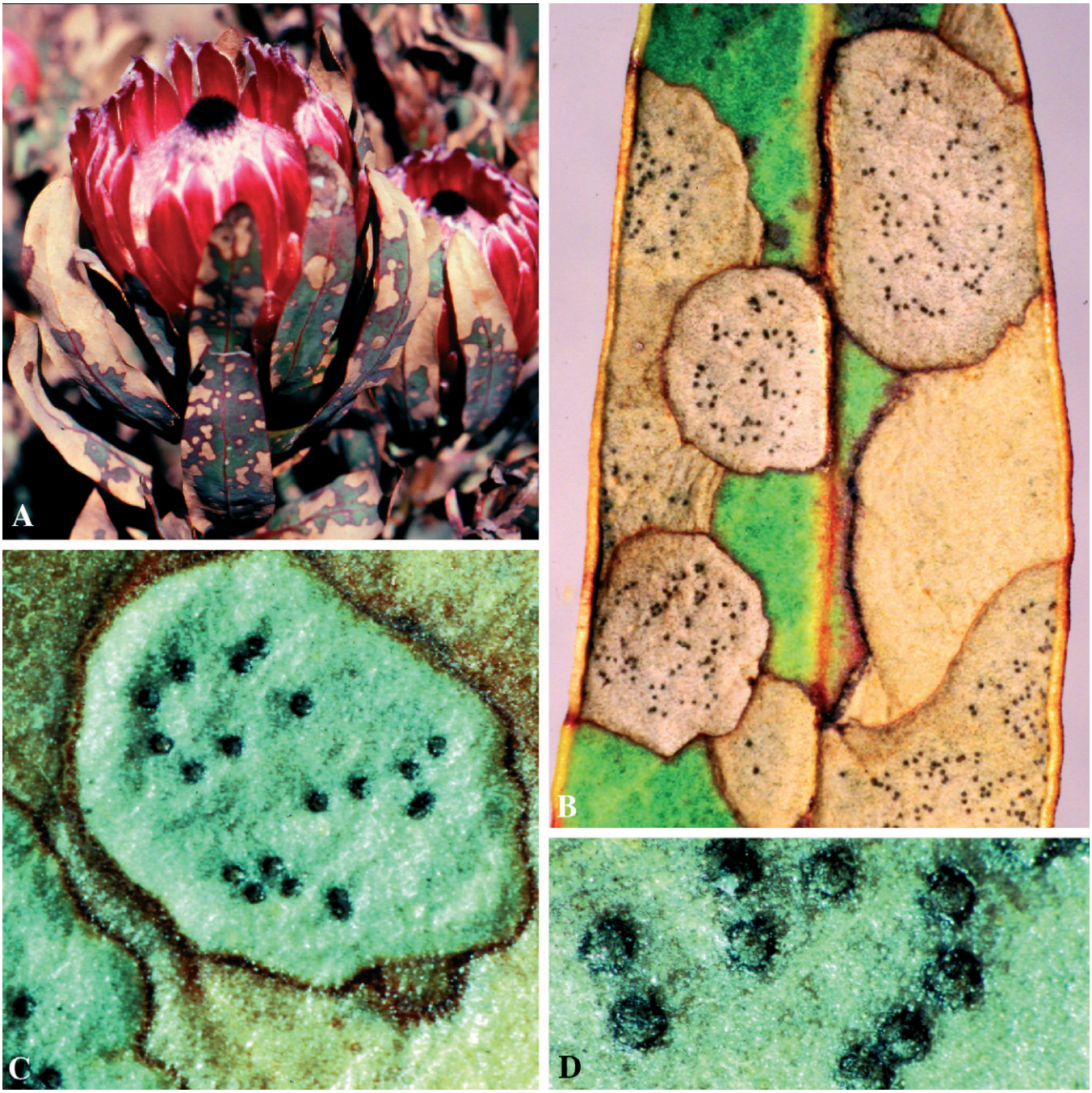


Plate 4A–D. Leptosphaeria leaf blight, caused by *Leptosphaeria protearum*. A–D. Leaf spots and fungal fruiting bodies.

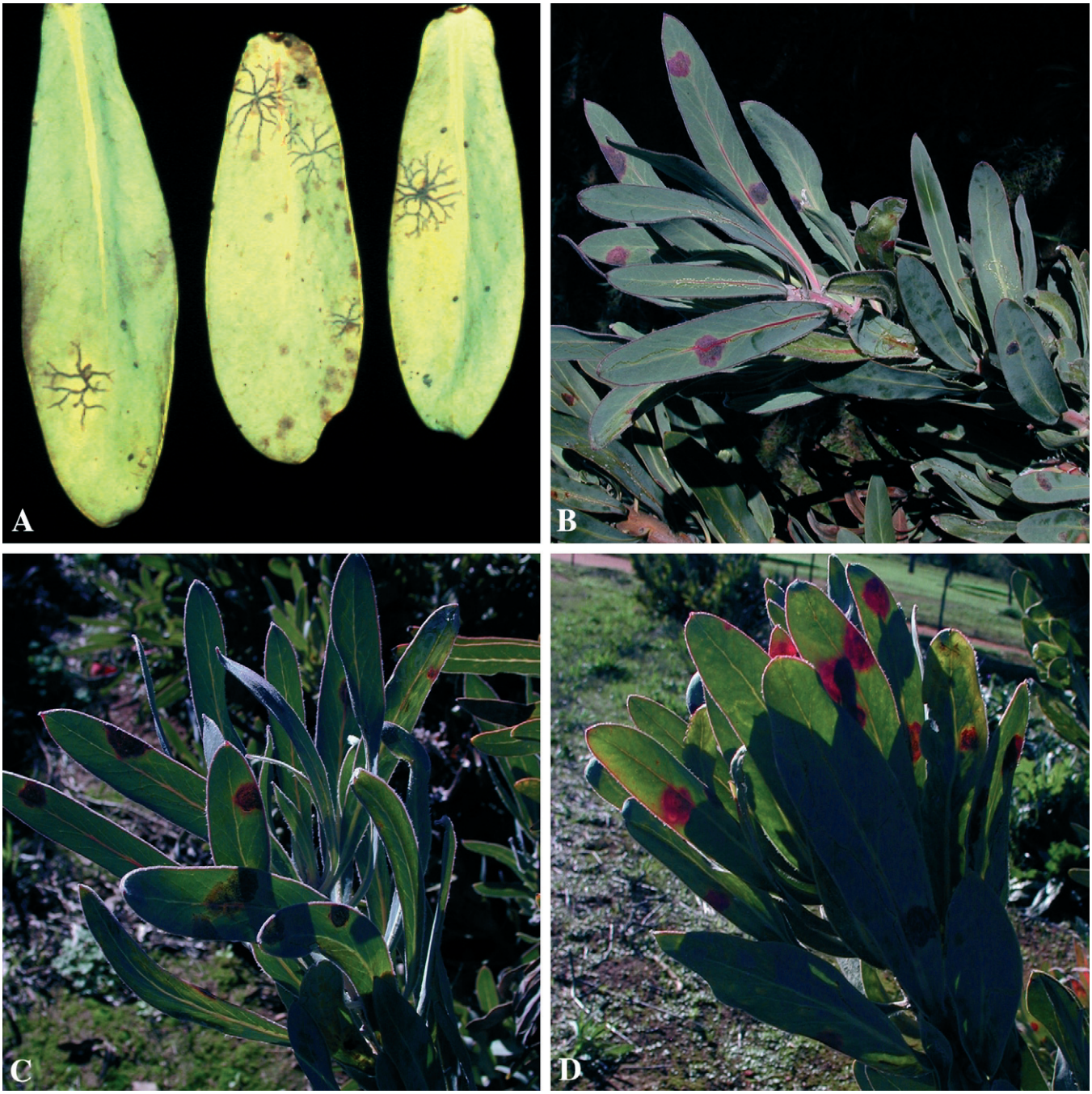


Plate 5A–D. Mycosphaerella leaf spot. A. Characteristic arrangement of fungal fruiting bodies of *Mycosphaerella fibrillosa*. B–D. Leaf spots of *Mycosphaerella proteae*.

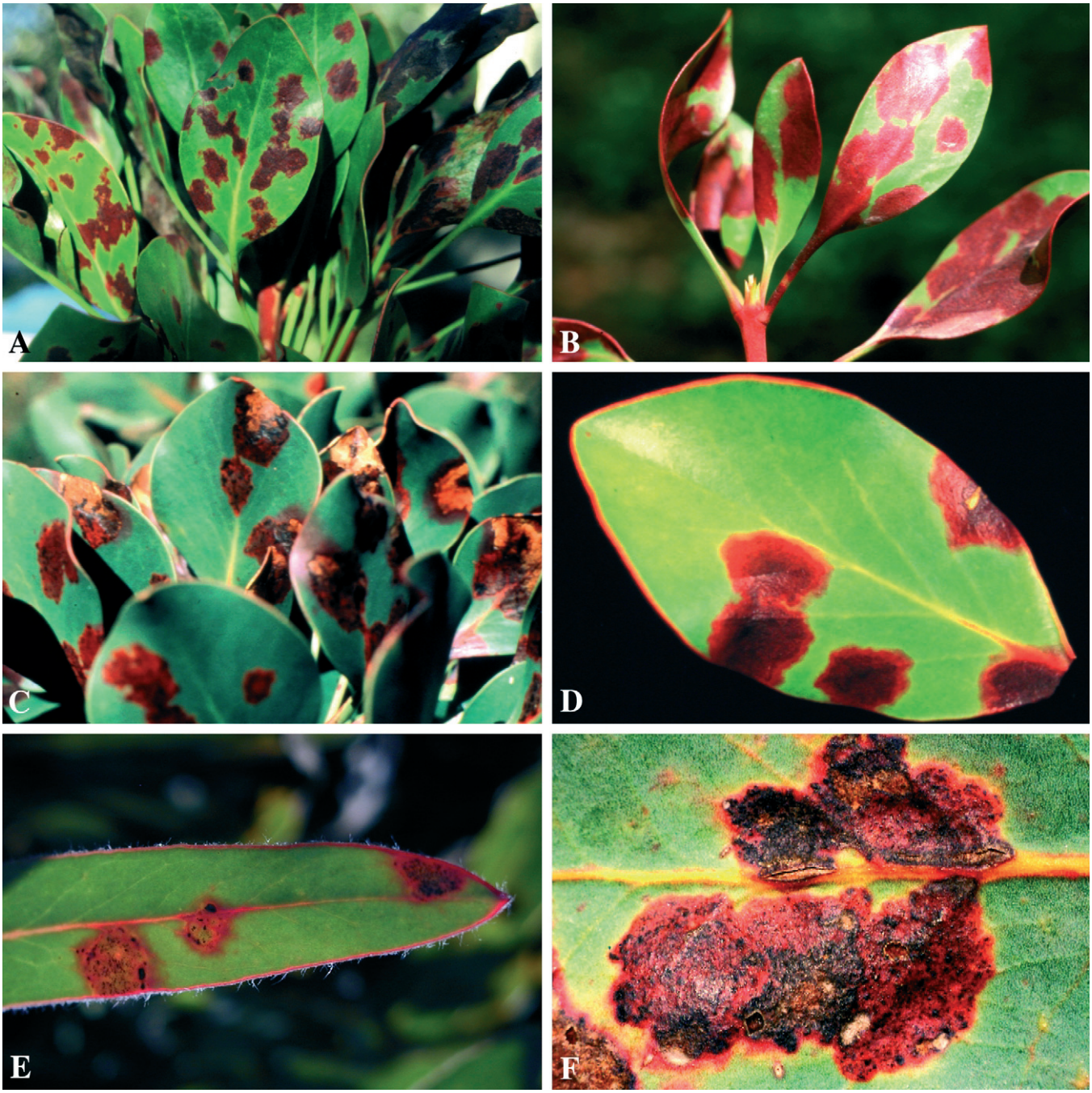


Plate 6A–F. Red-purple leaf spots of *Mycosphaerella proteae* on *Protea* spp.

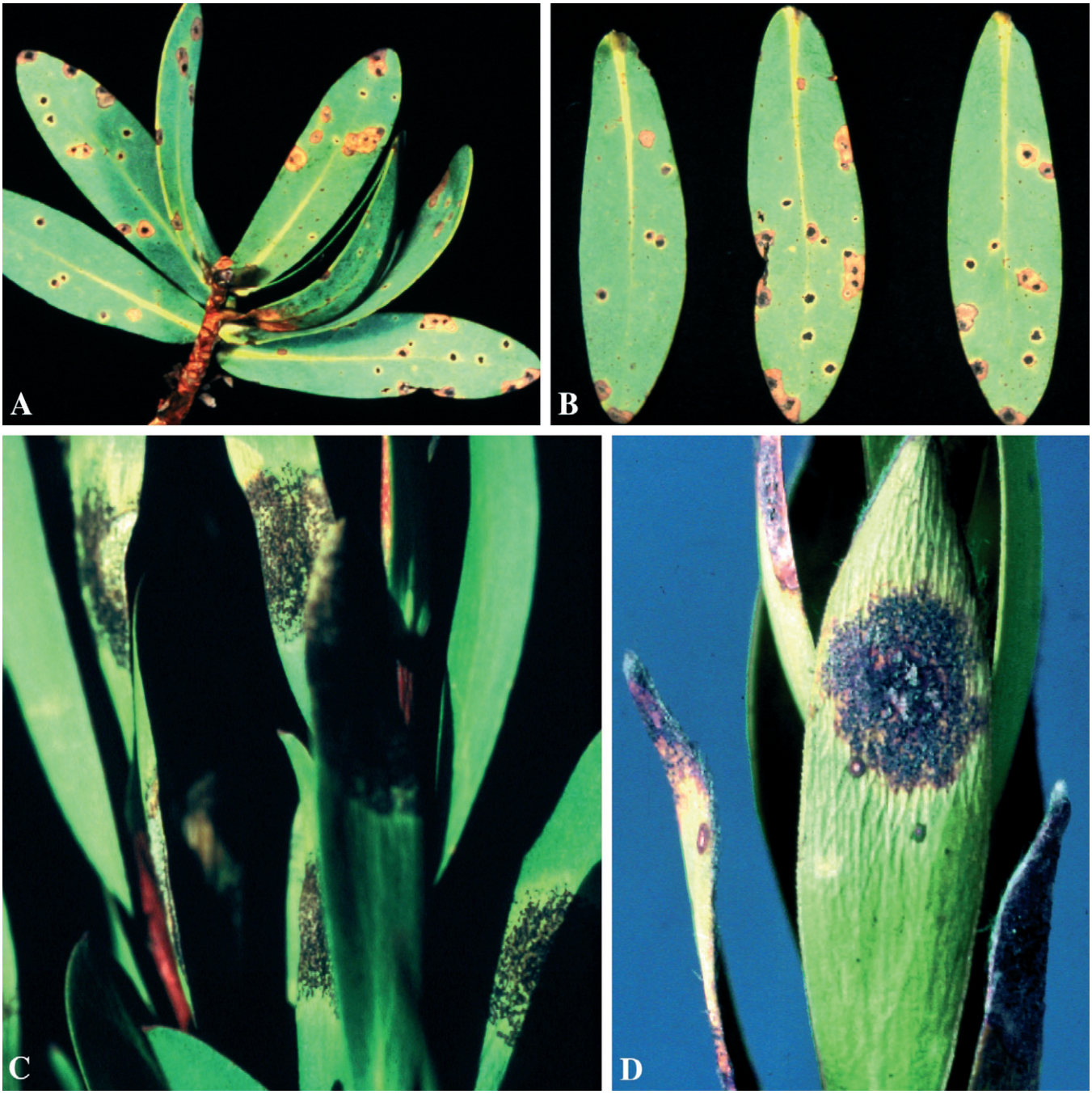


Plate 7A–D. Mycosphaerella and “Stigmina” leaf spots. A–B. *Mycosphaerella proteae-arboreae*. C–D. *Pseudocercospora protearum* var. *leucadendri*.

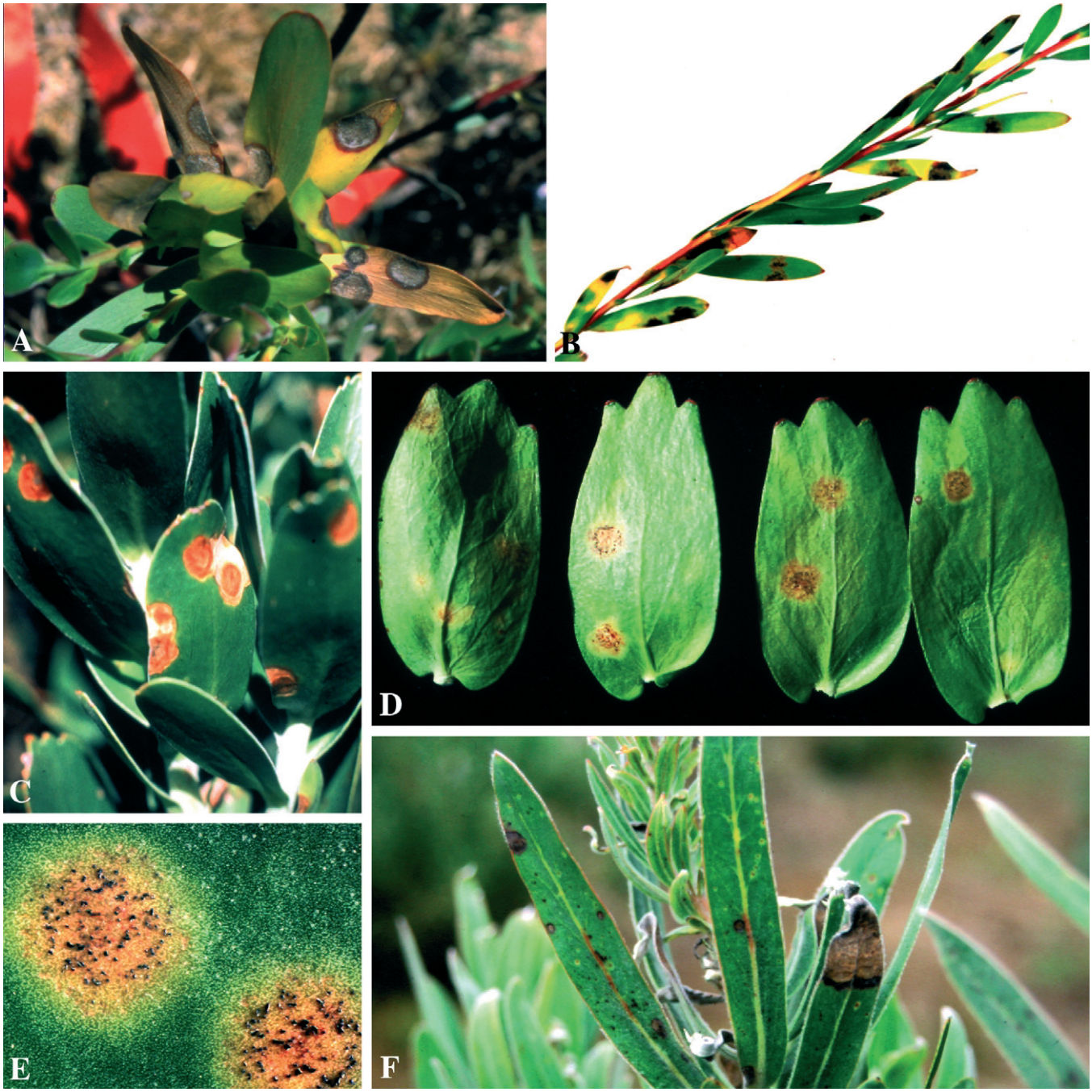


Plate 8A–F. Mycosphaerella and "Stigmina" leaf spots. A–B. *Pseudocercospora protearum* var. *leucadendri*. C–E. *Pseudocercospora protearum* var. *protearum*. F. *Mycosphaerella waimeana*.

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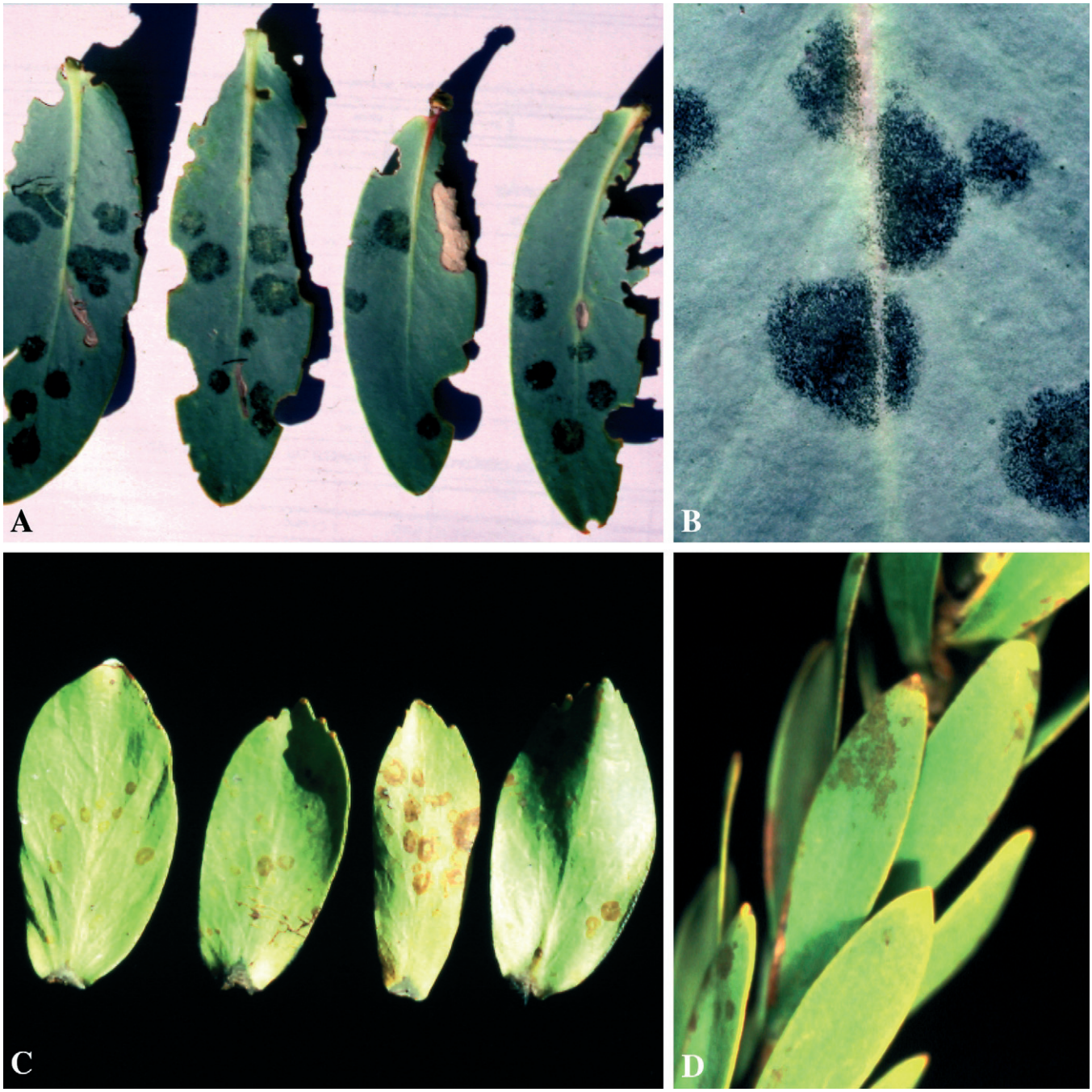

Plate 10A–D. Trimmatostroma and Vizella leaf spots. A–B. *Trimmatostroma macowanii*. C–D. *Vizella interrupta*.

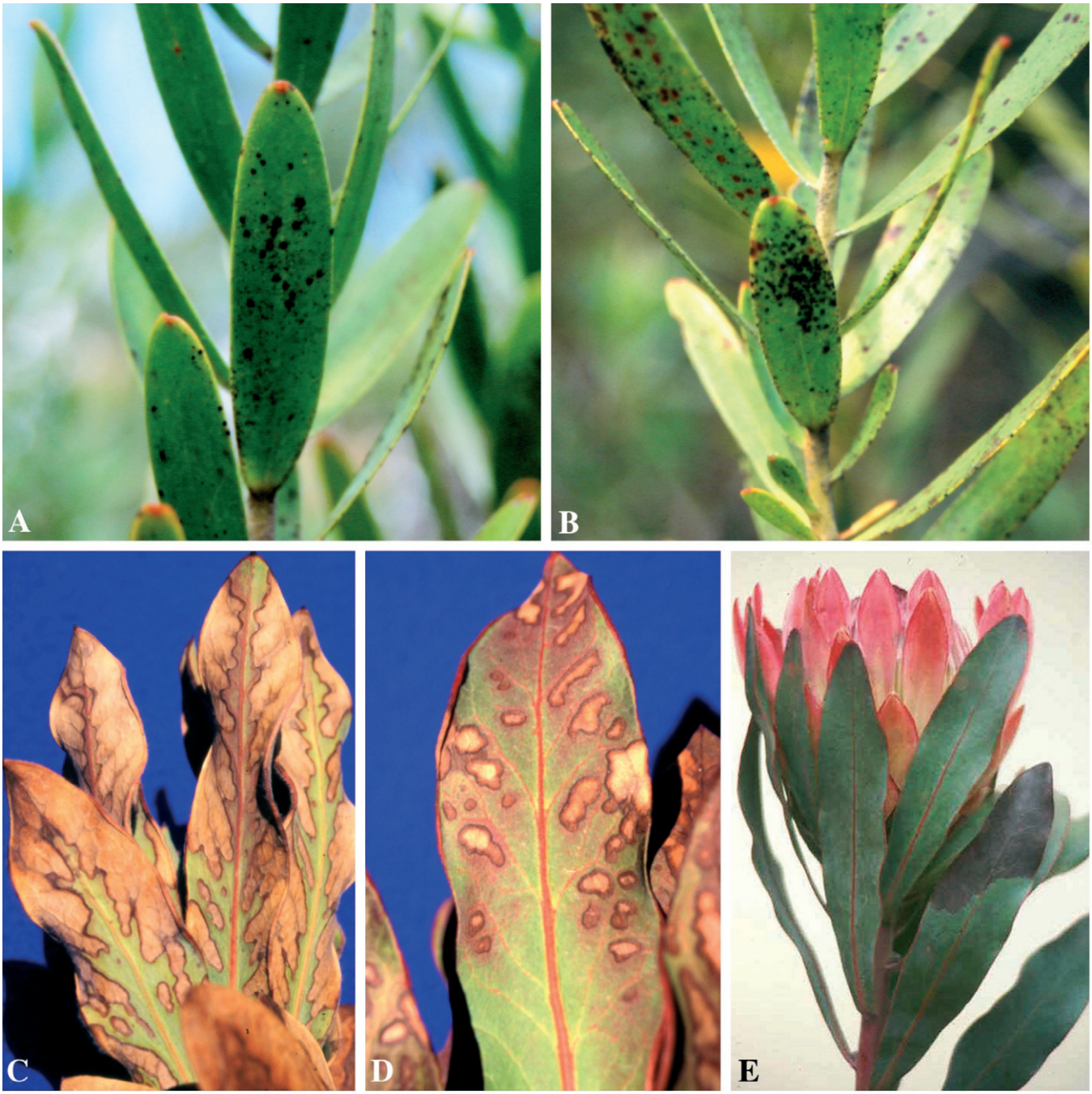

Plate 11A–E. Helicosingula leaf spot caused by *Helicosingula leucadendri* on *Leucadendron* (A–B), herbicide damage on a *Protea* sp. (C–D), and leaf blackening on leaves of a harvested *Protea* sp. (E).

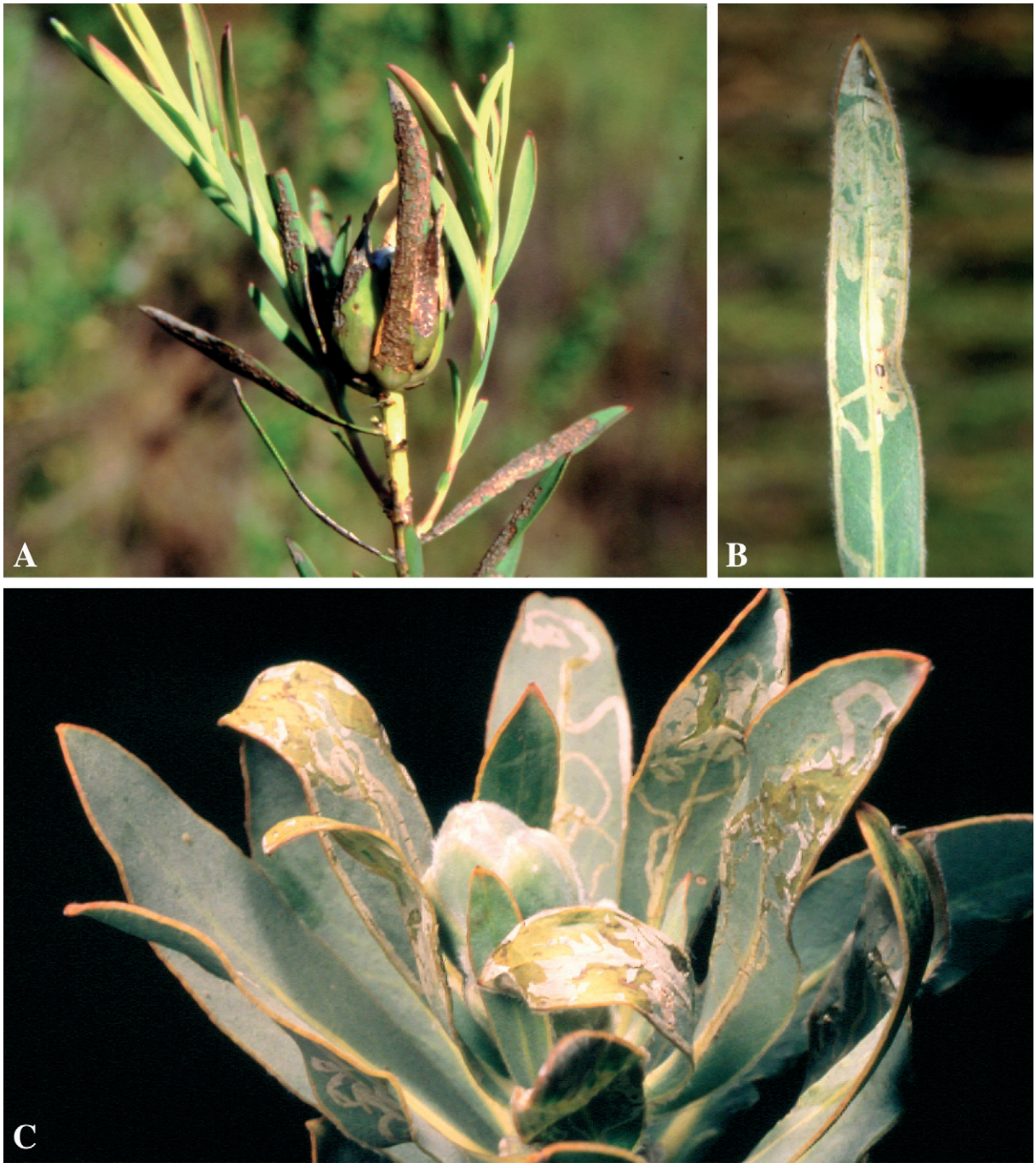

Plate 12A–C. Insect damage on *Proteaceae.*

Plate 13A–E. Witches' brooms on *Proteaceae;* symptoms associated with a phytoplasm.

Plate 14A–F. Botryosphaeria stem cankers, showing branch dieback (A–B, D), stem necrosis (right) (C), and stem lesions originating in attached, diseased leaves (E–F).

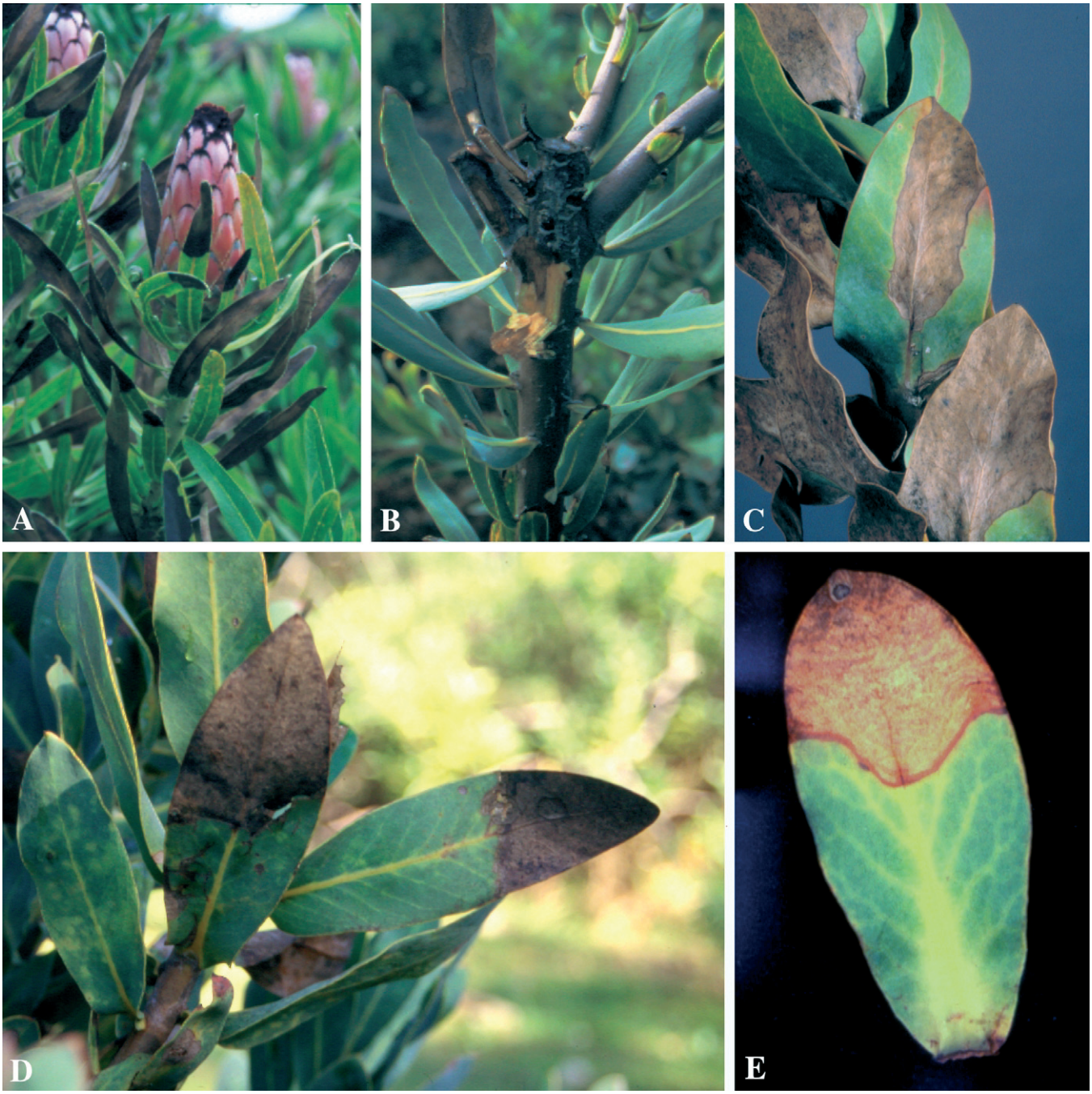

Plate15A–E. Botryosphaeria leaf necrosis (A & C) and stem canker associated with pruning wounds (B) and Saccharata leaf tip dieback, associated with *Saccharata proteae* (D–E).

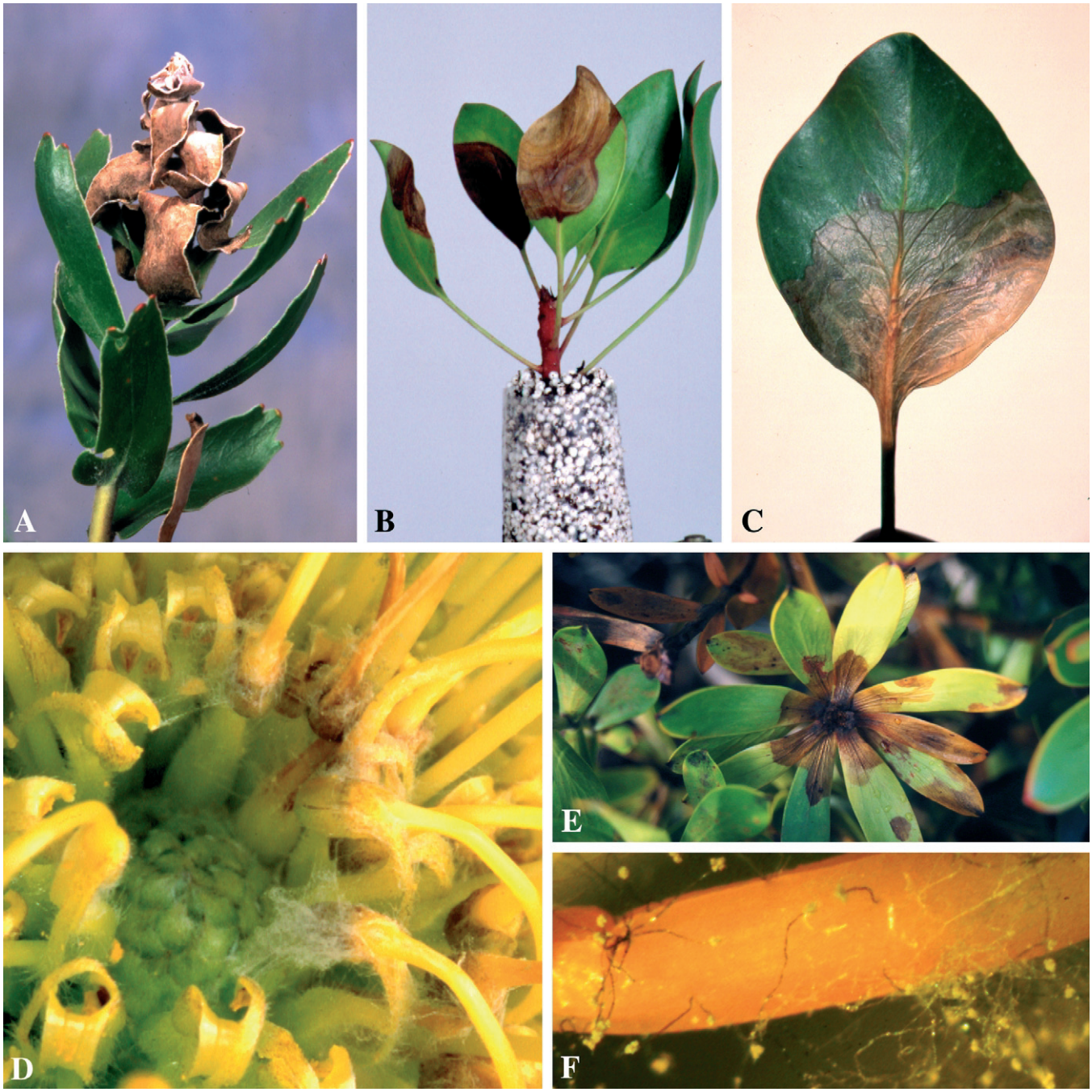

Plate 16A–F. Botrytis blight, caused by *Botrytis cinerea*. A. Shoot tip blight. B–C. Leaf lesions. D. Mycelium colonising flower parts. E. Botrytis blight, entering the stem and leaves through a wound where a flower had been broken off. F. Mycelium and spores on flower.

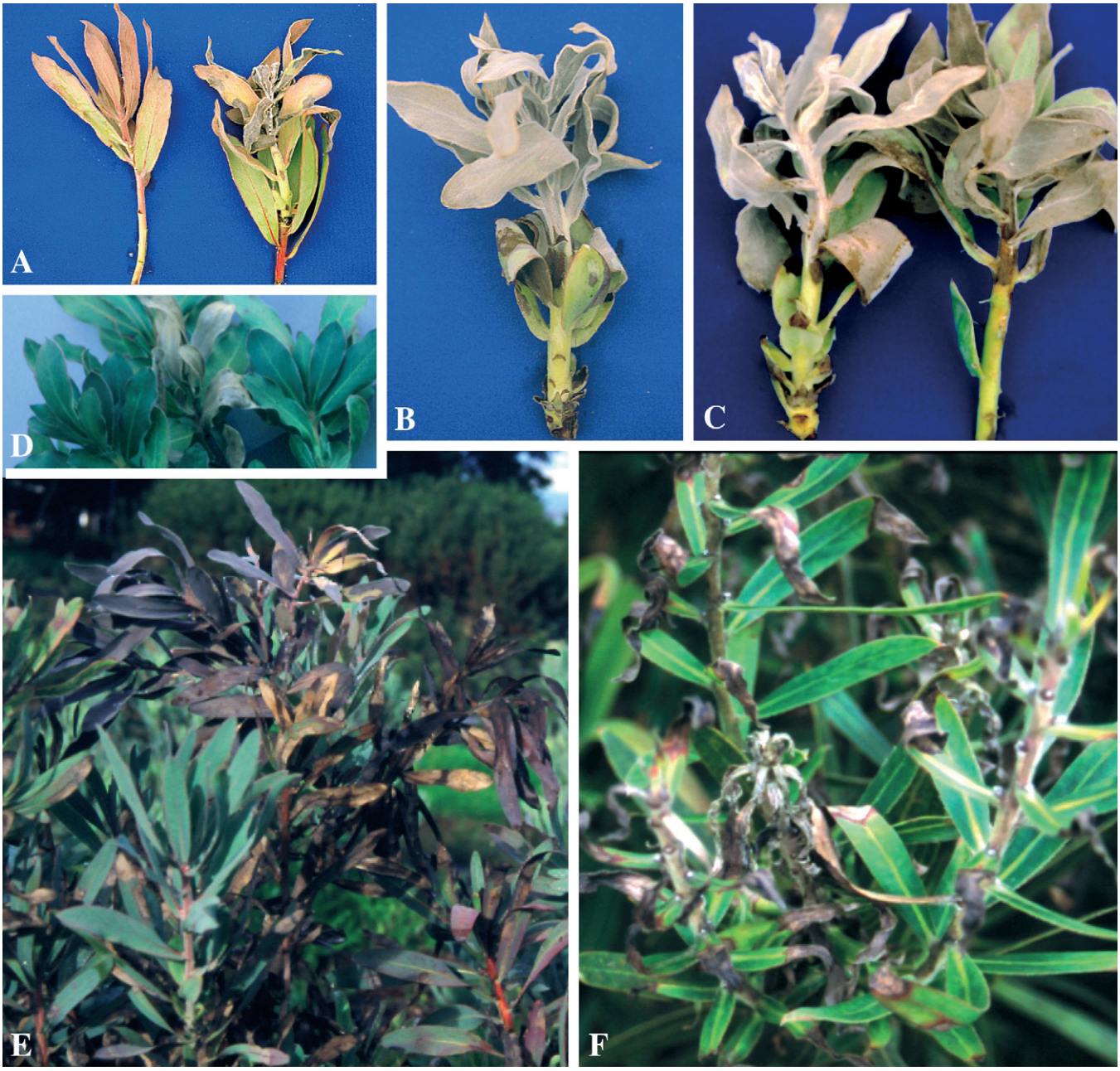

Plate 17A–F. Colletotrichum anthracnose on *Protea* spp.

Plate 18A–D. Colletotrichum anthracnose and Cylindrocladium root rot. A. Shepherd's crook symptom associated with Colletotrichum anthracnose. B. Colletotrichum stem canker. C. Cylindrocladium wilt. D. Cylindrocladium root rot.

Plate 19A–F. Drechslera blight, showing typical stem collapse, desiccation and canker (A, C) and leaf lesions on *Leucospermum* spp.

Plate 20A–F. Leaf spots and stem dieback associated with Drechslera blight on *Leucospermum* spp.

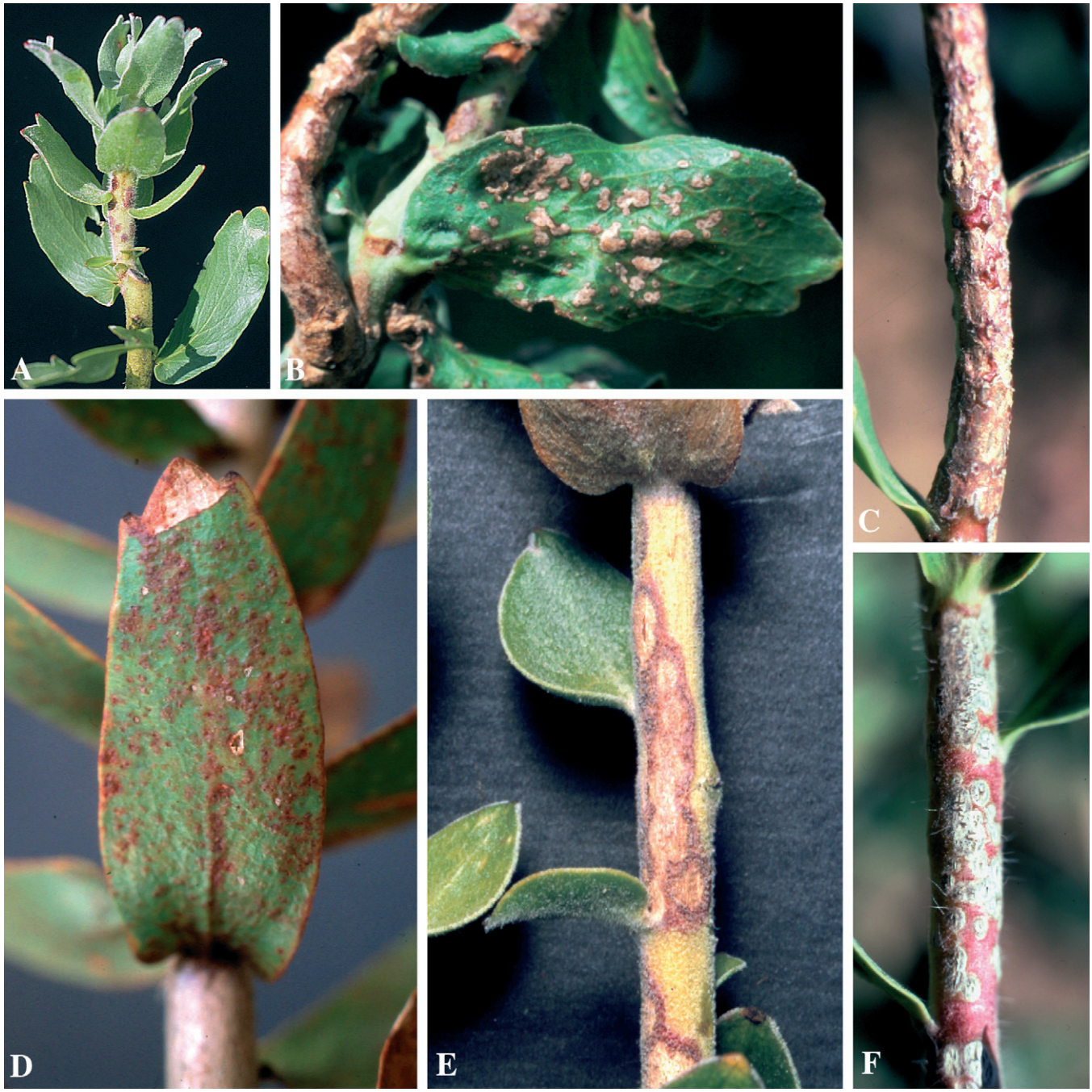

Plate 21A–F. Elsinoë scab disease, associated with *Elsinoë leucospermi* on *Leucospermum*, showing leaf and stem symptoms.

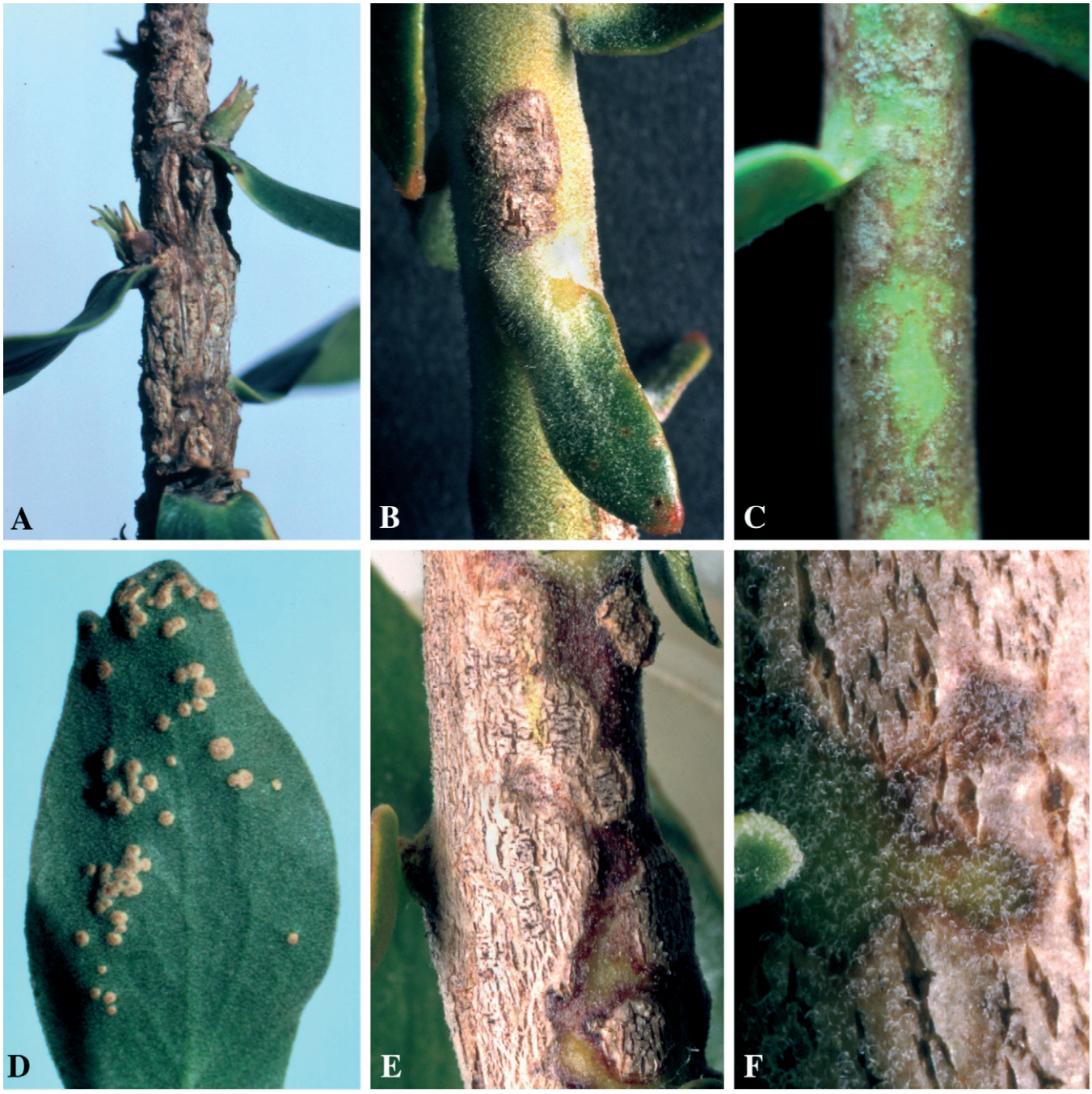

Plate 22A–F. Elsinoë scab disease, associated with *Elsinoë leucospermi* on *Leucospermum*, showing typical corky bark symptoms (A–C), leaf spots (D), and fungal fruiting bodies occurring in superficial cracks on the stems (E–F).

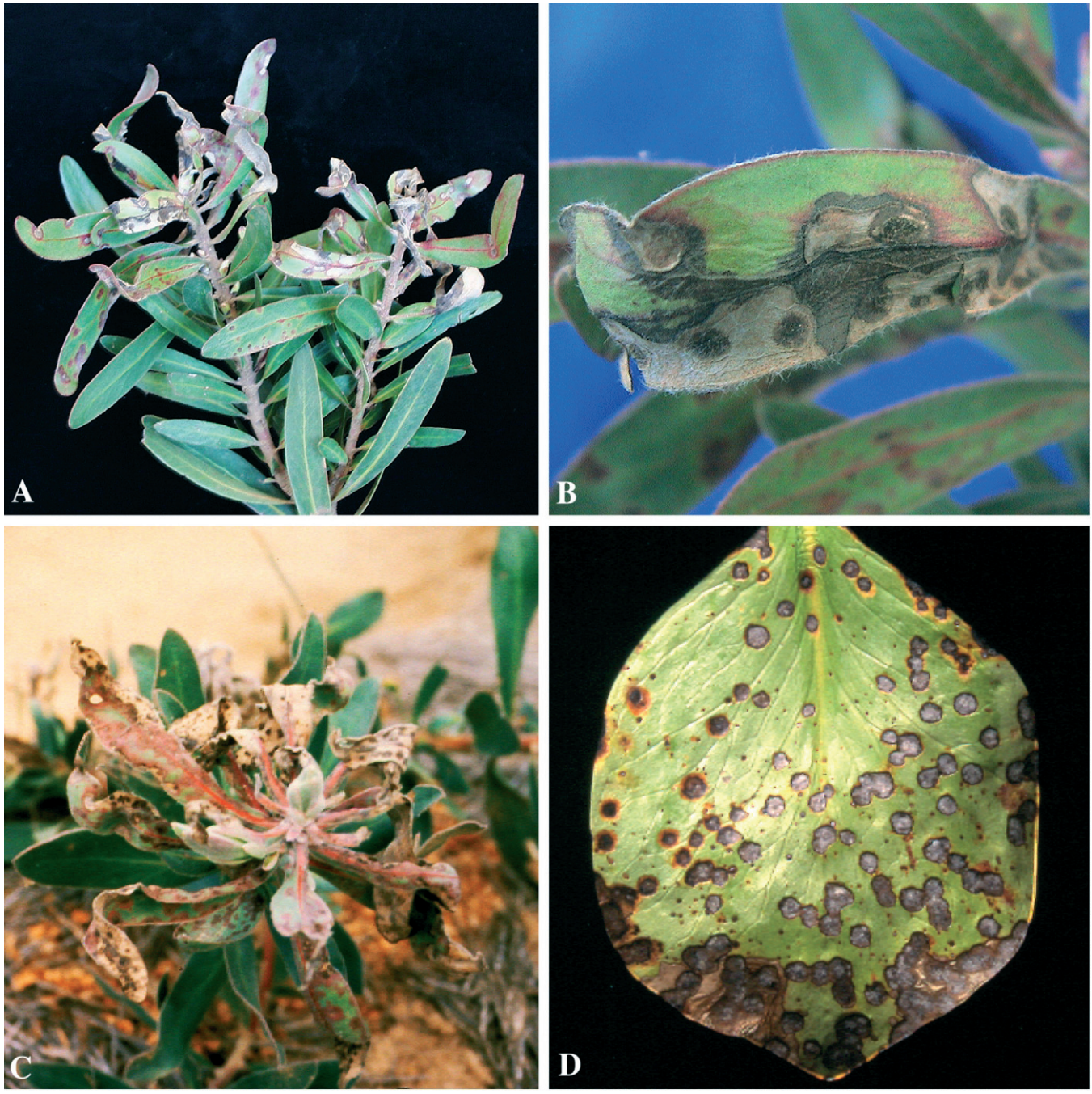

Plate 23A–D. Elsinoë scab diseases. A–C. *Sphaceloma protearum* on *Protea* sp. D. *Elsinoë proteae* on *Protea cynaroides*.

Plate 24A–D. Schizophyllum trunk rot, caused by *Schizophyllum commune*. A. Dieback on a *Protea* sp. B, D. Colonising stems via pruning wounds. C. Fungal fruiting bodies forming on the stem.

Plate 25A–C. Armillaria root rot, associated with *Armillaria luteobubalina*. A. Fungal fruiting bodies. B. Above ground symptoms caused by root rot. C. Rotted roots and stem, colonized by *A. luteobubalina*.

Plate 26A–E. Armillaria root rot, associated with *Armillaria mellea*. A, C. Fungal fruiting bodies. B. Fungal rhizomorphs forming in culture on malt extract agar. D, E. Root rot, and fungal rhizomorphs.

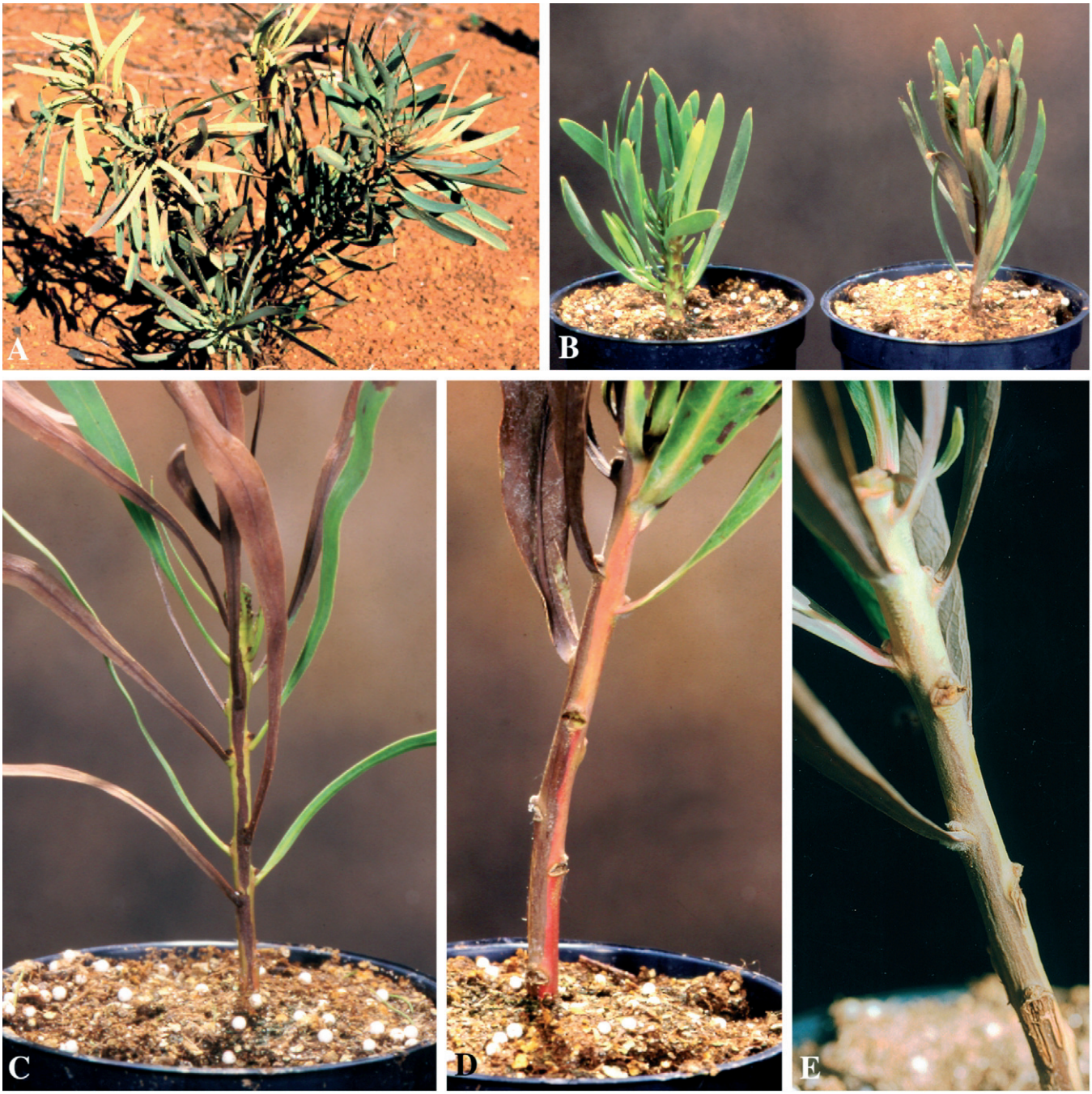

Plate 27A–E. Fusarium wilt caused by *Fusarium oxysporum* on *Protea* spp. A. Wilt as observed in the field. B. Inoculated plants showing diseased (right), and healthy control (left). C–D. Stem lesions, with tissue discoloration running up on side of the stem.

Plate 28A–E. Fusarium wilt caused by *Fusarium oxysporum* on *Protea* spp. A. basal stem discoloration. B. Wilted plant. C–E. Stem lesions and tissue discoloration.

Plate 29A–D. Phytophthora root rot, caused by *Phytophthora cinnamomi*. A–D. wilted, yellowing symptoms associated with root rot.

Plate 30A–D. Dead and wilted bushes associated with *Phytophthora cinnamomi* infection.

Plate 31A–D. Dead, yellowing and wilted bushes associated with Phytophthora root rot

Plate 32A–D. Phytophthora and Rosellinia root rot. A–B. Wilt symptoms caused by *Phytophthora cinnamomi*. C. Stem discoloration associated with Rosellinia root rot. D. Root decay caused by *Rosellinia* infection.

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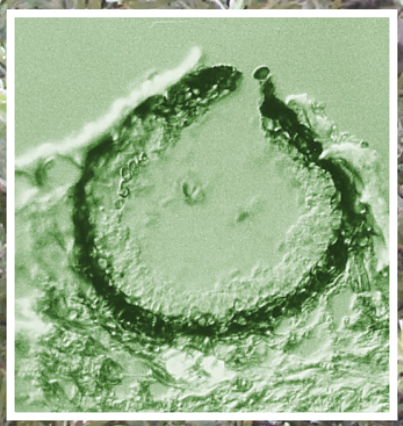

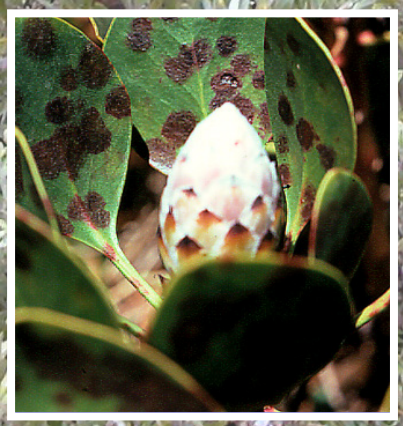

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