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FOLIICOLOUS AND CAULICOLOUS FUNGI ASSOCIATED WITH PROTEACEAE CULTIVATED IN CALIFORNIA

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ABSTRACT

Proteaceae, cultivated in California for the cut-flower industry, were assessed for the occurrence of leaf and stem pathogens. Although fields and nurseries were relatively disease free, several unrecorded pathogens were collected. The most important of these are *Coleroa senniana, Botryosphaeria dothidea* and *Elsinoë leucospermi*, which can cause significant losses for producers in California, and also *Batcheloromyces leucospermi, Botryosphaeria proteae* and *Coniothyrium leucospermi,* which are generally regarded to be of lesser economic importance. New species described include *Camarosporium* sp., *Coniothyrium septatum*, *Elsinoë fecunda*, *Mycosphaerella leucospermi, Mycosphaerella* sp. and *Stigmina californiae*. *Didymosphaeria futilis* was recorded for the first time associated with proteaceous hosts. A compilation of all fungi recorded on proteas cultivated for the cut-flower industry in the U.S.A. (excluding Hawaii) is given. It is suggested that further collections of pathogens should be made at different times of the year, to obtain an overall impression of the status of proteaceous diseases in California.

KEY WORDS: Fungal pathogens, phytosanitary, systematics.

INTRODUCTION

In the U.S.A., *Proteaceae* (proteas) are cultivated only in two states, California and Hawaii. The climate and soils in these regions are

suitable for this crop and there is a growing market in the U.S.A. and abroad for these products (Perry 1998). There are no indigenous *Proteaceae* in the U.S.A., as *Proteaceae* is a southern hemisphere plant family, restricted to Asia, Australasia, southern Africa and Central and South America (Rebelo 1995). This has advantages and disadvantages for the protea cut-flower industry in the U.S.A. The disadvantages are that the acquisition of new genetic material for the development of novel cultivars is restricted. Most new cultivars of proteas are derived from South Africa and Australia (Middelmann 1998). However, the advantages are that the plantations are relatively disease free (Perry 1998), unlike their South African counterparts, and that providing phytosanitary practices are thorough, they should remain that way.

Proteas form an important component of the cut-flower industry in California (Middelmann 1998). Until the present study, however, there had been no scientific and systematic sampling of protea diseases in the U.S.A. Thus, the aim of this study was to carry out a scientific survey of the diseases associated with proteas cultivated in California. These data are combined with that of previous records from the U.S.A. An overall impression of the status of plantations in California is given and the most important findings are discussed.

METHODS AND MATERIALS

Symptomatic leaf and stem samples of cultivated proteaceous hosts (*Grevillea* R. Br. ex Knight, *Leucospermum* R. Br., *Leucadendron* R. Br. and *Protea* L.) were collected and surveyed for the presence of fungal fruiting structures. Microscopic examination was carried out on material mounted in water and lactophenol, respectively, and measurements made at x1000 magnification. In cases where measurements were made from mounts in lactophenol, this is stated. The 95% confidence intervals were determined from 30 observations and the minimum and maximum ranges given in parentheses. Herbarium specimens are lodged at PREM and HKU(M), and reference cultures are maintained in the culture collection of the Department of Plant Pathology, University of Stellenbosch (STE-U). However, facilities were not available for making isolations for the majority of the specimens at the time of collection, and specimens were treated by quarantine officers to kill all fungi and insects present.

TAXONOMY

Camarosporium sp.

Figs. 1–11

Leaf spots circular to irregular, necrotic, sunken, grey-brown with a raised dark brown margin, up to 25 mm diam. Conidiomata amphigenous, pycnidial, nonerumpent but raising host surface slightly, dark brown, 90–170 µm diam. (Fig. 1); in section, substomatal, subepidermal, globose with a central, non-papillate ostiole, reduced stroma consisting of brown fungal hyphae in hosts cells surrounding ostiole, conidiogenous cells peripheral, approximately 270–300 µm high × 250–300 µm diam. (Fig. 2). Peridium comprising an outer stratum of thick-walled, small-celled, pale brown textura angularis becoming thin-walled inwardly; with the innermost cells thin-walled and hyaline, 20–25(–45) µm diam. (Fig. 2). Conidiophores reduced to conidiogenous cells. Conidiogenous cells subglobose, hyaline, determinate to indeterminate, 6–9 × 5–8 µm; conidiogenesis holoblastic, with infrequent percurrent proliferation, producing up to three wide annellations (Figs. 3 and 4). 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 (Figs. 5, 7 and 8). Germination of the conidium was by the development of a single germ tube, produced from the basal cell of the conidium (Fig. 6). *In vitro*: Conidiogenous cells, pyriform to globose, hyaline, 7–12 × 7–9 µm (Figs. 9 and 10). Conidia initially hyaline, becoming medium red-brown with maturity, 0–1(–3) septate, thinner-walled, smooth, 7–12 × 7–9 µm (Fig. 11).

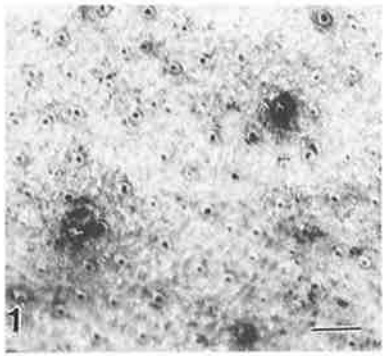
CULTURAL CHARACTERISTICS: On 2% malt extract agar (MEA; Biolab, Midrand, South Africa), supplemented with 0.1g/L Streptomycin sulphate at 25°C in the dark, colonies circular with undulate margins, buff (19"d) (Rayner 1970), often with darker patches visible. In reverse, brown vinaceous (84"'m), with broad concentric bands of buff (19"d) and brown vinaceous (84"'m). Moderately dense, felty to woolly aerial mycelium. Colonies reaching 60 mm diam. after 20 days.

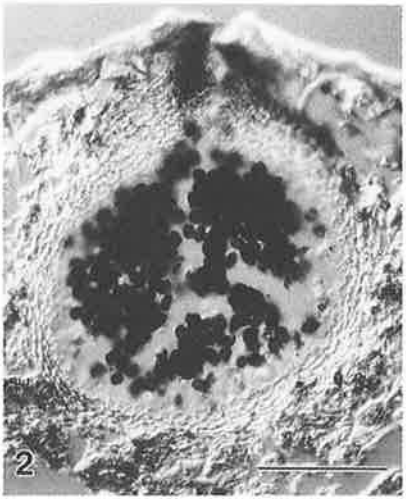
SPECIMENS EXAMINED: U.S.A.: California, Santa Barbara, leaf spot on *Protea cynaroides* (L.) L., 8 Apr. 1998, L. Swart (JT 396, PREM 56474, STE-U 3040); California, Santa Barbara, leaf spot on *Leucospermum cordifolium* (Salisb. ex Knight) Fourc., 8 Apr. 1998, L. Swart (JT 469, PREM 56473).

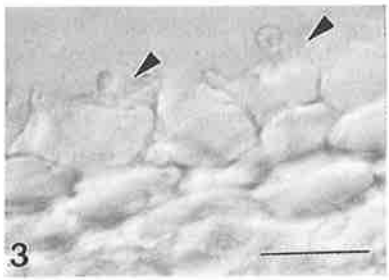
TELEOMORPH: Unknown.

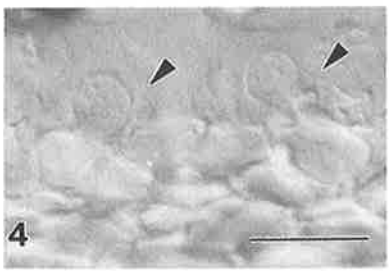
HOST RANGE: *Protea cynaroides* and *Leucospermum cordifolium*.

GEOGRAPHIC DISTRIBUTION: U.S.A. (California).


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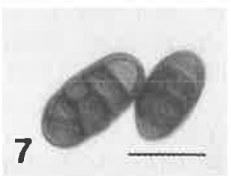

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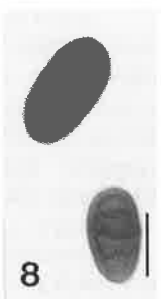

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NOTES: *Camarosporium* Schulzer is a widespread genus comprising approximately 100 species (Hawksworth et al. 1995), which represent anamorphs of *Cucurbitaria* Gray, *Leptosphaeria* Ces. & De Not. and *Pleospora* Rabenh. ex Ces & De Not. (*Cucurbitariaceae, Dothideales*) (Hawksworth et al. 1995). These collections were identified as *Camarosporium* according to Sutton (1980), as they form unilocular pycnidia with annellidic conidiogenous cells and muriform, euseptate conidia. They were also compared to other similar genera such as those discussed by Ramaley (1995, 1997) and Crous and Palm (1999).

Camarosporium sp. is not described to species level, as Sutton (1980) notes that *Camarosporium* is in need of revision, which should not involve any approach based on host specificity. As no revision has been carried out, and it is unfeasible to compare this species with all the other *Camarosporium* spp., no attempt is made to name these collections. However, it is illustrated and described here as cultures are available, which not only provide further characteristics for identification, but these cultures can be used in molecular studies which are likely to be important in the revision of this genus. It is possible that these collections represent the anamorph of one of the *Leptosphaeria* and *Pleospora* spp. which were also recorded in the study, and of which connections in culture could not be confirmed.

Coniothyrium septatum Joa.E.Taylor & Crous, sp. nov.

Figs. 12–17

Conidiomata pycnidialia, subepidermalia, amphigena, discreta, atrobrunnea 90–130 µm diam.; in sectione globosa ad subglobosa, ostiolata, 130–180(–230) µm alta × (150–)130–160(–200) µm diam. Peridium ex exterioribus stratis cellularum brunnearum texturae angularis, introrsum pallescens et hyalinum factum, (12–)14–23 (–32) µm. Conidogenae cellulae hyalinae, discretae, laeves, pyriformes ad ampulliformes, determinatae vel indeterminatae, 4–6(–7) × 3–5(–6) µm; conidiogenesis holoblastica, 1–3-plo enteroblastice et percurrenter proliferantes. Conidia fusiformia interdum ad septum angustata, pallide ad atro luteo-brunnea, parietibus crassis, laevia, (0–)1-medium septum, apice obtuso, base obtusirotundata ad truncata, (8–)10.5–11.5(–13) × (4.5–)5–5.5(–6) µm.

ETYMOLOGY: Referring to the medianly euseptate spore of this species.

Figs. 1–11. *Camarosporium* sp. (PREM 56474) Fig. 1. Conidiomata on host substrate. Fig. 2. Conidioma in cross section. Figs. 3 and 4. Conidiogenous cells showing wide phialides (arrowed). Figs. 5, 7 and 8. Conidia *in vivo*. Fig. 6. Germinating conidium. Figs. 9 and 10. Conidiogenous cells *in vitro*. Fig. 11. Conidia *in vitro*. Scale bars: 1 and 2 = 100 µm; 3–8 and 11 = 10 µm; 9 and 10 = 5 µm.

Leaf spots amphigenous, irregular, grey to light brown with a raised, dark brown border, mainly associated with leaf margins, 3–17 mm. Conidiomata pycnidial, subepidermal, substomatal, separate, visible as dark brown spots, 90–130 µm (Fig. 12); in section, globose to subglobose, with a central apapillate ostiole, 130–180(–230) µm high × (150–)130–160(–200) µm diam. (Fig. 15). Peridium consisting of two strata of cells in a textura angularis, outer stratum of thick-walled brown cells, becoming thinner walled and hyaline inwardly, (12–)14–23(–32) µm, often thicker surrounding ostiole (Fig. 17). Conidiophores reduced to conidiogenous cells. Conidiogenous cells hyaline, discrete, smooth, pyriform to ampulliform, determinate to indeterminate, 4–6(–7) × 3–5(–6) µm (Figs. 13 and 14); conidiogenesis holoblastic, proliferating 1–3 times enteroblastically and percurrently. Conidia fusiform, occasionally narrowing at the septum, thick-walled, pale to dark yellow-brown, smooth to finely verruculose, euseptate, (0–)1-median septum, apex obtuse, base truncate, (8–)10.5–11.5(–13) × (4.5–)5–5.5(–6) µm (Figs. 16).

CULTURAL CHARACTERISTICS: On MEA at 25° in the dark, colonies circular with entire margins, white on both surfaces, becoming olivaceous (23'"b) in the centre. Sparse, fine cottony aerial mycelium. Colonies slow growing, reaching 20 mm diam. after 28 days.

HOLOTYPE: U.S.A.: California, Santa Barbara, leaf spot on *Leucospermum cordifolium*, 8 Apr. 1998, L. Swart (JT 455, PREM 56499).

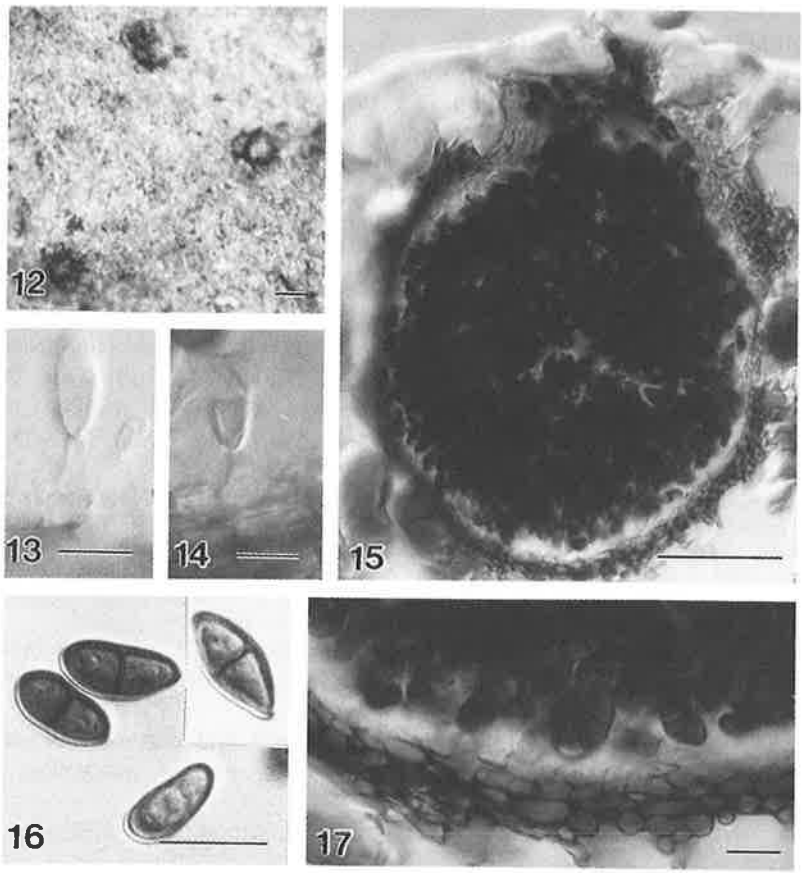
ADDITIONAL SPECIMENS EXAMINED: U.S.A.: California, Santa Barbara, leaf spot on *Protea laurifolia* Thunb., 8 Apr. 1998, L. Swart (JT 495, PREM 56501). CANARY ISLANDS: Tenerife, Proteas da Canarias, leaf spot on *Leucospermum* 'High Gold', 5 Apr. 2000, J.E. Taylor & S. Denman (JT1145, PREM 56864, STE-U 3737-3739); Tenerife, La Laguna University Experimental Farm, leaf spot on *Protea* 'Susara', 5 Apr. 2000, J.E. Taylor & S. Denman (JT1154, PREM 56865, STE-U 3740-3742).

TELEOMORPH: Unknown.

HOST RANGE: *Leucospermum cordifolium, Leucospermum* 'High Gold', *Protea laurifolia* and *Protea* 'Susara'.

GEOGRAPHIC DISTRIBUTION: U.S.A. (California) and Canary Islands (Tenerife).

NOTES: *Coniothyrium* spp. are commonly associated with necrotic leaf spots of proteas (Swart et al. 1998, J.E. Taylor and P.W. Crous, unpubl. data). Although no isolates were obtained from the Californian material, cultures were obtained from collections made in Tenerife. *Coniothyrium septatum* is very distinctive in having predominantly 1-septate conidia, differing from other species of *Coniothyrium* Corda, and especially from those known from proteas (Swart et al. 1998, J.E. Taylor and P.W. Crous, unpubl. data).



Figs. 12–17. *Coniothyrium septatum* (holotype). Fig. 12. Conidiomata on host substrate. Figs. 13 and 14. Conidiogenous cells. Fig. 15. Conidioma in cross section. Fig. 16. Conidia. Fig. 17. Peridium. Scale bars: 12 = 100 µm; 15 = 50 µm; 13, 16 and 17 = 10 µm; 14 = 5 µm.

Morphologically it most closely resembles *Coniothyrium nitidae* Crous & S. Denman, although the conidial dimensions of the latter are smaller (6.5–)8–9(–11) x 3–4(–4.5) µm (Swart et al. 1998).

Didymosphaeria futilis (Berk. & Broome) Rehm, Hedwigia 18: 167 (1879). Figs. 18–24

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. (Fig. 20). Ascomata amphigenous, immersed to semi-immersed, non-erumpent, black, singular, scattered, 180–320 µm diam. (Fig. 22); 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 (Fig. 18). Peridium comprising two strata of textura intricata, an outer stratum of mid brown, thick-walled cells, becoming hyaline and thin-walled inwardly, 22–30 µm at the sides and thicker at the base, 20–50 µm (Fig. 19). Pseudoparaphyses trabecular, numerous, anastomising in between the asci, 1–2 µm (Figs. 24). 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 (Figs. 24). Ascospores slightly overlapping, uniseriate, ellipsoidal, medium red-brown, median distoseptate, fine spinulose ornamentation, (7.5–)8.5–10.5(–12) × (3.5–)4–4.5(–6) µm (Figs. 21 and 23).

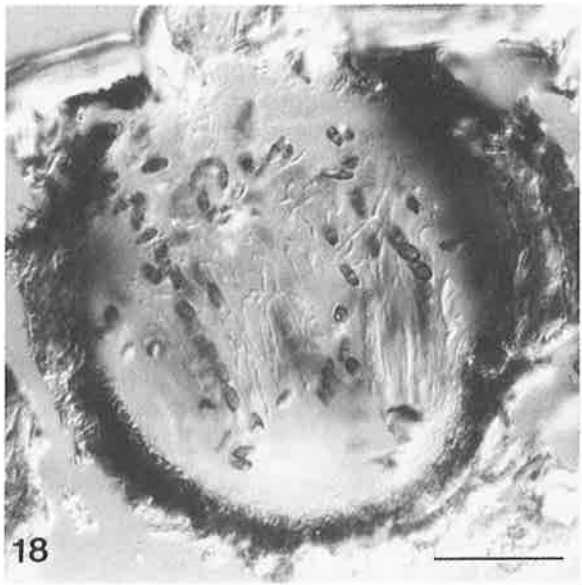
SPECIMENS EXAMINED: U.S.A.: California, UC Santa Cruz, Arboretum, *Protea magnifica* Link, 4 Apr. 1998, L. Swart (JT485, PREM 56502); SOUTH AFRICA: Western Cape, Gouriqua, Reins Nature Reserve, leaf spots on *Protea susannae* E. Phillips, 8 Apr. 1998, L. Dreyer (JT705, PREM 56548); Western Cape, Kirstenbosch, leaf spots of *Protea* sp., Sept. 1932, I.B. Pole-Evans (PREM 26414); Western Cape, on stem of *Leucadendron* sp, 27 May 1999, L. Swart (JT829, PREM 56549).

ANAMORPH: Phoma-like coelomycete and *Fusicladiella* Höhn. synanamorph (Aptroot 1995a), though none observed in these collections.

HOST RANGE: In and on stems, wood and leaves of various plants (Aptroot 1995a); *Leucadendron* sp., *Protea magnifica, P. susannae* and *Protea* sp.

GEOGRAPHIC DISTRIBUTION: Cosmopolitan (Aptroot 1995a).

Figs. 18–24. *Didymosphaeria futilis* (PREM 26414). Fig. 18. Ascoma in cross section. Fig. 19. Peridium. Fig. 20. Lesions on leaves of *Protea* sp. Figs. 21 and 23. Ascospores. Fig. 22. Ascomata on host substrate. Fig. 24. Ascus. Scale bars: 22 = 100 µm; 18 = 50 µm; 19, 21, 23 and 24 = 10 µm.


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NOTES: *Didymosphaeria futilis* appears to be commonly associated with lesions of *Proteaceae*. Although we suspect that this species is a saprobe (Aptroot 1995a, b), *Didymosphaeria* Fuckel is still listed on the U.S. Actionable Pathogen List for proteas (Mary Palm, pers. comm.). As no comprehensive description or illustrations could be found for this fungus, it is described here in full for the benefit of quarantine officers and plant pathologists studying protea diseases.

Elsinoë fecunda Joa.E.Taylor & Crous, sp. nov.

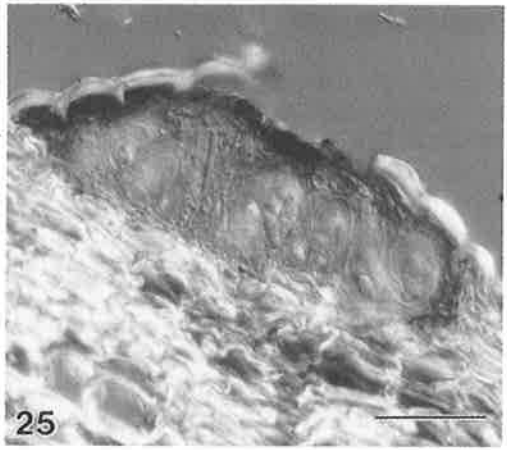
Figs. 25–31

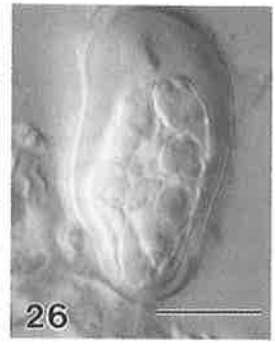
Ascomata dispersa, separata; in sectione pulvinata, intra epidermide, unilocularia vel multilocularia 125–175 µm diam. × 40–65 µm lata. Peridium compositum ex textura angulare, pseudoparenchymatica pallido vel medio brunnea, 7–18 µm. Asci diffusi irregulariter ubique in ascomate, saccati, clavati et obclavati cum extremis rotundatis, crasso pariete, octospori, sessiles, hyalini, (20–)26–28(–33) × (12–)15–16.5(–20) µm. Ascospori hyalini, late ellipsoidei cum extremis rotundatis vel obtusis, cum 1–4 septis transversalibus et 0–2 septis verticalibus, interdum constricti ad septum medianum, (10–)11.5–12.5(–15) × (4–)4.5–5(–5.5) µm.

ETYMOLOGY: From the Latin *fecundus* meaning 'prolific', in reference to the abundant production of ascomata of this *Elsinoë* species.

Lesions visible as regularly scattered small black specks on the twigs and some leaves (Fig. 30); typical corky and scab-like lesions not observed. Ascomata separate and singular, circular to irregular, 70–200 µm diam. (Fig. 30); 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 (Fig. 25). Peridium composed of medium to dark brown pseudoparenchymatic textura angularis, becoming paler and thin-walled inwardly and extending between asci, 7–18 µm (Fig. 28). 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 (Figs. 26 and 27). Ascospores multiseriate, hyaline, broadly ellipsoid with rounded to obtuse ends, widest just above first formed septum, 1–4 transversely septate, and 1–2 vertical septa, oblique septa rare, occasionally constricted at median septum, (10–)11.5–12.5(–15) × (4–)4.5–5(–5.5) µm (Figs. 29 and 31).

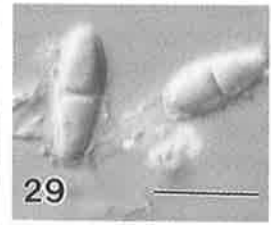
Figs. 25–31. *Elsinoë fecunda* (holotype). Fig. 25. Ascoma in cross section. Figs. 26 and 28. Asci. Fig. 27. Peridium. Figs. 29 and 31. Ascospores with sheath (arrowed in 31). Fig. 30. Ascomata on host substrate. Scale bars: 30 = 100 µm; 25 = 30 µm; 26–29 and 31 = 10 µm.


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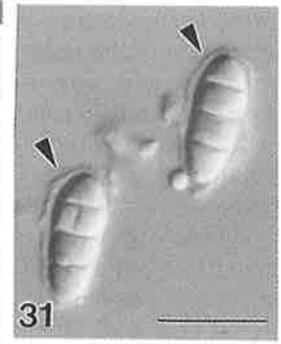

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HOLOTYPE: U.S.A.: California, San Diego, leaf spot on *Protea scolymocephala* (L.) Reichard, 12 Apr. 1998, L. Swart (JT 390, PREM 56503).

ANAMORPH: *Sphaceloma* state not observed.

HOST RANGE: *Protea scolymocephala.*

GEOGRAPHIC DISTRIBUTION: U.S.A. (California).

NOTES: There are over 40 species of *Elsinoë* Racib., which occur mainly in warmer parts of the world (Hawksworth et al. 1995). *Elsinoë* is a member of the *Elsinoaceae* (*Dothideales*) and produces a *Sphaceloma* de Bary anamorph. Members of this genus are biotrophic or necrotrophic on plants, causing scab diseases of fruits, leaves and stems of many cultivated hosts (Arx 1987). Host specificity is unclear at this stage as many species have been described, but are indistinguishable from each other by morphological characters (Arx 1987) or symptoms. Molecular studies are presently addressing this problem, and at least four distinct species of *Elsinoë* (or the *Sphaceloma* anamorph) have been described from proteaceous hosts (Benic and Knox-Davies 1983; Swart et al. 2000a).

Elsinoë spp. are regarded as serious pathogens of cultivated proteas in parts of Australia (Pascoe et al. 1995) and southern Africa (Swart et al. 2000a) and is thought to be distributed via apparently healthy nursery material. This type of distribution has possibly also occurred on an international scale, therefore all new disease records are regarded as of great importance to the protea industry.

This collection is not considered to be conspecific with any of the previously described species from *Proteaceae* (Benic and Knox-Davies 1983; Swart et al. 2000a). Its disease symptoms differ markedly from the characteristic 'scab' symptoms associated with *Sphaceloma protearum* L. Swart & Crous. Of the other species isolated from *Proteaceae*, *Elsinoë fecunda* most closely resembles *Elsinoë leucospermi* L. Swart & Crous, but can be distinguished in that ascospores of the latter species lack a sheath. Disease symptoms are also much less severe than those associated with *E. leucospermi* (Benic and Knox-Davies 1983).

The origin of *E. fecunda* is unclear, as *P. scolymocephala* has been introduced into California. Therefore, it is possible that this fungus was derived from another host. Molecular studies have shown that *Elsinoë* spp. on *Proteaceae* have wide host ranges within the family (Swart et al. 2000a). Whether some species of *Elsinoë* are truly plurivorous, however, is uncertain at this stage.

This was not the only record of scab disease on proteas in California. *Elsinoë leucospermi* was isolated from diseased *Leucospermum* spp. (Table 1). In addition, a severe scab disease of

Protea mundii Klotzsch was also observed. However, only the morphologically conserved anamorph was present, so the identity of the species on this host remains uncertain.

Mycosphaerella leucospermi Crous & Joa.E.Taylor, sp. nov.

Figs. 32–34

Maculae amphigenae, circulares ad irregulares, morboscentes. Mycelium internum et externum, septatum, medio brunneum, verruculosum, usque ad 5 µm latum. Ascomata amphigena, sub stomate, intra epidermide vel sub epidermide, erumpescentia, globosa ad subglobosa, ostiolata. Paries compositus ex 2–3 stratis texturae angularis medio brunneis. Asci octospori, sine paraphyse, fasciculati, subsessiles, obovoidei, recti vel leviter incurvati, 40–64 × 8–20 µm. Ascosporae biseriatae ad multiseriatae, guttulatae, cum parietibus crassis, rectae ad leviter incurvatae, fusiformes-ellipsoideae, cum extremis obtusis, latissimae ad medium cellulae apicalis, decrescentes in utraque extrema sed magis manifeste in inferiorem extremum, pallide brunneae et constrictae ad septum factae; ascosparae brunneae et subtiliter verruculosae post dimissum factae, (15–)17–20 × (4–)5–6(–7) µm.

ETYMOLOGY: In reference to the host, *Leucospermum,* from which the type is recorded.

Leaf spots amphigenous, subcircular to irregular, 3–10 mm diam., grey, surrounded by a thin, raised, brown border. 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 wide and 130 µm high (Fig. 32); apical ostiole up to 20 µm wide; 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 (Fig. 34). 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 lower end, becoming pale brown and constricted at septum; ascospores become brown and finely verruculose after discharge, (15–)17–20 × (4–)5–6(–7) µm (in lactophenol) (Fig. 33).

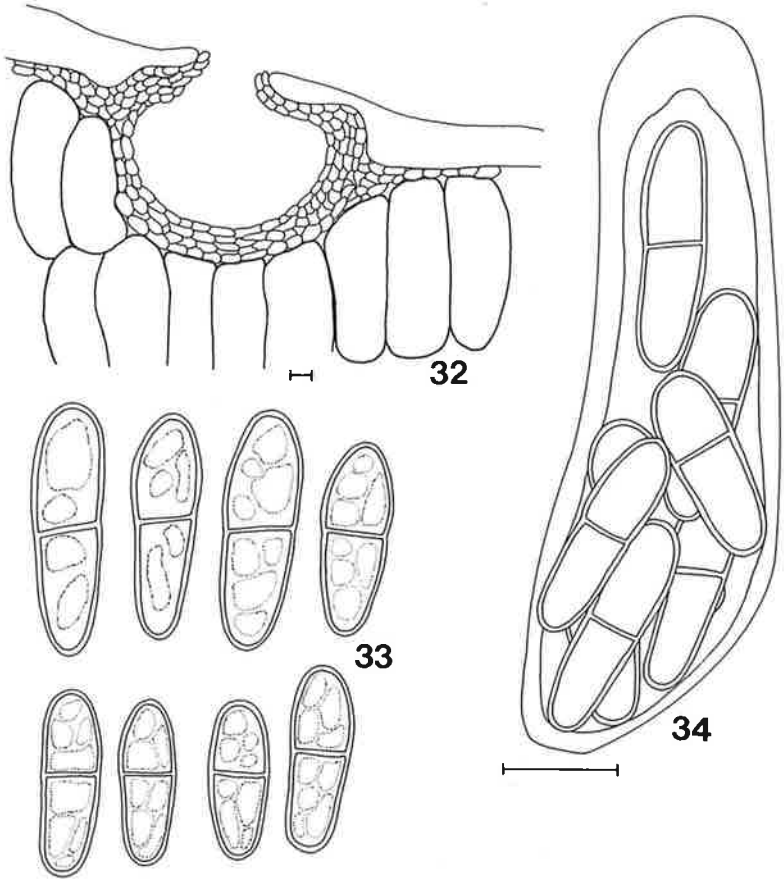
HOLOTYPE: U.S.A.: California, Santa Barbara, on leaf spot on *Leucospermum cordifolium,* 8 Apr. 1998, L. Swart (JT454, PREM 56524).

ADDITIONAL SPECIMENS EXAMINED: U.S.A.: California, Santa Barbara, on leaf spot on *Protea laurifolia,* 8 Apr. 1998, L. Swart (JT493, PREM 56526); *ibid.,* on leaf spot on *Protea cynaroides* (JT501, PREM 56525).

ANAMORPH: Unknown.

HOST RANGE: *Leucospermum cordifolium, Protea laurifolia* and *P. cynaroides.*

GEOGRAPHIC DISTRIBUTION: U.S.A. (California).



Figs. 32–34. *Mycosphaerella leucospermi* (holotype). Fig. 32. Conidioma in cross section. Fig. 33. Ascospores. Fig. 34. Ascus. Scale bars: 32–34 = 10 µm.

NOTES: These collections represent a distinctive species of *Mycosphaerella* pathogenic to *Proteaceae*, and although the anamorph is lacking and cultures of this pathogen are not available, it is considered to be characteristic enough to describe as new. Using the key to *Mycosphaerella* spp. occurring on these host genera (Taylor and Crous 2000), *M. leucospermi* is found to most closely resemble *M. jonkershoekensis* P.S. van Wyk, Marasas & Knox-Dav.

(Van Wyk et al. 1975; Taylor and Crous 1998). The ascospores of *M. jonkershoekensis,* however, differ in morphology in having a longer lower cell, and are narrower (10.5–)18–19.5(–23) × (4–)4.5–5(–6) µm (Taylor and Crous 1998). It is possible that *M. leucospermi* is derived from another host, as these proteas are introduced into California. Further collections need to be made to determine the anamorph connection and for cultures to be made available for molecular studies.

Mycosphaerella sp.

Figs. 35–38

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. 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 (Fig. 36); apical ostiole 10–15 µm wide; wall consisting of 3–4 layers of brown textura angularis, up to 10 µm thick. Asci aparaphysate, fasciculate, bitunicate, subsessile, obovoid to ellipsoid, straight to incurved, 8-spored, 28–50 × 8–11 µm (Fig. 38). 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, not constricted at the septum, thick-walled, (9–)10–11(–12) × 3–3.5 µm (in lactophenol) (Fig. 37).

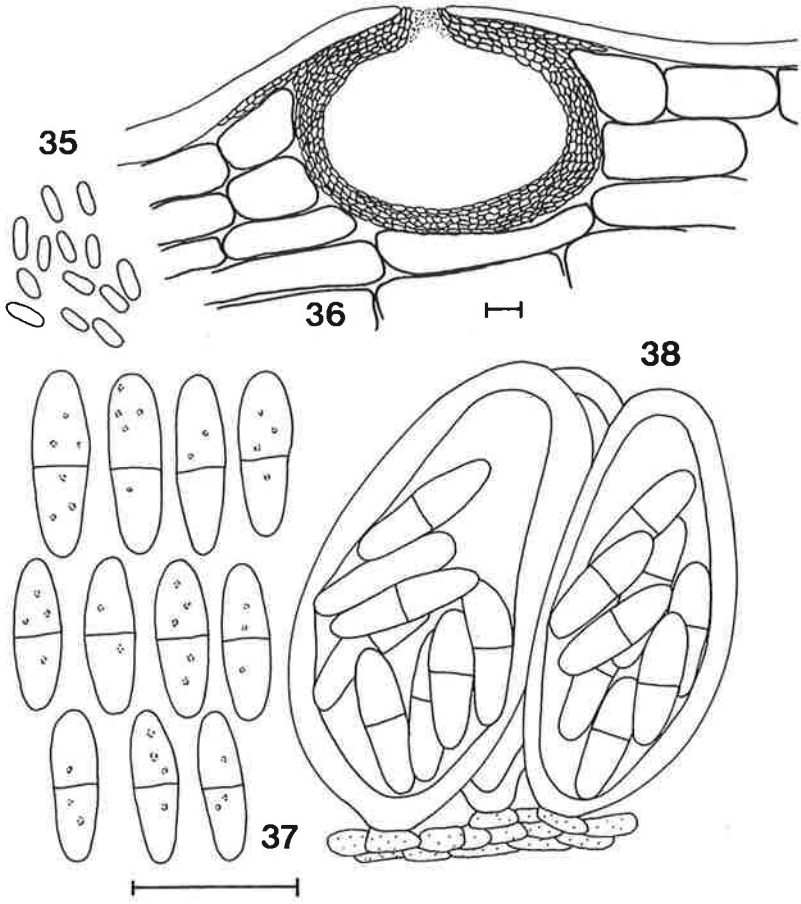
SPECIMENS EXAMINED: U.S.A.: California, San Diego, on leaf spot of *Protea cynaroides*, 12 Apr. 1998, L. Swart (JT286, PREM 56522); California, Arboretum, UC Santa Cruz, on leaf spot of *Protea eximia* (Salisb. ex Knight) Fourc., 4 Apr. 1998, L. Swart (JT439, PREM 56523); California, Santa Barbara, on leaf spot on *Leucospermum cordifolium*, 8 Apr. 1998, L. Swart (JT 452, PREM 56521).

ANAMORPH: Unknown.

HOST RANGE: *Leucospermum cordifolium, Protea eximia* and *P. cynaroides.*

GEOGRAPHIC DISTRIBUTION: U.S.A. (California).

NOTES: These collections represent a species of *Mycosphaerella*, that in the absence of cultures and anamorph details, would be difficult to distinguish from the complex recognised by Taylor and Crous (2000). For this reason no specific name has been appointed. In dimensions, this pathogen is similar to *Mycosphaerella bellula* Crous & M.J. Wingf.(Crous and Wingfield 1993; Taylor and Crous 1998). However, the ascospores are not constricted at the septum, are slightly narrower and lack a distinctive sheath (Taylor and



Figs. 35–38. *Mycosphaerella* sp. (PREM 56522). Fig. 35. Spermatia. Fig. 36. Conidioma in cross section. Fig. 37. Ascospores. Fig. 38. Asci. Scale bars: 35–38 = 10 µm.

Crous 1998), and could possibly represent yet another species. A description of this pathogen is given here in anticipation of future collections, when the information necessary for identification or full description may be available.

Stigmina californiae Joa.E.Taylor & Crous, sp. nov.

Figs. 39–46

Sporodochia amphigena, numerosa, atro-brunnea ad nigra, punctiformia, (70–)80–140(–200) µm, consociata cum maculis morboscentibus luteis ad pallide brunneis et depressis, cum marginibus extantibus, 4–25 mm diam. Mycelium superficiale, rubro-brunneum, cum parietibus crassis, verruculosum, constrictum ad septa, reticulatum et crustam quasi stratum infra et inter sporodochia formans, 7–9(–10) µm. Stromata ex stomatibus emergentia, formantia superficiales massas sporodociorum contiguorum. Conidiophora cylindrica, decrescentia ad cellulas conidiogenas, in fasciculis laxis, ex stromate exorientia, macronemata, recta vel flexuosa, praecipue simplicia, rubro-brunnea, tenuiter verruculosa et cum striis, (8–)14–24(–60) × (5–)6–6.5(–7) µm. Cellulae conidiogenae cylindricae, medio rubro-brunneae, subtiliter verruculosae, indeterminatae cum 0–4 annellatis irregularibus et asperis (5–)9.5–13(–17) × (4–)5–6 µm. Conidia cylindrica, fusiformia vel obclavata, cum base truncata et decrescentia ad apicem rotundatem, recta, luteo-brunnea, subtiliter verruculosa, 0-3 distoseptata, (11–)14–16(–19) × (3–)5–5.5(–7) µm.

ETYMOLOGY: In reference to the place where this fungus was first collected.

Sporodochia amphigenous, numerous, dark brown to black, punctiform, (70–)80–140(–200) µm, associated with yellow to pale brown, sunken, necrotic leaf spots, with raised borders, 4–25 mm diam. (Fig. 39). Mycelium superficial, red-brown, thick-walled, finely verrucose, constricted at the septa, reticulate and forming a crust-like layer beneath and in between sporodochia, 7–9(–10) µm (Fig. 41). Stromata emergent from stomata, forming superficial masses of adjoining sporodochia (Fig. 41). Conidiophores cylindrical, tapering to the conidiogenous cells, in loose fascicles, arising form 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 (Fig. 46). Conidiogenous cells cylindrical, medium red-brown, finely verrucose, indeterminate with 0–4 irregular, roughened annellations (5–)9.5–13(–17) × (4–)5–6 µm (Figs. 44 and 45). 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 (Figs. 40, 42 and 43).

HOLOTYPE: U.S.A.: California, Santa Barbara, leaf spot on *Protea* 'Pink Ice', Apr. 1998, L. Swart (JT 383, PREM 56541).

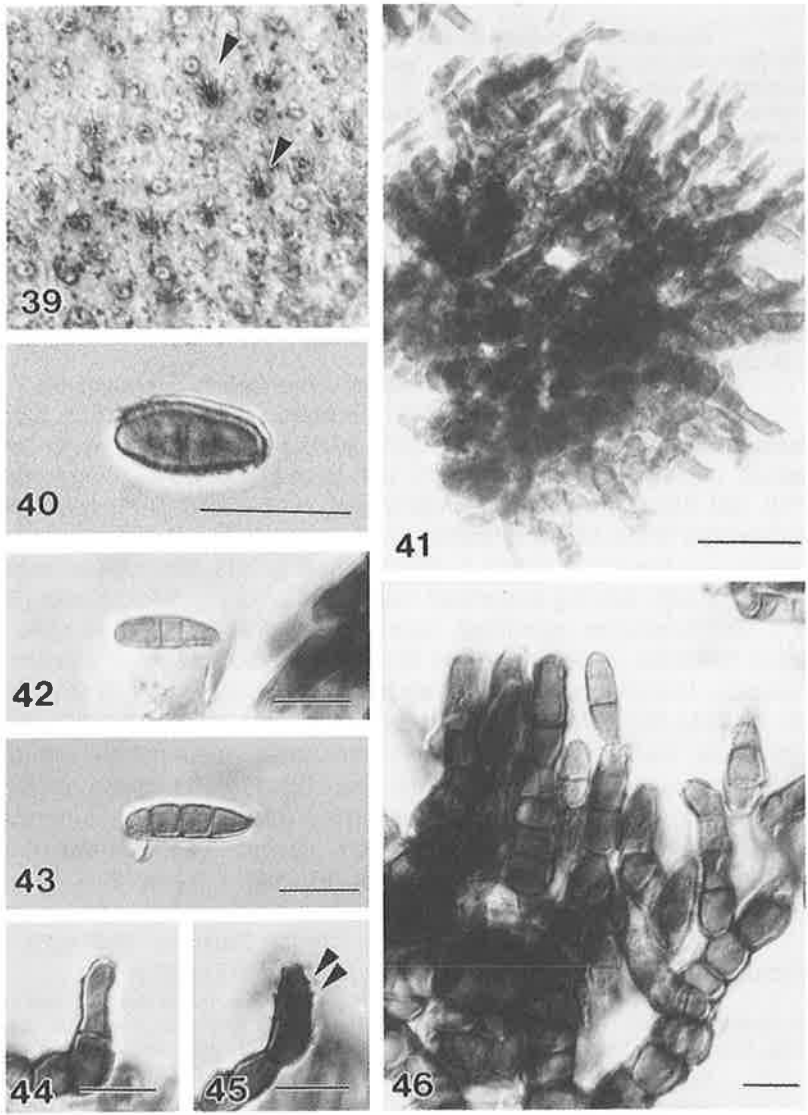
ADDITIONAL SPECIMENS EXAMINED: U.S.A. California, Santa Barbara, leaf spot on *Protea laurifolia*, 8 Apr. 1998, L. Swart (JT 491, PREM 56542); California, Santa Cruz, leaf spot on *Protea magnifica*, 5 Apr. 1998, L. Swart (JT 472, PREM 56543).

TELEOMORPH: Unknown.

HOST RANGE: *Protea laurifolia, Protea magnifica* and *Protea* 'Pink Ice'.

GEOGRAPHIC DISTRIBUTION: U.S.A. (California).

NOTES: The widespread genus *Stigmina* Sacc. has recently been reviewed by Sutton (1989). The collections above subscribe to this generic delimitation, and differ from allied genera (Braun et al. 1999).



Figs. 39–46. *Stigmina californiae* (holotype). Fig. 39. Sporodochia on host substrate. Figs. 40, 42 and 43. Conidia. Fig. 41. Sporodochium and superficial mycelium. Figs. 44 and 45. Conidiogenous cells showing annellides (arrowed). Fig. 46. Conidiophores and conidiogenous cells. Scale bars: 41 = 30 µm; 39 and 40, 42–46 = 10 µm.

The spores are distoseptate, and therefore these collections belong in *Stigmina,* rather than *Scolecostigmina* U. Braun, which has pigmented, euseptate, scolecosporous conidia (Braun et al. 1999). These collections do not correspond to any other previously described species of *Stigmina sensu stricto* as the spore length is smaller (Hughes 1953; Ellis 1971, 1976; Sutton and Pascoe 1989).

Three varieties of *Cercostigmina protearum* (Cooke) U. Braun & Crous have been described from proteaceous hosts in South Africa (Crous and Braun 1994, 1996), as well as three species of *Batcheloromyces* Marasas, P.S. van Wyk & Knox-Dav. (Marasas et al. 1975; Van Wyk et al. 1985; Taylor et al. 1999). *Batcheloromyces* is restricted to proteaceous hosts in South Africa [although one species, *B. leucospermi*, was collected in California (Table 1)], and was included in generic synonymy of *Stigmina* by Sutton and Pascoe (1989), but not by Taylor et al. (1999). However, none of the species of the aforementioned genera correspond to the collections made in California.

DISCUSSION

This study provides baseline data on the status of diseases of proteas in California (Table 1). It also summarizes the data collected on proteas from other states, excluding Hawaii, where proteas are cultivated (Farr et al. 1989) and of specimens intercepted by quarantine (Table 1). All of the identified fungi are deposited at PREM, unless otherwise stated. The associated symptoms and the occurrence of the disease in South Africa are also noted (Table 1).

An overall impression of protea plantations in the protea production regions of California suggests that they show generally little symptoms of disease. In the few plantations, however, where the most devastating diseases of proteas in California are present, the diseases result in significant losses of stems and even entire plants. Plants that are stressed due to nutritional factors are more prominent (Perry 1998), although these stressed plants are also more susceptible to diseases (Schoeneweiss 1975). So far the most serious diseases recorded in California have been Botrytis blight of flowers, and root diseases such as quick die-back (probably caused by species of *Phytophthora* de Bary and/or *Pythium* Pringsh.) (Perry 1998).

In this study of mainly leaf and stem diseases, Botryosphaeria stem canker caused by *Botryosphaeria dothidea* was the most devastating disease found on older *Protea* and *Leucadendron* plants, that were probably more subjected to stress. In some regions (central and southern parts of California) and on certain farms, entire plantations were destroyed by the canker pathogen. This cosmopolitan pathogen

with its very wide host range (Brown and Hendrix 1981), probably spread from other host plants nearby. In South Africa, this is also probably the most important canker causing pathogen (Von Broembsen and Van der Merwe 1990) affecting woody stems, and research into integrated disease management programmes is currently being undertaken (S. Denman, pers. comm.).

Botryosphaeria proteae caused leaf spots on various *Protea* species, as did a currently unidentified *Fusicoccum* sp., but neither were as devastating as *B. dothidea*. *Botryosphaeria proteae* has recently been treated by Denman, Crous and Wingfield (1999), and was possibly introduced to California as an endophyte, as it is commonly isolated from surface sterilized leaves of *Protea* and *Leucospermum* spp. (Swart et al. 2000b). *Fusicoccum* sp. has been found associated with leaf spots of proteas in South Africa and other areas where proteas are cultivated. Currently, molecular studies are being carried out in order to determine the correct identity of this pathogen (S. Denman, pers. comm.). It is possible that it is either a common widespread pathogen, or has been distributed endophytically on seed, as it has also been isolated in endophyte studies (J.E. Taylor, unpubl. data). This pathogen broadly resembles *Fusicoccum aesculi,* but in culture it differs in that it produces grey and woolly mycelium, compared to the former, of which the culture is black in colour and the mycelium is only woolly at the edges. In addition, the conidia of *Fusicoccum* sp. differ as they do not taper to the apices, but are oblong with a fairly broad obtuse apex, narrowing slightly to a truncate base with a minute basal frill and are wider (22–) 27–30(–33) × (7–)8.5–9.5(–11) µm, compared to 18–25(–30) × 4–4.5(–5) µm for *F. aesculi* (Crous and Palm 2000).

Coleroa leaf spot is the most common leaf spot pathogen (Taylor and Crous 1998), occurring widely on most of the *Protea* cultivars and species in California. Although this pathogen generally affects older growth (Von Broembsen 1989), it can cause financial losses for the producer as it can affect the aesthetic value of the flower, especially when infections are severe. Other common leaf spot pathogens recorded in South Africa, such as *Batcheloromyces* spp. (with the exception of a few collections of *B. leucospermi*) (Taylor et al. 1999), *Cercostigmina* spp. (Crous and Braun 1994), *Leptosphaeria protearum* Syd. & P. Syd., *Teratosphaeria* spp. and *Vizella interrupta* (G. Winter) S. Hughes (Taylor and Crous 1998) were not collected in California. However, there were present other pathogens associated with necrotic leaf spots on *Protea* and *Leucospermum* unrecorded in South Africa, such as those new species reported here, but which appear to be of lesser importance.

In California Elsinoë scab disease can be a very important and devastating disease on *Leucospermum cordifolium* and *Protea mundii*. Heavy losses occurred in one nursery in the early 1980's due to scab disease on *Leucospermum* spp. caused by *Elsinoë leucospermi* (Swart et al. 2000a). The disease has a very limited distribution in California, only occurring in two plantations and one nursery on *L. cordifolium*. The species of *Elsinoë* causing scab symptoms on *P. mundii* is as yet unidentified, and was also detected in one nursery. However, the fact that this disease is found in nurseries can have significant implications for the Californian protea producers since the disease can spread throughout the Californian protea production area by means of infected cuttings or plants (Von Broembsen 1989). The spread of scab infected nursery material already had devastating consequences in Australia (Pascoe et al. 1995) and Zimbabwe (L. Swart, pers. obs.). A number of other protea hosts also displayed symptoms of Elsinoë scab disease (*Leucadendron* 'Silvan Red', *Leucadendron discolor* E. Phillips & Hutch., *Protea cynaroides, Protea magnifica* and *Protea* sp.), but the identity of the causal organisms could not be verified.

The limited range of pathogens and saprophytes recorded on protea plants from California can be partly ascribed to the fact that plant material for identification was only collected once during the spring of 1998. The occurrence of some diseases can be affected by the prevailing climate, for instance, specific climatic conditions are necessary for the development of Botrytis blight, Drechslera blight and Colletotrichum anthracnose (Von Broembsen 1989). Therefore, the absence of these pathogens from the collections made in this study is no indication of their status in plantations in California. More collections need to be made during different seasons of the year in order to be able to obtain a more realistic impression of the range of fungal pathogens associated with proteas in California.

The implications of these findings, and the impact they may have on the status of these fungi as actionable pathogens, is difficult to assess. Unfortunately, the issues in question go beyond the scope of the present study. However, this research does represent the initial stage required by the Sanitary and Phytosanitary Agreement (World Trade Organisation 1994) in the assessment of risk to plant health and the determination of the appropriate level of phytosanitary measures. This must take into account the available scientific evidence, of which prior to this study, there was none.

Table 1: Microfungi recorded from diseased *Proteaceae* hosts. Includes records collected in California during Apr. 1998, records listed in Farr et al. (1989)* and records from quarantine interceptions †.

Pathogen	Host Range	Symptoms	Status in South Africa	State Collected	Reference
Alternaria sp.	*Protea cynaroides*	leaf spot	-	CA	PREM 56463
Anthostomella spartii Berl. & Voglino	*Protea eximia*	leaf spot	N	CA	PREM 56464
Armillaria tabescens (Scop.) Dennis, Orton & Hora	*Grevillea robusta* A. Cunn. ex R. Br.*	root rot	N	FL	(Alfieri et al. 1984)
Batcheloromyces leucospermi J.E. Taylor & Crous	*Protea* sp.	leaf spot	Y	CA	PREM 56465
Botryodiplodia sp.	*Grevillea robusta* †		-	FL	BPI 625084B
Botryosphaeria dothidea (Moug. ex Fr.) Ces. & De Not. (*Fusicoccum aesculi* Corda)	*Leucadendron* 'Silvan Red' *Leucadendron discolor* *Protea cynaroides* *Protea cynaroides* 'Silver Mink' *Protea eximia* *Protea laurifolia* *Protea magnifica* *Protea obtusifolia* H. Buek ex Meisn. *Protea* sp.	Severe pathogen causing leaf spots and stem cankers	Y	CA	PREM 56466 PREM 56467 PREM 56468 PREM 56505 PREM 56469 PREM 56470 PREM 56506 PREM 56507 PREM 56508
Botryosphaeria proteae (Wakefield) S. Denman & Crous (*Fusicoccum proteae* S. Denman & Crous)	*Protea* 'Queen Mink' *Protea cynaroides* *Protea eximia*	leaf spot	Y	CA	PREM 56509 PREM 56471 PREM 56510
Botryosphaeria quercuum (Schwabe) Sacc.	*Grevillea robusta* *	die-back, gumming	-	FL	(Alfieri et al. 1984)
Botryosphaeria sp.	*Grevillea robusta* † *Grevillea* sp. †	-	-	FL	BPI 625084A,B BPI 625082

Botryotinia fuckeliana (de Bary) Whetzel (*Botrytis cinerea* Pers.)	*Protea cynaroides*	blight	Y	CA	PREM 56472
Camarosporium sp.	*Leucospermum cordifolium* *Protea cynaroides*	leaf spot	N	CA	PREM 56473 PREM 56474
Cladosporium spp.	*Leucospermum cordifolium* *Protea* 'Pink Ice' *Protea* 'Queen Mink' *Protea cynaroides* 'Silver Mink' *Protea magnifica* *Protea repens* (L.) L.	leaf spot	-	CA	PREM 56475 PREM 56476 PREM 56477 PREM 56478 PREM 56479 PREM 56480
Coleroa senniana (Sacc.) E. Mull. & Arx	*Leucospermum cordifolium* *Protea* 'May Day' *Protea* 'Pink Ice' *Protea* 'Queen Mink' *Protea* 'White Owl' *Protea laurifolia* *Protea laurifolia* x *neriifolia* *Protea magnifica* *Protea neriifolia* R. Br. *Protea neriifolia* x *magnifica* *Protea obtusifolia* *Protea pudens* Rourke *Protea* sp.	Severe leaf spot pathogen. Biotrophic, sometimes causing necrotic lesions	Y	CA	PREM 56481 PREM 56482 PREM 56483 PREM 56484 PREM 56485 PREM 56486 PREM 56487 PREM 56488 PREM 56489 PREM 56490 PREM 56491 PREM 56492 PREM 56493
Coniothyrium leucospermi Crous & S. Denman	*Protea neriifolia* *Protea obtusifolia* *Protea* sp.	leaf spot	Y	CA	PREM 56495 PREM 56496 PREM 56497
Coniothyrium septatum	*Leucospermum cordifolium* *Protea laurifolia*	leaf spot	N	CA	PREM 56499 PREM 56501
Coniothyrium spp.	*Leucospermum cordifolium* *Protea cynaroides*	leaf spot	-	CA	PREM 56498 PREM 56500

Corticum sp.	*Grevillea robusta* †		-	FL	BPI 1101248
Didymosphaeria futilis	*Protea magnifica*	leaf spot	Y	CA	PREM 56502
Diplodia sp.	*Grevillea robusta** *Grevillea* sp.*	die-back, gumming*	-	FL	(Anonymous 1960; Alfieri et al. 1984)
Elsinoë leucospermi	*Leucospermum cordifolium* *Leucospermum cordifolium* var. Vlam *Leucospermum. glabrum* *Leucospermum. reflexum* var. Luteum	scab	Y	CA	STE-U 2042 STE-U 2041 STE-U 2039 STE-U 2040
Elsinoë fecunda	*Protea scolymocephala*	scab	N	CA	PREM 56503
Fusarium sp.	*Protea cynaroides* *Grevillea robusta**	root rot*	-	CA, FL	PREM 56504 (Alfieri et al. 1984)
Fusicoccum sp.	*Protea compacta* *Protea cynaroides* *Protea eximia* *Protea laurifolia*	leaf spot	Y	CA	PREM 56511 PREM 56512 PREM 56513 PREM 56514
Fusicoccum proteae	See *Botryosphaeria proteae*				
Fusicoccum aesculi	See *Botryosphaeria dothidea*				
Ganoderma zonatum Murrill	*Grevillea robusta**	root rot	-	FL	(Alfieri et al. 1984)
Ganoderma brownii Murrill	*Hakea* sp.*	on wood	-	CA	(Gilbertson and McHenry 1969)
Glomerella cingulata (Stoneman) Spauld. & H. Schrenk (*Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc.)	*Protea cynaroides*	anthracnose	Y	CA	PREM 56494
Kabatiella sp.	*Protea cynaroides*	leaf spot	-	CA	PREM 56515

Leptosphaeria cf. *lonicera* (Fautrey) L. Holm	*Protea magnifica* *Protea neriifolia* x *magnifica* *Protea* sp.	leaf spot	-	CA	PREM 56516 PREM 56517 PREM 56518
Lophiostoma fuckelii Sacc.	*Protea cynaroides* *Protea laurifolia* x *neriifolia*	leaf spot	Y	CA	PREM 56519 PREM 56520
Mycosphaerella leucospermi	*Leucospermum cordifolium* *Protea cynaroides* *Protea laurifolia*	leaf spot	N	CA	PREM 56521 PREM 56522 PREM 56523
Mycosphaerella sp.	*Leucospermum cordifolium* *Protea cynaroides* *Protea eximia*	leaf spot	-	CA	PREM 56524 PREM 56525 PREM 56526
Nectria pseudotrichia Berk. & M.A. Curtis	*Grevillea robusta* † *Grevillea* sp.*		-	FL	BPI 552941 (Petrak 1953)
Penicillium sp.	*Protea cynaroides*	leaf spot	-	CA	PREM 56527
Pestalotiopsis spp.	*Leucospermum cordifolium* *Protea cynaroides* 'Silver Mink'	leaf spot	-	CA	HKU(M)10419 HKU(M)10418
Phomopsis sp.	*Protea cynaroides*	leaf spot	-	CA	PREM 56528
Phymatotrichopsis omnivora (Duggar) Hennebert	*Grevillea robusta** *Grevillea* sp.*	root rot	-	AZ	(Anonymous 1960)
Phytothphora sp.	*Grevillea robusta**	root rot	-	FL	(Alfieri et al. 1984)
Pleospora herbarum (Pers.) Rabenh.	*Leucospermum cordifolium* *Protea* cv. Pink Ice *Protea cynaroides* *Protea magnifica* *Protea* sp.	leaf spot	Y		PREM 56529 PREM 56530 PREM 56531 PREM 56532 PREM 56533
Pleospora spp.	*Protea laurfolia* *Protea magnifica*	leaf spot	-		PREM 56534 PREM 56535

Pyrenochaeta-like sp.	*Grevillia* 'Misty Pink'	leaf spot	-	CA	PREM 56536
Pythium sp.	*Grevillea robusta** *Banksia media* * *Hakea petiolaris**	root rot	-	FL	(Alfieri et al. 1984)
Ramularia sp.	*Grevillea robusta**	leaf spot	-	FL	(Alfieri et al. 1984)
Rhizoctonia solani Kühn.	*Grevillea robusta**	basal stem rot	Y	FL	(Alfieri et al. 1984)
Rhytidhysteron rufulum (Spreng.: Fr.) Speg. (* as *Tryblidiella rufula* (Spreng.: Fr.) Sacc.)	*Grevillea robusta* †* *Grevillea* sp. †		-	FL	(Petrak 1953) BPI 648633 BPI 648219 BPI 648514 BPI 648515 BPI 648212
Sarcostroma grevillieae (Loos) Nag Raj	*Grevillia* 'Misty Pink'	leaf spot	-	CA	PREM 56537
Seimatosporium sp.	*Protea* sp.	leaf spot	-	CA	PREM 56538
Seridium cf. *cardinale* (W.W. Wagener) B. Sutton & I.A.S. Gibson	*Protea* sp.	leaf spot	-	CA	PREM 56539
Seridium sp	*Leucospermum cordifolium*	leaf spot	-	CA	PREM 56540
Stigmina californiae	*Protea* 'Pink Ice' *Protea laurifolia* *Protea magnifica*	leaf spot	-	CA	PREM 56541 PREM 56542 PREM 56543
Truncatella angustata (Pers.: Link) S. Hughes	*Leucospermum cordifolium*	leaf spot	Y	CA	PREM 56544
Zetiasplonza cf. *cordylines* (Speg.) Nag Raj	*Leucospermum cordifolium*	leaf spot	-	CA	PREM 56545

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