Pestalotiopsis leaf spot disease of Proteaceae in Zimbabwe

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

Keywords: *Leucospermum*, pathogen, *Pestalotiopsis*, Protea, South Africa.

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Introduction

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

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

Materials and Methods

Isolation

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

Identification

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

Inoculation

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

Results

Symptom development

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

Taxonomy

***Pestalotiopsis* sp.,** Figures 1 and 2

Leaf spots irregular, necrotic, associated with leaf margins or causing tip die-back; slightly sunken, pale brown with reddish brown margins that are mostly raised and distinct, rarely diffuse, 2–35 mm (Figure 1a). *Conidiomata* amphigenous, pycnidioid to acervular, immersed, becoming erumpent, unilocular, dark brown to black, dehiscence by irregular splits in the apical wall and overlying host tissue, scattered, (100–)195–240(–400) µm (Figure 1b); pyriform or conical in section, base applanate, intra-epidermal in origin (125–)138–165(–180) µm wide and (125–)138–165(–180) µm high (Figure 1c). *Peridium* comprising two strata of *textura angularis*, an outer stratum of pale brown, thick-walled cells becoming hyaline inwardly, apical and lateral walls composed of slightly compressed, thinner-walled cells; thicker basal wall (13–)17–21(–23) µm, thinner apical wall (7–)11–17(–19) µm (Figure 1d). *Conidiophores* peripheral, reduced to conidiogenous cells, invested in mucus. *Conidiogenous cells* discrete, ampulliform,

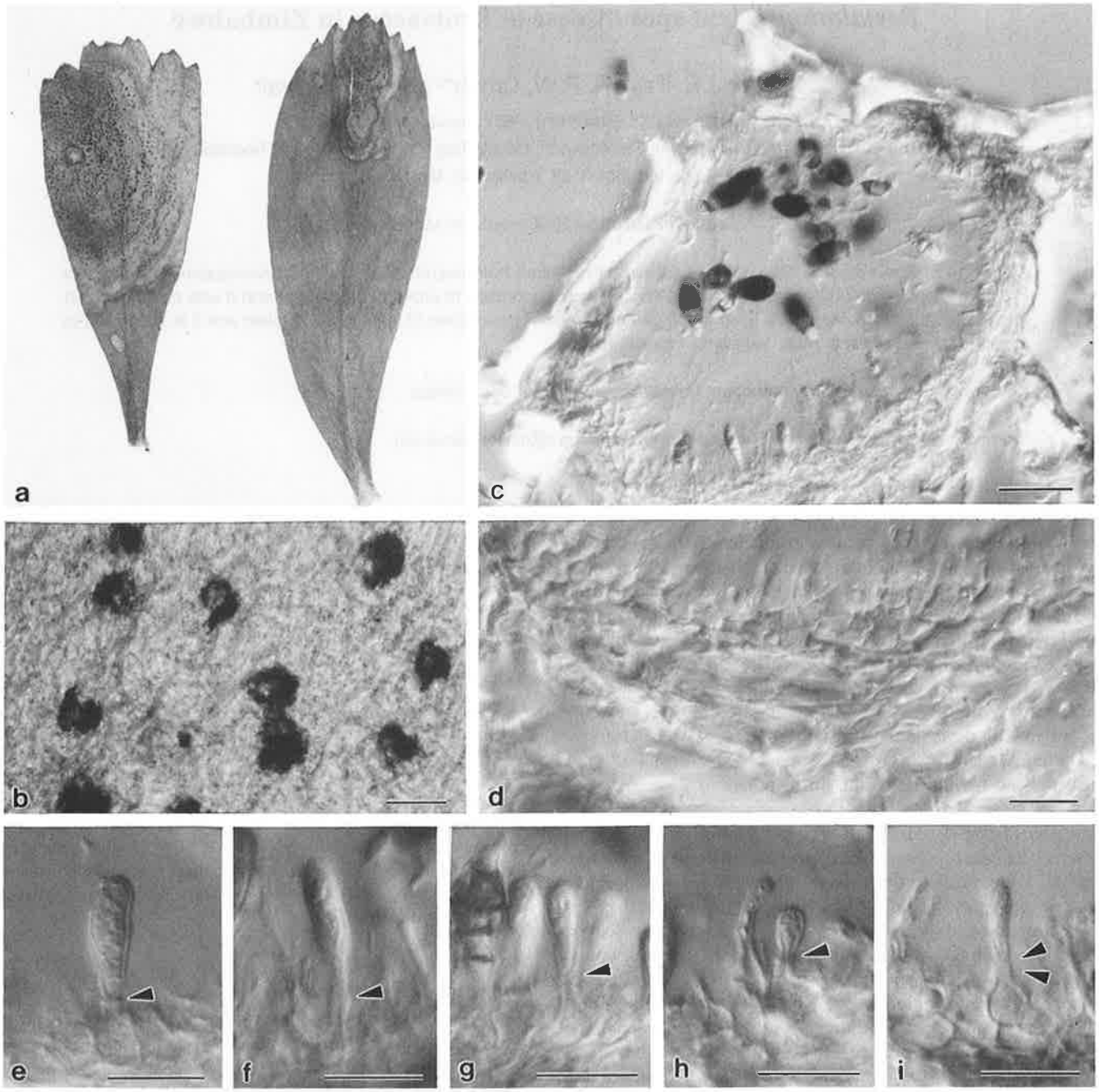

Figure 1 *Pestalotiopsis* sp. (PREM 56186). **a**. Lesions on living leaves of *Leucospermum cuneiforme*. **b**. Erumpent conidiomata on leaf surface (Scale bar = 200 µm). **c**. Transverse section of a conidioma (Scale bar = 20 µm). **d**. Peridium (Scale bar = 10 µm) **e–i**. Conidiogenous cells showing one or two enteroblastic, percurrent proliferations (arrowed) (Scale bar = 10 µm).

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 (Figure 1 e–i, Figure 2b). *Conidia* ellipsoidal to obovoid, euseptate, 4-septate, the second and third septa often darkened and indistinct, cells unequal, without constrictions at the septa, versicoloured, bearing appendages; basal cell obconic with a truncate base, bearing minute marginal frills, hyaline below, thin-walled, (3.5–)5–6(–7.5) × 4–4.5(–5) µm; second cell subcylindrical, pale brown, verruculose, third and fourth cells doliiform to subcylindrical, dark red-brown, verruculose, combined dimensions of median cells (14–)16–17(–18) × (6.5–)8–9(–10) µm [length of second cell from base (4–)5–5.5(–6) µm; central cell (4–)5–6(–7) µm; fourth cell (4–)5.5–6(–7) µm] apical cell subconical, hyaline, collapsed at maturity, thin-walled, smooth, (3–)3.5–4.5(–6) × (3–)3.5–4(–5) µm; appendages tubular, branched or not, straight to flexuous; 2–4 appendages arising apically, tip rounded, (15–)26–32(–43) µm long; basal appendage occasionally absent, filiform, flexuous, slender, centric, (2–)4.5–6(–9) µm (Figure 2a).

Morphological characteristics *in vitro*: *Conidia* ellipsoidal to obovoid, 4-euseptate, the second and third septa often darkened and indistinct, cells unequal, without constrictions at septa, versicoloured, bearing appendages; basal cell obconic with a truncate base, bearing minute marginal frills, hyaline below, thin-walled, (4–)5–6(–8) × 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)

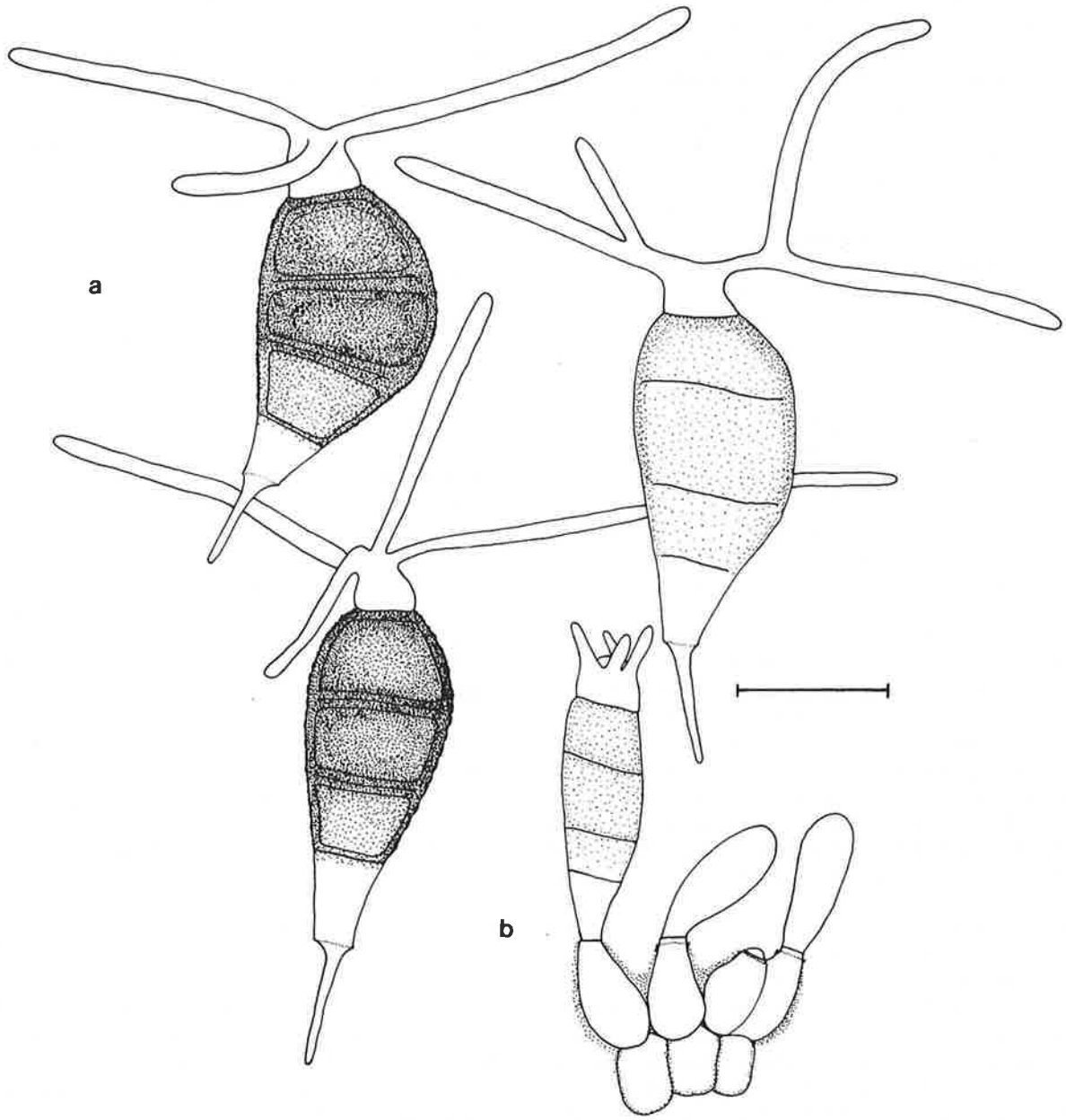

Figure 2 Diagramatic representation of *Pestalotiopsis* sp. (PREM 56186). **a**. Conidia. **b**. Conidiogenous cells showing up to two enteroblastic, percurrent proliferations. Scale bar = 10 µm.

µm long; basal appendage occasionally absent, filiform, flexuous, slender, centric, (2–)3–3.5(–5) µm.

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

Material examined

SOUTH AFRICA: Stellenbosch, Protea Heights Farm, on a living leaf of *Protea* sp., J. Taylor, 6 Mar. 1998, JT 158, PREM 56187, culture STE-U 1749; ZIMBABWE: Harare, Aveley Farm, on living leaves of *Leucospermum cuneiforme* cv. Sunbird, L. Swart, 6 Mar. 1998, JT212, PREM 56186, culture STE-U 1765; ibid, Banket, Mariondale Farm, 9 Mar. 1998, JT213, PREM 56188, culture STE-U 1777; ibid, Juliasdale, Zorora Farm, *Leucospermum vestitum*, 5 Mar. 1998, JT203, PREM 56189, culture STE-U 1783; Karoi, Glenellen Farm, on living leaves of *Protea eximia* (Salisb. ex Knight) Fourc., L. Swart, 10 Mar. 1998, JT211, PREM 56190, culture STEU 1779

Host range

Leucospermum cuneiforme cv. Sunbird, *Leucospermum glabrum* × *tottum* cv. Scarlet Ribbon, *Leucospermum vestitum*, *Protea eximia*, *Protea* sp.

Known distribution

South Africa, Zimbabwe.

Discussion

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

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

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

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

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

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

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

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