

## *Passalora perplexa*, an important pleoanamorphic leaf blight pathogen of *Acacia crassicarpa* in Australia and Indonesia

Vyrna C. Beilharz<sup>1\*</sup>, Ian G. Pascoe<sup>1</sup>, Michael J. Wingfield<sup>2</sup>, Budi Tjahjono<sup>3</sup> and Pedro W. Crous<sup>4</sup>

<sup>1</sup>Primary Industries Research Victoria, Department of Primary Industries, Knoxfield, Private Bag 15, Ferntree Gully Delivery Centre, Victoria 3156, Australia; <sup>2</sup>Forestry and Agricultural Biotechnology Institute, Faculty of Natural and Agricultural Sciences, University of Pretoria, Pretoria, South Africa; <sup>3</sup>PT Riau Andalan Pulp and Paper, P.O. Box 1080, Pekanbaru-Riau, Sumatra, Indonesia; <sup>4</sup>Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands

\*Correspondence: Vyrna C. Beilharz, vyrna.beilharz@dpi.vic.gov.au

**Abstract:** *Passalora perplexa* is described from lesions on blighted phyllodes of *Acacia crassicarpa* growing in northern Australia and Indonesia. The fungus develops two distinct conidiomatal synanamorphs from the same stroma in nature, one external and sporodochial (Type 1), the other internal and coelomycetous (Type 2). A third synanamorph (Type 3) develops as resting spores within cells of Type 2 conidia in culture. Type 1 conidiophores and conidia are consistent with *Passalora sensu lato*, with pigmented conidiophores and conidia and thickened, darkened, refractive scars. The conidiophores are initially caespitose and stromatal, but later sporodochial and generated by the outer cell layer of more or less protuberant stromata. Type 2 conidia are smaller, paler, cylindrical and mostly 1-septate. They have unthickened scars and are formed on short, 0–1-septate conidiophores which line a central cavity that develops within the same stroma. In culture, conidial cells of Type 2 conidia may eventually release an inner, hyaline propagule (Type 3 conidia) that possibly acts as a resting spore. The connection between Type 1 and Type 2 synanamorphs has been confirmed in culture via single-conidial isolates. Sequence data derived from the ribosomal DNA ITS region (ITS1, ITS2) and the 5.8S gene, show that *P. perplexa* is an anamorph of *Mycosphaerella*, closely allied with other species of *Passalora*. *Passalora perplexa* is a severe pathogen of *Acacia crassicarpa* in Indonesian plantations and has become a serious constraint to plantation development with this species.

**Taxonomic novelty:** *Passalora perplexa* Beilharz, Pascoe, M.J. Wingf. & Crous sp. nov.

**Key words:** *Acacia crassicarpa*, *Mycosphaerella*, *Passalora*, phyllode blight, synanamorph, systematics.

### INTRODUCTION

*Acacia crassicarpa* Benth. (*Leguminosae*, *Mimosaceae*) is indigenous to New Guinea and the tropical northern regions of Australia, including the coastal areas of far north Queensland, the off-shore islands to the north of Cape York Peninsula, and Melville Island near Darwin, NT. Along with *A. aulacocarpa* A. Cunn. ex Benth., *A. auriculiformis* A. Cunn. ex Benth. and *A. mangium* Willd., *A. crassicarpa* has become an important plantation species in South-East Asia. Thus, plantations have been established in northern Australia to meet the demand for seed of particular provenances of these three species (Old *et al.* 1997).

During an investigation into diseases which might pose a threat to these plantations, an unidentified cercosporoid pathogen was found on blighted phyllodes of *A. crassicarpa* (Old *et al.* 1997, 2000) (Figs 1–6). A similar collection from Melville Island had earlier been referred to by Yuan (1996) as *Pseudocercospora* sp., based on his observations of the external sporulation. It was similarly referred to as an “undescribed sp. aff. *Pseudocercospora*” by Old *et al.* (1997) and, following the first sighting of a putative

synanamorph, as a “gen. indet., aff. *Pseudocercospora*” by Cannon *et al.* (1997). This sporodochial, cercosporoid conidial type is referred to as Type 1. Subsequent examination of some of this material confirmed the presence of a coelomycetous synanamorph occupying a central locule within the stromata of sectioned sporodochial conidiomata, which is subsequently referred to as Type 2. Both morphs were clearly derived from cells of the same stroma, and the connection was confirmed via cultural studies, so the possibility of hyperparasitism was ruled out. DNA sequence data of the ITS region (ITS1, ITS2) and the 5.8S gene confirmed that this species is a *Mycosphaerella* Johanson anamorph. More recently, the pathogen was recollected from plantation-grown *A. crassicarpa* in Indonesia, where it causes a severe foliar blight disease. Sequence data derived from the ITS region confirmed that it is the same pathogen as that occurring in Australia (Crous *et al.* 2004, this volume).

Numerous pleoanamorphic fungi have been described. The provision of names for these fungi with multiple states has been discussed in some detail (Carmichael 1981, Gams 1982, Hennebert 1987, Minter 1987, Seifert & Samuels 2000). We have been

unable to find any combination of synanamorphs that would equate with the cercosporoid fungus infecting phyllodes of *A. crassicarpa* and described here. The correct or most logical means of treating the nomenclature of pleoanamorphic fungi remains somewhat subjective, and has not been defined in the Code of Botanical Nomenclature. The well-known generic name *Passalora* Fr. *sensu* Crous & Braun (2003) is correct for the hyphomycetous synanamorph, and it is particularly useful in this case because most colonies of the fungus show liberal and conspicuous sporulation of this morph, whereas the cryptic Type 2 synanamorph is less likely to be observed, and is unlikely to be found in isolation. Gams (1982) suggested that the anamorph form with the greatest differentiation should have priority (unless it is rare), a view which further supports the application of the name *Passalora* to the fungus on *A. crassicarpa*.

The conidia of the Type 1 and Type 2 synanamorphs of the cercosporoid fungus from *A. crassicarpa*, although easily distinguished, show some similarity in morphology. The Type 2 conidia are somewhat cercosporoid in type and reminiscent of some species of *Colletogloeum* Petr., but the conidiomata do not fit with the acervular conidiomata of that genus. Critical differences between the two types of conidia, including pigmentation, can be linked to their relative positions in relation to the host tissue. For example, the thickened hila of Type 1 conidia are probably associated with their readiness to secede (Beilharz 1994). In contrast, the broader, unthickened hila of Type 2 conidia are more appropriate to passive release following breakdown of overlying fungal and host tissues.

*Acacia crassicarpa* is a species that has become increasingly important in plantations in various parts of South-East Asia, where it is grown specifically for the production of pulp. The relatively recent outbreak of a serious leaf blight disease caused by a cercosporoid fungus has demanded an appropriate taxonomic treatment of this organism. This study represents a collaborative effort by a number of research groups who have an interest in this fungus and the disease with which it is associated. Here, we describe a potentially devastating, newly recognised disease of *A. crassicarpa*, and describe the causal organism, a novel pleoanamorphic species of *Passalora*.

## MATERIALS AND METHODS

### Isolates

At VPRI, cultures were derived from the Australian specimen VPRI 21125 by the following means. Naturally-produced Type 1 conidia were lifted from lesions *en masse* with a fine needle and jab-inoculated on to 2 % potato-dextrose agar (PDA; Difco) plates emended with achromycin (0.05 mg/mL) (PDA+A). Similarly

harvested Type 1 conidia were also suspended in a drop of water containing a trace of Tween 80, streaked out on to 2 % tap water agar and transferred individually to PDA+A the following day, after germinating. Type 2 conidia formed *in vitro* in PDA cultures derived from individual Type 1 conidia were used to provide single-conidial isolates as described above. In addition, whole, pale, smooth protuberant stromata lacking external conidiophores and putatively containing Type 2 conidia, were lifted from the phyllode surface with a fine, sterile needle and placed directly onto PDA+A. All PDA+A cultures were transferred to PDA after 3–7 d and grown on for up to 2 mo in the dark at 22 °C. The choice of the various forms of inoculum was determined by the ease with which they could be harvested from infected phyllodes or cultures. For example, Type 1 conidia were abundant on the natural substrate, but occurred in comparatively small numbers in culture, where they were liable to be contaminated with Type 2 conidia. Type 2 conidia were abundant in wet masses in culture; in nature, however, they tended to remain aggregated and often could not be completely freed from excised conidiomata, despite the application of pressure on coverslips or attempts to tease the elements apart in a drop of water on a microscope slide. Type 3 conidia were not seen in 2-mo-old cultures on PDA, and Type 2 conidia from these cultures germinated normally on fresh agar plates. The Australian specimens and a dried culture of VPRI 21125 have been deposited in herb. VPRI, Knoxfield, Victoria, Australia.

At CBS, single Type 1 conidial isolates were derived from Indonesian specimens and cultivated on 2 % malt extract agar (MEA; Difco) as described by Crous (1998). Colonies sporulated on MEA after 1–2 mo incubation on the laboratory bench in daylight at room temperature, forming conidiomata containing Type 1 and Type 2 conidia. After 3 mo incubation on MEA, conidiomata with Type 2 conidia were observed to also give rise to Type 3 conidia, a form observed only in culture. Specimens and cultures have been deposited in the herbarium and culture collection of CBS in Utrecht, the Netherlands.

A phylogeny of the cercosporoid fungi occurring on *Acacia*, including *Passalora perplexa*, is presented elsewhere in this volume (Crous *et al.* 2004).

### Morphology

Slide preparations were made in lactic acid and 50 examples of each structure were measured under a ×100 oil immersion lens using Olympus BH-2 (VPRI) or Zeiss Axioskop (CBS) light microscopes. The 95 % confidence intervals were also determined for conidial dimensions, with the extremes in conidium length and width given in parentheses. Colony colour was determined on 2 % MEA after 3 mo at 25 °C in the dark using the colour designations of Rayner (1970).



**Figs 1–6.** *Passalora perplexa*. 1. Trees showing defoliation. 2–6. Symptoms associated with *Crassicaarpa* leaf blight.

## RESULTS

### Disease symptoms

Lesions occur primarily on the phyllodes of *A. crassicaarpa* but they can also form on the petioles and young shoots. Phyllode lesions are initially small and typically elliptical, and are surrounded by a distinct chlorotic halo (Figs 1–6). On freshly formed lesions, fascicles of grey-brown conidiophores and dense olivaceous spore masses can easily be seen. Lesions formed at the edges of phyllodes or abutting primary veins can cause severe malformation and curling of the phyllodes (Figs 2–6). Infections are often severe, causing the dramatic malformation of the apical portions of young (1–2-yr-old) trees (Fig. 1).

### Taxonomy

Sequences obtained for the ITS region in the laboratories of both VPRI and CBS, confirmed that the Australian and Indonesian specimens represented the same taxon. The DNA sequence analyses also showed that the fungus is an anamorph of *Mycosphaerella*, clustering with *Cercospora loranthi* McAlpine (Crous *et al.* 2004, fig. 1), which is a species of *Passalora*. These relationships have been discussed elsewhere (V.C. Beilharz, in press). Sequences of *P. perplexa* have been deposited in GenBank, and the alignment of sequence data in TreeBASE (Crous *et al.* 2004).

The habit, morphology, pigmentation and scar characteristics of Type 1 conidiophores and conidia

are characteristic of the genus *Passalora*. This observation is consistent with the results of the DNA-based comparisons. Currently there are no species of *Passalora* known from *Acacia* (Crous & Braun 2003), and hence this species with its pigmented Type 1 conidia and thickened, darkened, refractive conidial hila can be described as new. Prior to the discovery of additional coelomycetes resembling the Type 2 synanamorph and their affiliations being established, it would be inappropriate to provide a separate generic name for the Type 2 synanamorph or to name this synanamorph.

***Passalora perplexa*** Beilharz, Pascoe, M.J. Wingf. & Crous, **sp. nov.** MycoBank MB500123. Figs 7–27.

*Etymology:* Named because of the unusual combination of conidial synanamorphs.

Fungus pleoanamorphic conidia generis *Passalorae* et coelomycitica formans. Conidiophora solitaria vel laxe aggregata, pallide vel medio-brunnea, levia vel verruculosa, subcylindrica, ramosa vel simplicia, pluriseptata, sympodialiter proliferantia, 15–80(–116)  $\mu\text{m}$  longa, 3–5  $\mu\text{m}$  lata. Cellulae conidiogenae terminales, verruculosae vel rugosae, simplices, subcylindricae, apicem rotundatum versus angustatae, 15–20  $\times$  3–4  $\mu\text{m}$ ; cicatrices modice inspissatae et fuscatae, refringentes, 1–2  $\mu\text{m}$  diam. Conidia solitaria, pallide olivacea vel medio-brunnea, levia vel eximie verruculosa, recta vel curvata, anguste obclavata vel subcylindri-

ca, sursum obtusa, ad basim longe obconice subtruncata, (1–)3–9(–13)-septata, (16–)50–100(–153)  $\mu\text{m}$  longa, 2.5–5.5  $\mu\text{m}$  lata, hilo modice inspissato et fuscato, refringente, 1–2  $\mu\text{m}$  diam.

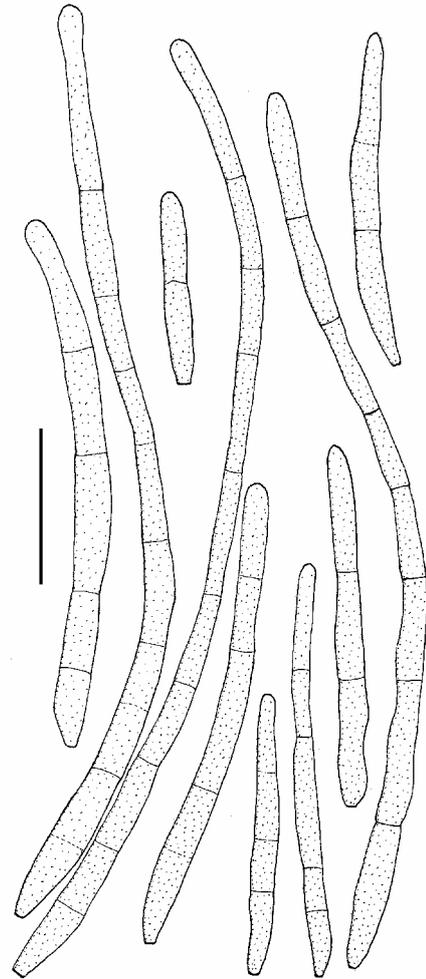
*Holotype*: Indonesia, South Sumatra, Kerinci, Herb. CBS 9907 **holotype**, on phyllodes of *Acacia crassicarpa*, Feb. 2004, M.J. Wingfield, ex-type cultures CBS 116363 = STE-U 11147–11149.

Pleomorphic, producing stromatic conidiomata on phyllodes of *Acacia crassicarpa*. *Leaf spots* hologenous, initially pale brown, orbicular and non-necrotic, becoming medium brown, necrotic, elongated, narrowly ellipsoidal to sub-circular, with an inconspicuous border, often distorted and wrinkled and causing distortion of the phyllode, limited by main secondary veins, to at least 15 mm long, 2–5 mm wide. *Mycelium* internal, consisting of smooth, branched, septate, brown hyphae, 3–4  $\mu\text{m}$  wide. *Stromata* medium brown throughout or ranging from brown in the exposed apical cells to hyaline or sub-hyaline in the deeper tissues, initiated in the substomatal cavity, usually becoming erumpent, protuberant and pulvinate, composed of *textura angularis*, 50–80  $\mu\text{m}$  wide, 50–90  $\mu\text{m}$  high. *Conidiomata* amphigenous, eustromatic, comprising either (a) pale-yellowish, fleshy, protuberant stromata containing Type 2 conidia but, at least initially, bearing no Type 1 conidiophores; (b) brown immersed or erumpent stromata bearing Type 1 conidiophores but, at least initially, containing no Type 2 conidia, or (c) mature brown conidiomata, up to 80  $\mu\text{m}$  diam, 60  $\mu\text{m}$  high, bearing numerous Type 1 conidiophores and containing numerous Type 2 conidia.

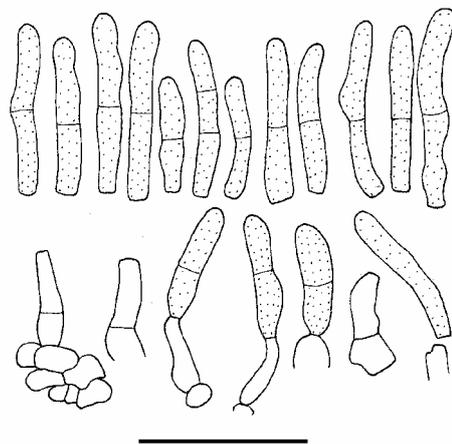
*Type 1 synanamorph*: *Conidiophores* occasionally solitary, usually aggregated in loose fascicles arising from the upper cells of a brown stroma, up to at least 62 in number, pale to medium brown, smooth towards the base and often becoming rugose towards the apex with age, subcylindrical, branched or unbranched, walls slightly thickened, straight to variously curved or geniculate-sinuous, having a basal septum and 0–11 additional septa; proliferation sympodial, with endohyphal regeneration or proliferation also commonly exhibited, 15–80(–116)  $\mu\text{m}$  long, 3–5  $\mu\text{m}$  wide. *Conidiogenous cells* terminal, verruculose or rugose, unbranched, subcylindrical, tapering to rounded apices proliferating sympodially, 15–20  $\times$  3–4  $\mu\text{m}$ .

*Conidiogenous scars* slightly thickened and darkened, refractive, flat against the side of the conidiophore, on short pegs or on sloping shoulders following proliferation of the conidiogenous cell, sometimes protruberant, often somewhat disguised by the dark, rugose wall of mature conidiophores but clearly seen on paler, more newly generated conidiogenous cells, 1–2  $\mu\text{m}$  diam. *Conidia* solitary, pale olivaceous to medium brown, dry, smooth, rarely finely verruculose, straight or curved, narrowly obclavate to sub-cylindrical,

tapering gradually to an obtuse apex and to a rounded or long-obconically-subtruncate base, often constricted at one or more septa or with an otherwise uneven edge-line, (1–)3–9(–13)-septate, (16–)50–100(–153)  $\mu\text{m}$  long, and 4–4.5(–5.5)  $\mu\text{m}$  wide *in vivo* (Australian specimens), or (2.5–)3–4  $\mu\text{m}$  wide (Indonesian specimens). Secondary conidiation was occasionally seen. Hila slightly but distinctly thickened and darkened, refractive, 1–2  $\mu\text{m}$  diam.



**Fig. 7.** Conidia of *Passalora perplexa* *in vivo*. Scale bar = 10  $\mu\text{m}$ .



**Fig. 8.** Type 2 conidia and conidiophores of *Passalora perplexa* *in vivo*. Scale bar = 10  $\mu\text{m}$ .

*Type 2 synanamorph*: *Conidiophores* reduced, hyaline to sub-hyaline, 0–1-septate, lining a single, initially ill-defined cavity, which develops within either a substomatal or protuberant stroma exactly as described above. *Conidia* initially hyaline and inconspicuous, later pale olivaceous,  $\pm$  cylindrical, barely if at all tapering to the apex or the base, sometimes swollen at the apex or broadening to the base, occasionally constricted, smooth, (0–)1(–3) septate, (12–)15–21(–25)  $\mu\text{m}$  long, 2.5–4  $\mu\text{m}$  wide; hila broad, truncate to slightly convex, not darkened, unthickened, non-refractive, 2–2.5  $\mu\text{m}$  diam. No pore or slit has been detected that would allow ready release of Type 2 conidia. On the other hand, the contents of certain old conidiomata have become exposed by the apparent breakdown and peeling back of both fungal and host tissues. These conidiomata eventually resemble acervuli, although they are no longer actively sporulating. It is possible that Type 2 conidia depend on tissue breakdown for their dissemination, and that insects or other animals may aid their dispersal.

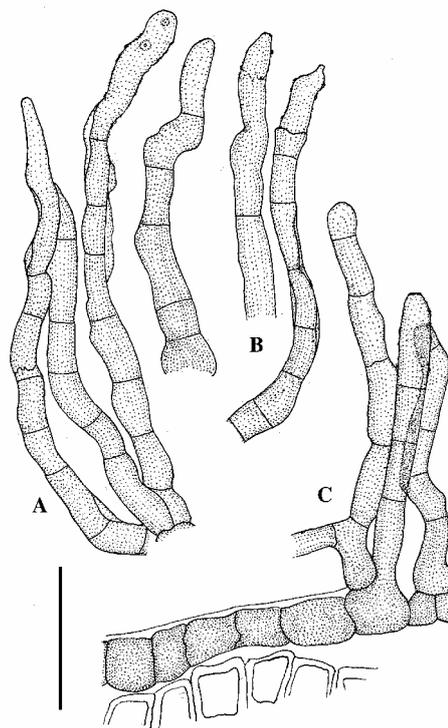
*Type 3 synanamorph*: After 1 mo, Type 2 conidia from 3-mo-old MEA cultures exposed to daylight develop thick-walled hyphal swellings (reminiscent of chlamydo-spores that develop in conidial cells of certain *Fusarium* spp.); these inner propagule cells eventually burst free from the cells of the Type 2 conidia, frequently still having pigmented remnants of the conidial wall attached to their hyaline walls. Type 3 conidia are 6–20  $\times$  4–6  $\mu\text{m}$ , 0(–1)-septate, ellipsoid and hyaline. Type 3 conidia did not develop in 2-mo-old PDA cultures grown mostly in the dark.

*Cultural characteristics*: Colonies slow-growing, reaching up to 20 mm diam after 3 mo on 2 % MEA at 25 °C under near-UV light; colonies erumpent, margins feathery, irregular; outer region (surface) sepia (15" k) due to submerged, radiating mycelium; inner region whitish to cream, with slimy sporodochial spore masses, fuscous black (7" k); central region with moderate hazel (17" i) aerial mycelium; reverse brown-vinaceous (5" m).

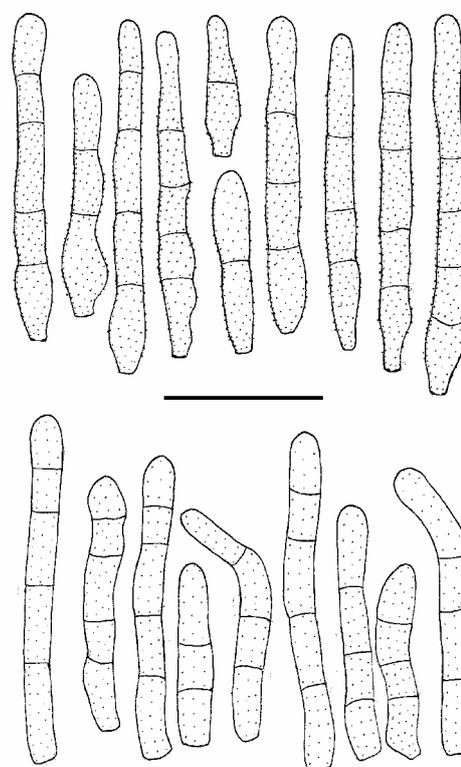
*Substrate and distribution*: Pathogenic to phyllodes of *Acacia crassicarpa*; Australia, Indonesia.

*Additional specimens examined*: All on phyllodes of *Acacia crassicarpa*, **Indonesia**, Southern Sumatra, Kerinci, Feb. 2004, M.J. Wingfield, herb. CBS 9908, 9909, 9911, cultures derived from CBS 9911, CBS 116364 = STE-U 11150–11151. **Australia**, Queensland, Cooktown–Cape Tribulation Hwy, 4 km W of Bloomfield, 8 Apr. 1995, K.M. Old (ex DFR 257) (CSIRO Forestry and Forest Products herbarium, Canberra Australia), VPRI 20902; 5 km W of Bloomfield, 8 Apr. 1995, K.M. Old (ex DFR 252), VPRI 20904; Ingham, Shell trial site, Lannercost State Forest, 3 Apr. 1995, K.M. Old (ex DFR 255), VPRI 20905; Edmund Kennedy National Park, K.M. Old (ex DFR 305), 4 Apr. 1995, VPRI 20906; Ingham, Shell trial site, Lannercost State Forest, K.M. Old (#6), 9 Apr. 1996, VPRI 21125; N.T., Yapilika, Melville Island, 17 May 1994, Z.Q. Yuan,

(ex DFR 162), VPRI 20901; Melville Island, plantation provenance, 17 Sept. 1992, K.M. Old (ex DFR 138), VPRI 20903.



**Fig. 9.** Type 1 conidiophores of *Passalora perplexa* *in vivo*. A. Conidiophores emerging from stomata. B. Sporodochial conidiophores. C. Conidiophores arising from a subcuticular hypha. Scale bar = 10  $\mu\text{m}$ .



**Fig. 10.** Conidia of *Passalora perplexa* *in vitro* on PDA. Type 1 conidia (top), and Type 2 conidia (bottom). Scale bar = 10  $\mu\text{m}$ .

*Notes:* In culture, colonies sporulate on MEA after 1–2 mo, forming sporodochia situated on globose conidiomata that are pycnidoid but lacking a clear ostiole. Conidia formed on sporodochia are of Type 1. A conidial type intermediate between Type 1 and Type 2 was also observed. These conidia were initially hyaline, becoming medium brown, smooth, cylindrical to subcylindrical, apex obtuse, base obconically subtruncate with a darkened (not thickened and refractive as in Type 1) scar, and a minute marginal frill,  $20\text{--}35 \times 3\text{--}6 \mu\text{m}$ , (1–)3(–4)-septate. Inside the conidiomata Type 2 conidia were formed. After 3 mo, these conidia were observed to give rise to Type 3 conidia which were 0(–1)-septate, ellipsoid and hyaline.

Both Type 1 and Type 2 conidia were also generated in cultures on PDA held in the dark for 2 mo. When the superficial mycelium was lifted away from 2.5-mo-old colonies, wet, dark masses of Type 2 conidia were found in small, well-defined cavities in the mycelium. The conidia resembled naturally produced Type 2 conidia in shape, pigmentation and scar characteristics, but were longer, broader, and up to 5-septate. Type 2 conidia were sparsely present in mounts of both superficial hyphae and the fine, feathery mycelium at the more or less flat colony margins. They had been produced terminally on lateral conidiophores or hyphae of indeterminate length. Type 1 conidia produced *in vitro* were smooth to verruculose (the latter especially towards the base), occasionally verrucose, shorter and fewer-septate than those produced *in vivo*, but similar in shape and width and exhibiting the narrow, darkened, slightly thickened hila of conidia produced *in vivo*. The outer wall layer of these Type 1 conidia was often slightly retracted from the hilum. Cottony mycelium from the colony surfaces often contained a few small, dense hyphal aggregates composed largely of clusters of short conidiogenous cells producing Type 2 conidia. These conidia tended to be straighter than Type 2 conidia produced *en masse*, whether *in vivo* or *in vitro*.

## DISCUSSION

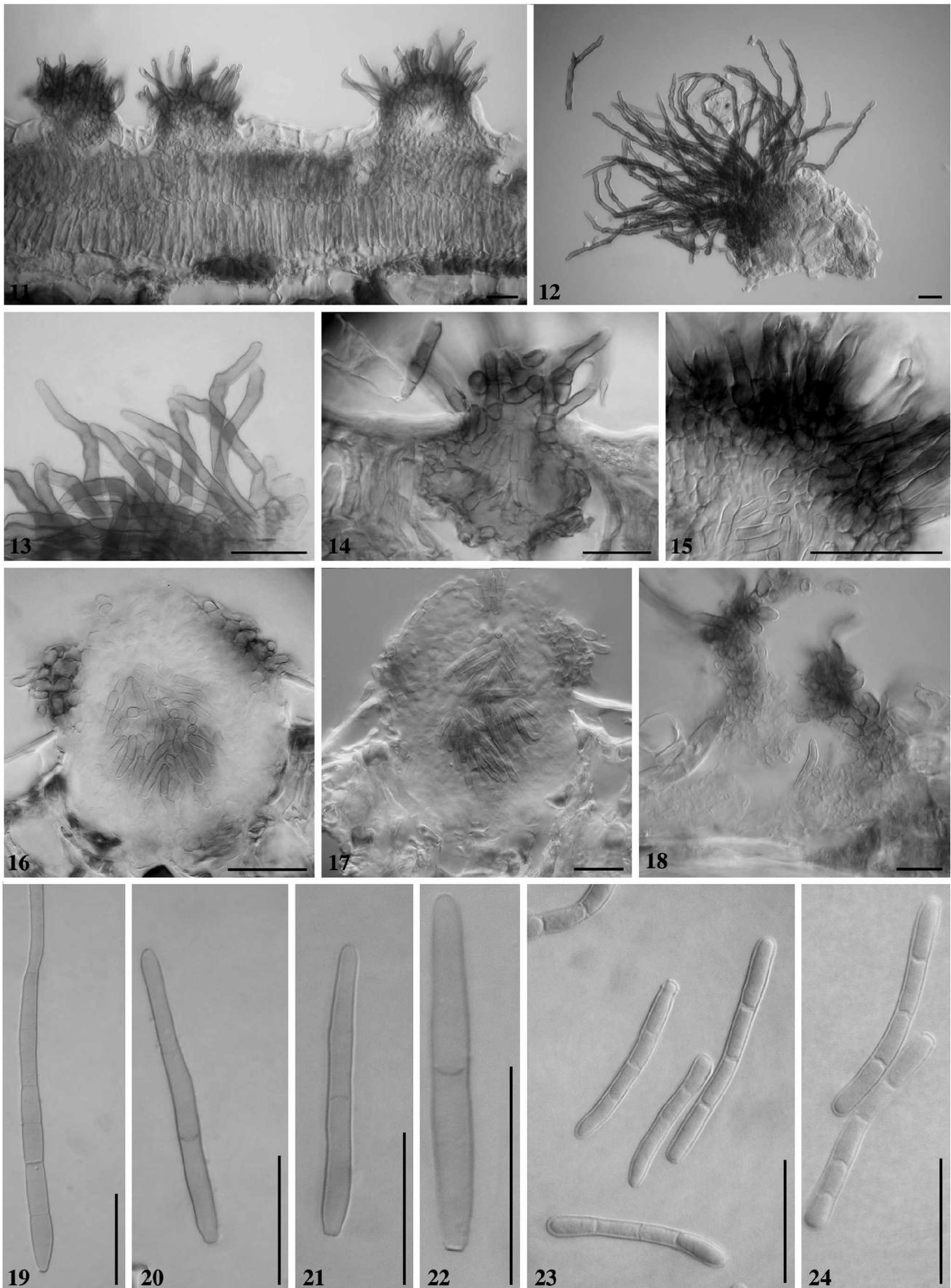
In this study, we have provided a name for the important fungal pathogen that causes leaf blight specifically on *Acacia crassicarpa*. *Passalora perplexa* is present both in Australia, where it is apparently native, and in the extensive plantations in Indonesia to which it has spread. In plantations, the disease associated with this fungus can be very severe and it is likely to provide significant challenges for forestry companies that plant *A. crassicarpa*. Having a name for the

pathogen that causes *Crassicarpa* leaf blight is an important step towards the recognition of the disease and the development of management strategies to deal with it.

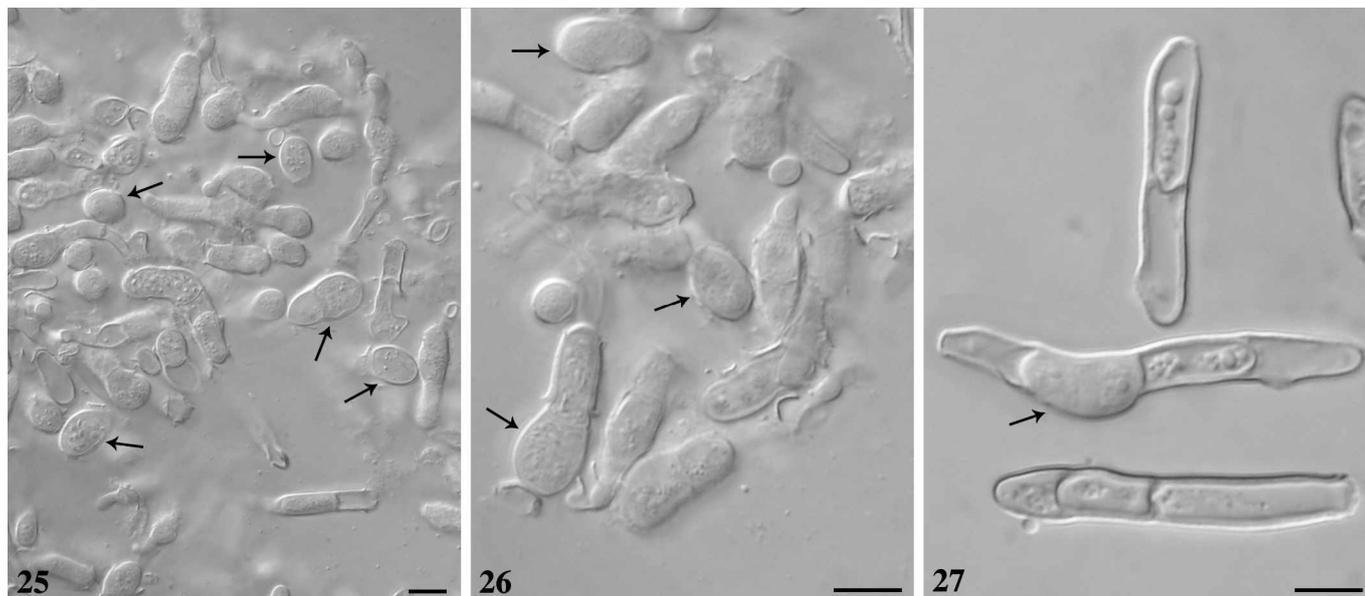
*Crassicarpa* leaf blight was first noted on Melville Island, Northern Territory, Australia in 1996 (Yuan 1996). There has been some confusion regarding the taxonomy of this fungus, particularly because very little work has been done on the taxonomy of leaf pathogens of tropical *Acacia* spp. Thus two fungi, a species of *Pseudocercospora* and a species of *Cercospora*, were recorded on the leaves of *A. crassicarpa* and *A. mangium* respectively. Although the fungi are readily distinguished, there has been confusion in the field regarding the causes of the two diseases. *Passalora perplexa*, described in this study, may be specific to *A. crassicarpa*, and certainly appears unable to infect *A. mangium*, which shows no signs of the disease even when planted in close proximity to heavily infected *A. crassicarpa* trees.

The pleoanamorphy displayed by *P. perplexa* is unusual in that one form could be characterised as a hyphomycete, a second form represents a coelomycete, and a third morph appears to represent a resting spore form. Because of this, care was taken to demonstrate unequivocally that Type 1 and Type 2 conidia did indeed belong to the same fungus. Careful characterisation of the relationship between morphs included single-spore culturing and connection of the various forms based on DNA sequence data. The conidiophores of the Type 1 and Type 2 morphs are generated, often concurrently, by cells of the same stroma. This appears to be a unique feature. Type 3 conidia were only observed in certain cultures, and we suspect that this conidial form is associated with the growth medium or conditions of incubation.

Different anamorphs in single fungal taxa often develop on conidiogenous cells having distinctly different morphological forms. These are often associated with different functions such as, for example, rain dispersal (conidia in wet slimy masses), wind-dispersal (conidia thin-walled, dry) and survival (conidia less numerous, larger, pigmented and thick-walled) (Carmichael 1981, Seifert & Samuels 2000). The conidia of the synanamorphs of *P. perplexa* differ in pigmentation and wall thickness, as well as in their mode of liberation. The hyphomycetous (Type 1) conidia are ideally suited to wind dispersal, and are typical of cercosporoid fungi. The coelomycetous (Type 2) conidia probably require moisture for dispersal, and the Type 3 conidia, which can form inside the conidial cells of Type 2 conidia in culture, are probably associated with longer term survival.



**Figs 11–24.** *Passalora perplexa*. 11. Vertical section through an array of conidiomata. 12. Particularly long Type 1 conidiophores on excised sporodochial conidioma. 13. Type 1 conidiophores on sporodochial conidioma. 14, 15. Vertical section through substomatal conidiomata showing Type 1 conidiophores and Type 2 conidia. 16–17. Vertical sections through conidiomata containing Type 2 conidia but lacking Type 1 conidiophores. 18. Vertical section through conidioma. 19. Type 1 conidium *in vivo*. 20–22. Type 1 conidia *in vitro*. 23, 24. Type 2 conidia *in vitro*. Scale bars = 10  $\mu$ m.



**Figs 25–27.** Type 2 conidia of *Passalora perplexa* forming Type 3 conidia (arrows) *in vitro*. Scale bars = 5  $\mu$ m.

There appears to be only one previously described pleoanamorphic cercosporoid fungus. Alcorn (1992) described *Parapithomyces clitoriae* Alcorn, which produced a *Pseudocercospora* Speg. synanamorph sporulating from epigenous, erumpent stromata on leaves of *Clitoria* sp. In contrast, the *Parapithomyces* morph sporulated from conidiophores borne on hyphogenous, superficial hyphae. Both spore types of this fungus were produced in culture from single *Pseudocercospora* sp. conidia. Intermediate spore types were also found, as indeed occurred in *P. perplexa*, in both instances emphasising the validity of the link between the respective synanamorphs. In contrast to *Passalora perplexa*, both anamorphs of *Parapithomyces clitoriae* were hyphomycetes and their conidiophores were indistinguishable from each other.

*Acacia crassicaarpa* has become one of the most widely planted plantation tree species in the tropics and various forestry companies depend on it for the production of pulp. Early plantings of this tree were virtually free of disease. Thus the wide-scale appearance of leaf blight caused by *P. perplexa*, particularly in Sumatra, is of considerable concern. Earlier records of Australian collections were referred to *Pseudocercospora* (Yuan 1996, Old *et al.* 1997), as the Type 2 synanamorph had not been observed. The present study represents the first detailed taxonomic evaluation of the causal agent of Crassicaarpa leaf blight.

The distribution of *P. perplexa* on native *A. crassicaarpa* in Australia suggests that it may be indigenous across the humid tropical north of Australia (Old *et al.* 1997) and that it has been accidentally introduced into Indonesia. Although there is no direct proof that this is the case, we believe that the pathogen has been moved with seed. This appears to be typical of *Mycosphaerella* spp. such as those on *Eucalyptus* leaves that have been widely distributed throughout the world, largely in the absence of any movement of plants. The fungi

might not specifically occur on seeds, but seed consignments often include fragments of leaves and fruits that bear fruiting structures of the pathogens. Great care should thus be taken in the future to prevent the movement of additional new and devastating pathogens of forest trees (Wingfield *et al.* 2001).

Virtually nothing is known regarding the biology of *P. perplexa*, and this alone represents an important constraint to efforts to control the leaf blight disease that it causes. The epidemiology of Crassicaarpa leaf blight will need to be elucidated in order that management strategies to reduce its impact may be implemented. The presence of healthy trees alongside severely blighted individuals suggests that substantial opportunity exists to breed and select for tolerance to this disease.

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