Chapter 3

New species, recombinations and descriptions

3.1 CERCOSPORIDIUM DUBOISIAE SP. NOV.

Lesions hologenous, spreading, more or less circular or irregular, commonly 25 mm diam. but often spreading over large areas of leaf, initially greasy and translucent green, becoming light brown with the development of pigmented conidiomata, and finally necrotic in the centre. Immersed mycelium abundant, broad, hyaline, sparsely septate, anastomosing, intercellular, 3.5-7 µm diam., forming a large mass of closely branched hyphae adjacent to and parallel with each vascular bundle. Stromata fully immersed, amphigenous, forming in green host tissue, scattered, substomatal, subspherical, discrete, compact, composed of textura globosa, hyaline to medium golden brown with the outer layers(s) darker, commonly 45 µm deep x 35-55 µm wide (epigenous stromata can be 70 µm deep), giving rise to a pigmented column of hyphae in the stomatal opening. Egress stomatal. Conidiophores short, plump, medium golden olivaceous, caespitose, divergent, up to 30 or more developing on swollen cells just outside the stoma, smooth to verruculose, straight to slightly curved or bent, continuous or once-branched, developing a crazed surface with age, ovoid to cylindrical, 03 septate, (15-) 24 (-34) µm long x 58.5 µm diam. Conidiogenous cells integrated, terminal, cylindrical, light brown, with a crazed surface. Conidiogenous loci 0-4, broad, thickened, sometimes protuberant, formed in association with up to 4 enteroblastic sympodial proliferations of the conidiogenous cell, 2-3 µm diam. Conidia dry, holoblastic, pale olivaceous, smooth to verruculose, cylindrical to obclavate or acicular, straight or slightly curved, 05 septate, tapered gradually to an obtuse apex 2.75-3.5 µm wide, and more abruptly to a truncate base with a slightly thickened and darkened scar (1.5-) 2.6 (-3.5) µm diam., (35-) 65 (101) x (2.5-) 3 (-4.5) µm.


Stomata occur on both surfaces of D. myoporoides leaves, and egress is always stomatal with no disruption of the guard cells.

Duboisia myoporoides (Solanaceae), a native of Australia and New Caledonia, is grown commercially, the leaves being a source of hyoscine which is used in medicinal drugs (Purdie, Symon & Haegi, 1982).

The large masses of hyphae associated with the vascular bundles were not found in any other host-pathogen combination dealt with in this study. The host cells are not invaded, but the prolific branching of the hyphae suggests that the contact between fungal and host cells in this region is maximised. The association may be relevant to the biotrophic nature of the pathogen during much of its development. Conidia were observed to anastomose.

The fungus on D. myoporoides has been placed in Cercosporidium, a diagnosis first suggested by Dr J. Walker in his notes on 20423 and later supported by Dr B. C. Sutton of the CMI, who examined specimen
VPRI 17402. It is noted, however, that the abscission scars on the conidiophores considerably less conspicuous than those on *Cercosporidium chaetomium*, the type species, as it is depicted in the literature. In addition, scars on conidia of the *Duboisia* fungus are variable in thickness, frequently limited to a thickened rim, and not always conspicuous, which is again at slight variance with the description of *C. chaetomium*. It is shown in Chapter 4 that slight scar thickening is often present on conidiophores and conidia of fungi which in every other way resemble *Pseudocercospora*, and which fit best in that genus. The incidence of occasional hyaline conidia, however, and the generally obvious conidial scars point to a diagnosis of *Cercosporidium* being more appropriate than *Pseudocercospora*.

Specimens examined

Fig. 3.1 Conidiomata, conidia and conidiophores of *Cercosporidium duboisiae*
3.2 PSEUDOCERCOSPORA HARDENBERGIAE SP. NOV.

Lesions hologenous, angular, necrotic, light brown in the centre with a darker brown margin, vein-limited, 1-3 mm diam., or more when lesions are confluent. Immersed mycelium intercellular, sub-hyaline, septate, branched, 2.5-4 μm diam. Stroma initials substomatal or subepidermal on the abaxial leaf surface and subepidermal on the adaxial surface. Stomatal stromata light brown, discrete or confluent, protuberant, 70-90 μm deep x 25-70 μm wide. Erumpent stromata light brown, scattered, discrete or occasionally confluent, protuberant, composed of textura angularis, 43-84 μm deep x 72-121 μm wide. Conidiophores short, initially caespitose, becoming sporodochial, very numerous, pale brown becoming paler towards the apex, straight, unbranched or once-branched low on the conidiophore, 0-1 (-2) septate, bulbous-based with a cylindrical neck 8-18 μm long, (14-) 23 (-35) μm x 44.5 μm wide near the base and 1.5-2 μm wide at the apex. Conidiogenous cells integrated, terminal, often with a pale to slightly conspicuous scar situated on a sloping shoulder at the base of the cylindrical neck, 8-22 μm long. Conidiogenous loci narrow, thin, inconspicuous or slightly dark, formed in association with at least one enteroblastic sympodial proliferation of the conidiogenous cell. Conidia dry, pale brown, smooth, obclavate, (1-) 3 (-6) septate, not constricted at the septa, aguttulate, tapering gradually to an obtuse apex and abruptly to an obconic or slightly rostrate base 1.5-2.5 μm diam., (22-) 46 (-68) x (3.5-) 4 (-5) μm.

Provisional type: On living leaves of Hardenbergia violacea (Scnee.) Stearn, 4 km S. of Camphora campsite turnoff, Mt Samaria, Vic., V. & R. Beilharz, 8 Feb. 1989, VPRI 15756.

Leaves of Hardenbergia violacea have stomata on the lower surfaces only.

Specimens examined

Fig. 3.2 *Pseudocercospora hardenbergiae* Conidia of (A) VPRI 16032, (B) 15756 and (C) 17473; D, conidiophores of VPRI 16032; E, conidiophores of VPRI 15756, several with attached, septate conidia; E, an erumpent hypogenous conidioma of VPRI 17473.
3.3 **Pseudocercospora kennediicola** Sp. Nov.

*Lesions* hologenous, necrotic, angular or subcircular to irregular, limited by major veins, pale brown with a darker brown margin, commonly 25 mm diam. but becoming much larger when confluent. *Immersed mycelium* very pale brown, branched, septate, intercellular, 1.75-2.5 µm diam. *Superficial mycelium* emerging from the stomata, pale brown, smooth, septate, lax, anastomosing, 2.25-3.0 µm diam. *External conidiophores* (Fig. 3.29) borne laterally on superficial mycelium, showing 0-2 percurrent enteroblastic proliferations, 32-38 µm long x ca 3 µm wide. *Stroma initials* substomatal or subepidermal on the abaxial leaf surface and subepidermal on the adaxial surface. *Substomatal stromata* light brown, ca 38 µm wide x 35 mm deep, discrete, composed of *textura angularis*, becoming erumpent. *Subepidermal stromata* medium brown, scattered, discrete, compact, composed of *textura angularis*, 55-72 µm wide x 29-58 µm deep. *Conidiophores* long, sporodochial, pale brown, cylindrical, erect, straight or slightly curved, 0-2 septate, (20-) 39 (-60) µm x 45 µm. *Conidiogenous cells* integrated, terminal, sometimes geniculate towards the apices, smooth, pale brown becoming paler towards the apices, 13-35 µm x 4-5 µm. *Conidiogenous loci* narrow, thin, pale or dark, formed in association with enteroblastic sympodial or pseudopercurrent proliferations of the conidiogenous cell. *Conidia* dry, holoblastic, pale brown, smooth, obclavate, straight or curved, (0-) 4 (-13) euseptate, not constricted at the septa, aguttulate, tapered gradually to an obtuse apex and more abruptly to an obconic or rostrate base (0.75-) 1.75 (-2) µm diam., (20-) 80 (-172) µm x (2.25-) 2.5-5 µm.


The leaves of the host species had stomata on their lower surfaces only.

*Pseudocercospora kennediicola* was distinguished from *Cercospora kennedyae* Cooke & Massee (see below) on the basis of its larger, darker, obclavate conidia and narrower scars (Fig. 3.3). There is little or no overlap in scar diameters between the two species, with 2 µm being the maximum scar diameter measured for conidia of *P. kennediicola* (Fig. 3.3) and the minimum scar diameter measured for conidia of *C kennedyae* (Fig. 3.4). *C. kennedyae* conidia are also narrower and shorter than those of *P. kennediicola*, although length variation was also seen between *P. kennediicola* specimens. Measurements of *C. kennedyae* were based on only 11 conidia from the type specimen, compared with 61 conidia from 5 specimens of *P. kennediicola*.

Specimens examined

Fig. 3.3 Hypogenous conidia, conidiophores and erumpent conidioma of

_Pseudocercospora kennediicola._
3.4 *PSEUDOCERCOSPORA KENNEDIAE* COMB. NOV.

*Cercospora kennedyae* Cooke & Massee. *Grevillea* 19, 90, 1891.

On leaves of *Kennedia prostrata* R. Br.

*Cercospora kennedyae* is recombined into *Pseudocercospora* on the basis of its slightly pigmented conidia and unthickened scars. The examination of a small portion of the type specimen showed lesions which were hologenous, necrotic, angular and vein-limited, but covering much of the leaf when confluent. Stomata were plentiful on both leaf surfaces. Numerous sporodochial conidiomata sporulated vigorously on the upper surface of the leaf. The brown stromata appeared to be initiated beneath the epidermis, to breach the epidermis in some way, and then to spread beneath the cuticle, which was eventually ruptured. Hypogenous conidiomata were caespitose (emerging through the stomata) and sparse. Conidia were cylindrical, and were attenuated only slightly, if at all, to their truncate, slightly flared bases which were (2-) 2.6 (-3.25) µm in diameter. Conidia (Fig. 3.4) measured (28-) 48 (-102) x (2.25-) 2.8 (-3.25) µm (11 conidia). The conidiophores resembled the conidia in their cylindrical shape and truncate, broad apices.

Specimen examined

*Kennedia prostrata* R. Br., Vic., *Martin*, No. 603, holotype of *Cercospora kennedyae* Cooke & Massee.
Fig. 3.4 Conidia of the type specimen of *Cercospora kennedyae* Cooke & Massee
3.5 **PSEUDOCERCOSPORA PLATYLOBII** SP. NOV.

Lesions hologenous, light to medium tan, angular, with indistinct or dark margins, weakly vein-limited, initially 1-2 mm diameter but sometimes spreading to 20 mm, rarely covering most of the leaf surface. Immersed mycelium hyaline to pale or medium olivaceous brown, branched, septate, anastomosing, intercellular, rarely within epidermal or mesophyll cells, (1.5-) 2.5-4.5 µm diam. Superficial mycelium sparse. Stromata amphigenous. Hypogenous stromata brown, discrete, irregular to subspherical, initiated in the substomatal cavities, eventually rupturing the stomata and becoming protuberant, up to at least 96 µm deep and 87 µm wide. Epigenous stromata (Figs 3.34, 3.35) brown, discrete, irregular to subspherical, initiated beneath the epidermis and becoming erumpent, up to 63 µm wide x 52 µm deep. Host cells are often incorporated into developing substomatal and subepidermal stromata. External conidiophores solitary, continuous, unbranched, 18-35 x 2.5-4 µm. Conidiophores initially caespitose (Fig. 3.36) but later sporodochial (Fig. 3.37) on the lower leaf surface, sporodochial (Fig 3.34) on the upper surface; pale olivaceous, straight or slightly flexuous, narrow and cylindrical with a bulbous base, unbranched or rarely once-branched, smooth, continuous (rarely very faintly 1-2 septate), (14-) 32 (-43) x 2-3 µm (the bulbous base can be 3 µm diam.), formed from upper cells of the stroma which become the basal cells of the conidiophores. Conidiogenous loci 1-3, broad, unthickened, non-protuberant, formed in association with 1-2 enteroblastic proliferations of the conidiogenous cell which leave the scars situated on sloping shoulders 710 µm apart. Conidia dry, silver to grey-brown in mass, holoblastic, pale olivaceous, smooth, acicular, sinuous, (1-) 5-8 (-10) euseptate, not restricted at the septa, aguttulate, tapered gradually to an obtuse apex and slightly, if at all, to a slightly flared and convex base 2-3 (-3.5) µm diam. with an inconspicuous, unthickened hilum, (23-) 80 (-126) x (2.75-) 3.4 (-5) µm.

Provisional type: On living leaves of *Platylobium formosum* Hook., in bush, Great Ocean Rd several km W of Apollo Bay, Vic., V. & R. Beilharz, 24 Nov. 1988, VPRI 17451.

The most striking features of *P. platylobii* are the sporodochial masses of persistent, pale, acicular conidia with very thin, unthickened scars borne on long, thin conidiophores of the same diameter (Figs 3.32, 3.33), the large protuberant conidiomata (Fig. 3.34) , and the consistent association of sporulation with stomata on the lower leaf surfaces (Fig. 6.7). The conidiomata are almost invariably discrete. Those pictured in Fig. 3.38 are unusual in that they have partly merged, but even so they remain separated by their well-defined walls. The species would have been placed in *Cercoseptoria* had this genus not recently been subsumed into *Pseudocercospora* (Deighton, 1987). The fungus closely resembles *Pseudocercospora pini-densiflorae* (Hori & Nambu) Deighton, (syn. *Cercoseptoria pini-densiflorae* (Hori & Nambu) Deighton), the anamorph of *Mycosphaerella gibsonii* Evans and the cause of Cercospora or brown needle blight of pine (Evans, 1984). The morphological similarity of the two species is emphasised by the presence of engulfed host cells within a conidiogenous stroma of *M. gibsonii* pictured in Evans' paper which resemble those seen here in *P. platylobii* (Fig. 6.1).

A *Mycosphaerella* perfect state (Fig. 3.6) was found on both leaf surfaces of several specimens of *P. formosum*, but the two states occurred only once in the same (hypogenous) lesion, in VPRI 17411.
The 2-loculate spermogonium seen in Fig. 3.39 was brown-walled, contained rod-shaped spermatia ca 1x2.5 µm, and in addition bore a small number of conidiophores typical of *P. platylobii*, some with terminal scars. A 3-loculate spermogonium was also seen.

The immersed mycelium of *P. platylobii* is almost entirely intercellular, but on several occasions small hyphal coils were seen in two or three adjacent cells of the upper or lower epidermis, relatively close to vascular bundles (Fig. 3.7).

Mature epigenous and hypogenous stromata were often large relative to leaf diameter, the depth of the immersed portion reaching two thirds of the leaf thickness. In attaining this size, the developing stroma spread between the nearby host cells, which became engulfed by the stroma and incorporated into it.

It has been noted in this study of *Pseudocercospora* species that where there is little or no constriction at the point of abscission (that is, the scar is broad relative to the width of the conidiogenous cell and the conidium), the scar is generally very thin and the conidia are persistent. *P. platylobii* falls into this category. Conidia often remain in tufts once separated from the conidiophores, and could conceivably be dispersed as such. This phenomenon was reported in *Pseudocercospora samuhaabeeja* (Verma et al., 1989).

Specimens examined


Fig. 3.5 *Pseudocercospora platylobii* Conidia scraped from hypogenous conidiomata of specimens (A) VPRI 15510 and (B) VPRI 17478; C, shed conidia lying on the abaxial leaf surface beside a conidioma of specimen VPRI 17472.
Fig. 3.6 *Pseudocercospora platylobii*. A, attached hypogenous conidia. Small arrows indicate the abscission septum delimiting the conidium on the left, and the position at which the abscission septum was about to form to delimit the conidium on the right (as indicated by a change in the appearance of the cytoplasm); B, hypogenous conidiophores, attached conidia and conidium initials. A very young conidium initial is typically broad and irregular (‘floppy’) in shape (broad arrow); C, epigenous conidiophores of VPRI 16451; D, an ascus and ascospores from specimen VPRI 13805.
Fig. 3.7 *Pseudocercospora platylobii*. A, two young, hypogenous conidiomata (VPRI 17451); B, a fully immersed hypogenous conidioma (conidiophore bases are arrowed) which has unusually pale, fragile, thin-walled internal cells which may be undergoing autolysis. The conidioma may be developing into a spermogonium; C, hyphal coils in epidermal cells over a vascular bundle. They may constitute a means by which the fungus circumnavigates the vascular tissue.
3.6 PSEUDOCERCOSPORA SP. ON HIBBERTIA ASPERA

Lesions hologenous, necrotic, light brown on the adaxial surface and often with a darker margin, paler brown on the abaxial surface. Mycelium immersed, pale, intercellular, sparingly septate, 1.75-3 µm diam. Stromata amphigenous but mostly epigenous, scattered. Epigenous conidiomata initiated beneath the epidermis, growing between the epidermal cells and often spreading a little beneath the cuticle, which it eventually ruptures. Conidiophores numerous, sporodochial, smooth, with a bulbous base and narrow, cylindrical neck, 0-1 septate. The basal part is pale olivaceous brown, 6-9 (-15) µm in length x 5-7 µm diam.; the neck part of the conidiophore is paler, 3-15 x 2.25 µm. Conidia cylindrical to acicular, straight or slightly curved, persistent, 0-4 faintly septate, not constricted at the septa, tapered gradually to an obtuse apex and little if at all to a truncate base ca 2.5 µm diam., 49-101 x 2.75-3.5 µm.


Stomata occurred only on the abaxial surfaces of H. aspera leaves.

The unusual toughness of H. aspera leaves made section cutting difficult, and no good section was prepared of a hypogenous conidioma. Hypogenous conidiomata were sporodochial, and about the same size as those on the upper leaf surface, but mode of development and egress are not known. Conidium dimensions given here are from only 4 conidia. Most conidiogenous cells had attached conidia (Fig. 3.8), the longest being 94 µm in length and 2-septate.

Specimens of this fungus were collected at Wilsons Promontory National Park on the same occasion that collections were made of Pseudocercospora platylobii on Platyllobium formosum and P. obtusangulum. It is not possible to distinguish the fungi occurring on Platyllobium and Hibbertia according to the shape or dimensions of the conidiomata, conidiophores or conidia. The only apparent difference was the greater pigmentation of the bulbous conidiophore bases in the Hibbertia fungus. Although it is unlikely that a species of Pseudocercospora would infect plant species in two different host families (Platyllobium is in the Leguminosae, Hibbertia in the Dilleniaceae), the Hibbertia fungus can not at this stage be distinguished from P. platylobii.

Specimens examined


Fig. 3.8 *Pseudocercospora* sp. on *Hibbertia aspera*  A. hypogenous conidia from specimen VPRI 17455; B, epigenous conidiophores, most of which have produced conidium initials which are not yet delimited by an abscission septum (VPRI 17454); C, attached, delimited hypogenous conidia (VPRI 17455); D, an erumpent, epigenous conidioma which was initiated beneath the epidermis and which has emerged by rupturing the epidermis and cuticle (VPRI 17454).
3.7 CERCSEPTORIA CHAMAESYCES (STEVENS & DALBEY) PETRAK.

The genus Cercoseptoria Petruk (syn. Septoriopsis Stevens & Dalbey) was redescribed by Deighton (1976), who distinguished it from Pseudocercospora on the basis of its subspherical substomatal stromata, densely fasciculate conidiophores and acicular conidia. Since that time, certain species of Pseudocercospora have been recombined into Cercoseptoria on the basis of the degree of attenuation towards the base of the conidium (Yen, 1981; Yen & Lim, 1980), Deighton (1987) was troubled by some of these recombinations, and consequently recombined Cercoseptoria into Pseudocercospora.

Portion of the type specimen of Cercoseptoria chamaesyces on Chamaesyce hypericifolia (probably Euphorbia glomerata) held by Herb. K was kindly loaned to me for examination. Deighton (1976) pointed out that it is not certain that this material is part of the type collection, because Stevens and Dalbey cited two collections, but that at least it is authentic for the name Septoriopsis chamaesyceae Stev. & Dalbey.

A tangential section taken from portion of a very small, immature lesion showed two whole conidia (a third was broken), and a few conidiogenous cells in the mouth of a stoma (Fig. 3.9). The conidia were very pale olivaceous, 32 x 2 µm with a scar 1.75 µm diam., and 29 x 2.25 µm with a scar 2 µm diam. Two conidia measured by Deighton (1976) from the same material were 2-2.5 µm wide, and their lengths were 49µm and 57.5 µm respectively. The conidia measured by Deighton tapered to a width of about 1 µm at the apex, but those seen here did not, possibly because they were immature and had not yet completed their apical extension. Because the very pale fungus was viewed against a background of host epidermis, I could have missed seeing some septa in the conidia.

Scars on 7 conidiogenous cells were (1.0-)1.5(-1.75 µm) in diameter. In discussing the generic disposition of Pseudocercospora correae, Sutton et al. (1987) mentioned that in P. correae the scars left on the conidiogenous cell are of similar width to the conidiogenous cell, whereas in C. chamaesyces and several other species of the genus they are much smaller, 'giving the junction between the developing conidium and the conidiogenous cell a distinctly constricted appearance'. They saw this as reason not to include the Correa fungus in Cercoseptoria.

The conidium initials drawn by Deighton (1976) were initiated at narrow conidiogenous loci very close to apices of bulbous conidiogenous cells, with the result that the scars left after secession were much narrower than their respective conidiogenous cells. Several conidiogenous cells shown in Figure 3.9, however, have developed distinct necks (apical extensions), possibly after one or more proliferations. The scars on those cells are much the same width as the portion of the conidiogenous cell on which they are borne. Similar conidiogenous cells were seen in young, hypogenous stromatal conidiomata of P. correae specimens VPRI 17425 and VPRI 16410. In more mature or larger conidiomata of P. correae the bulbous bases possibly become angular under pressure from neighbouring cells, while the necks have extended to become the conidiophores we recognise. In P. correae, then, Sutton et al., (1987) may have been comparing the width of the scar with that of the apical extension of the conidiogenous cell - often the only
recognisable part of the conidiophore - while in *C. chamaesyces* they may have been comparing scar width with that of the swollen basal cell, as necks had hardly developed.

Specimen examined

Fig. 3.9 Conidiogenous cells and conidia of *Cercoseptoria chamaesyces* (Stevens & Dalbey) Petrak
3.8 PSEUDOCERCOSPORA PULTENAEAE SP. NOV.

*Immersed mycelium* pale brown, branched, septate, occasionally anastomosing, intercellular, 2.5-4.5(-5.5) µm diam. *Superficial mycelium* present or absent, hypogenous, developing from the basal cells of caespituli or more rarely by apical extension of conidiophores, pale brown, branched, septate, occasionally anastomosing, patent, 3.5-4.5 µm diam. *Stromata* amphigenous. *Hypogenous stroma initiation* substomatal and later often also subepidermal. *Epigenous stroma initiation* subepidermal. *Substomatal stromata* (Figs 3.10, 3.47) sub-spherical, pale to olivaceous or dark brown, commonly 25µm diam. x 12µm deep, but up to 60µm diam. x 50µm deep when confluent, later also developing outside the stomata, which are not ruptured, the external portion eventually more or less matching the immersed portion in size. *Erumpent stromata* (Figs. 3.40, 3.42) amphigenous, the epigenous stroma invading and occupying several contiguous epidermal cells (Fig. 3.46); individual stroma size determined by the size of the epidermal cell, but up to ca 200 µm diam. x 180 µm deep when confluent. Egress by rupture of the outer epidermal wall and cuticle, or occasionally by rupture of the entire epidermal layer. Development of hypogenous stromata not determined. *Conidiophores* mononematous, moderately pale olivaceous brown, smooth, often branching near the base to form two widely divergent branches, occasionally anastomosing, divergent, (19 -)37(-64) µm x 3-4 µm wide and 02 septate; *stomatal conidiophores* caespitose (Figs 3.41, 3.47), velutinous; *stromatal conidiophores* sporodochial. *External conidiophores* solitary, lateral on external hyphae (Figs 3.41, 3.44). *Conidiogenous cells* integrated, terminal, ± cylindrical, straight or almost so, only slightly restricted at the conidium-delimiting septum, pale olivaceous brown, smooth, 630 µm long x 34 µm diam; proliferation enteroblastic sympodial (Fig. 5.6). *Conidiogenous loci* broad (Figs 3.10, 3.43), slightly convex, unthickened, non-protuberant, ca 3.5 µm diam. *Conidia* (Figs 3.10, 3.43) holoblastic, pale brown, smooth, guttulate, persistent, sub-cylindrical, straight, curved or occasionally bent, short conidia often barely tapering to the apex which may be swollen but longer conidia usually markedly tapered, (1-)3-5(-8) euseptate, 25-95 µm long x 2.5-7.5 µm wide at the widest point (which is usually 1/3 - 1/2 way from the base), 2.5-5.5 µm wide at the apex, and 3-4.5(-5.25) µm wide at the base.


Because the leaves of *P. daphnoides* have stomata on the lower surfaces only, stomatal egress can only be hypogenous, but erumpent egress through fissures in the leaf surface occurs from both leaf surfaces. Whether hypogenous sporulation is stomatal or erumpent may be governed by the climatic conditions. Specimen VPRI 17486, which displayed only velutinous hypogenous sporulation, was collected from large plants growing in a lush, sheltered forest during warm, wet conditions. In other collections, all from smaller plants growing on more exposed sites at Wilsons Promontory, both erumpent stromata and small patches of stomatal stromata usually occurred on the leaf undersurfaces. Starch grains were numerous in guard cells and mesophyll cells closely associated with the conidiomata in the specimen examined by means of the TEM (VPRI 17486), on which velutinous stomatal sporulation occurred on green host tissue. In mobilising assimilates and drawing them to the infected area, the fungus is acting as a biotroph at this
stage of its development. Immersed mycelium, sparse at this early stage, builds up prior to the emergence of erumpent conidiomata.

Conidium length can vary between specimens. For example, conidia from specimen VPRI 14760 are (26-46(-62) µm long in contrast with those from VPRI 14953 which are (43-61(-92) µm long. Scar diameter is almost the same in the two specimens, but the shorter conidia of VPRI 14760 have slightly broader apices (average 3.1 µm) than do the conidia of VPRI 14953 (average 2.8µm), which have undergone more apical extension. It is interesting that the short conidia of VPRI 14760 are 3-5(-6) septate while those of VPRI 14953 are only 1-3(-4) septate, an indication that the shorter conidia are no less mature than the longer ones from the other population. VPRI 14953 was the only specimen seen with long conidia.

Both conidiophores and conidia were seen to anastomose.

Specimens examined

Fig. 3.10 *Pseudocercospora pultenaeae*, hypogenous sporulation

Conidia from VPRI 14953 (A) and VPRI 14760 (B); C, branched conidiophore

and a fascicle of conidiophores emerging from a stoma in VPRI 17451
3.9 *PSEUDOCERCOSPORA ULURUENSIS* SP. NOV. ON *SANTALUM* SPP.

Lesions usually hologenous, occasionally amphigenous, circular, mauve, raised, 1-5 mm diam. or larger when confluent. Mycelium immersed, hyaline to pale olivaceous, smooth, intercellular, much branched, irregularly swollen, 3-5 mm diam. Stromata (Fig. 3.11) sub stomatal and subepidermal in origin. Sub stomatal stromata composed of loosely organised hyaline hyphae when young, later often becoming compact and unevenly pale olivaceous brown, subspherical, occurring in many substomatal cavities and giving rise to small groups of caespitose conidiophores which emerge through the stomata. Erumpent stromata larger, pale to medium olivaceous brown, developing from subspherical, hyaline, subepidermal stroma initials which invade the epidermal cells or grow between them before spreading a little beneath the cuticle which is eventually ruptured. Conidiophores comprising a bulbous base ca 12 µm long x 5-8 µm wide with a neck which is more or less straight when short (2-5 µm) but often curved when longer (up to 35 µm or more), unbranched, smooth, often very pale when stomatal and light olivaceous brown when sporodochial, initially 0.1 septate but probably becoming more septate with age. Conidiogenous cells integrated, terminal, ± cylindrical, smooth, showing enteroblastic regenerative growth. Conidiogenous loci broad, unthickened. Conidia cylindrical to obclavate, very pale olivaceous, smooth, irregularly guttulate, with an obtuse or swollen apex and tapering gradually or not at all to a truncate and flared or obconico-truncate base (1.5-)2(-3.5) µm, (14-)21-35(-39) x (3.25-)3.5-5 µm.


Leaves of *Santalum lanceolatum* have an abundance of deeply sunk stomata on both surfaces. The above description is based on specimens of *S. lanceolatum*, which exhibit unusual large, raised, mauve lesions. The Western Australian specimen of *S. spicatum*, which has not yet been fully investigated, shows brown, unraised lesions.

The only previous record of a cercosporoid fungus on a member of the Santalaceae appears to be that of *Cercospora santalacea* Gopinathan on leaves of *Osyris arborea* Wall. in India (Nair, 1964). Judging from Nair's publication, the conidia of the two fungi are quite different, those of *C. santalacea* being cylindrical to filiform and 27.5-70.5 µm long, while those on the Australian specimens (Fig. 3.11) are short, more or less cylindrical and 14-39 µm long (32 conidia). The widths of the conidia of the two fungi are much the same, but there is little overlap in length measurements. Although differing length measurements alone are insufficient reason to separate taxa in the cercosporoid fungi, the difference here is associated with differences in conidium shape. I have recently been loaned two newly collected specimens of *Pseudocercospora* on *Santalum lanceolatum* and one on *S. spicatum* (R. Br.) DC from different localities in Western Australia, by the Western Australian Herbarium. Although I have not yet had the opportunity to examine these specimens in detail, I have confirmed that the conidia are in each case of the same type as those described here, which suggests that those illustrated here are of mature shape and size and not immature conidia of *C. santalacea*. More specimens need to be examined, including a specimen of
Pseudocercospora on Santalum lanceolatum known to be held at Herb. BRIP, a newly collected specimen from the Kimberly, W.A., and the type specimen of C. santalaceae, before the name Pseudocercospora uluruensis is published.

Specimens examined

Fig. 3.11 Pseudocercospora uluruensis  A, conidia; B, conidiophores; C, stomatal conidioma; D, erumpent conidioma with a piece of cuticle resting on top; E, adjacent erumpent conidiomata.
3.10 PSEUDOCERCOSPORA LORANTHI McALPINE COMB. NOV.

_Cercospora loranthi_ McAlpine. Proc. Linnean Soc. NSW 28: 96. 1903

The following expanded description is based in part on that of Hansford (1954) for _Cercospora loranthi_ McAlpine.

Lesions amphigenous, initially visible as a white fleck 0.2-0.25 mm diam., later becoming rounded, raised, and up to 2 mm diam., initially grey, becoming olivaceous brown when sporulation is profuse. Immersed mycelium pale, branched (often lobed), septate, intercellular, 1.5-2.5 µm diam., causing localised hyperplasia in the vicinity of each young conidioma, and later a more general hyperplasia, initially substomatal and subepidermal, but spreading as sheets of hyphae or pseudoparenchymatous masses beneath the cuticle and epidermis and into the mesophyll. External mycelium emerging from stomata, sparse, bearing simple, aseptate, conidiophores. Stromata pale to medium olivaceous brown, initially discrete, punctiform, and uniformly scattered, later merging and emerging through fissures in the surface of the lesion. Egress stomatal from substomatal stromata and erumpent from subcuticular and subepidermal stromata. Subepidermal stromata up to 60 µm thick often crush the epidermal cells and can cause the epidermal layer to slough off; single conidiophores may then form on the surface of the exposed mesophyll tissue. External conidiophores continuous or with a basal septum, 8-18 µm long x 3.5-5.75 µm wide. Conidiophores (Fig. 3.13) very numerous, sporodochial, largely covering the surfaces of mature lesions, olivaceous brown, straight, ±cylindrical, unbranched or branched once near the base (Figs 5.13, 5.14), smooth when young and becoming rugulose with age, 0-3 septate, formed from upper cells of the stroma, often two from one cell, frequently showing successive regenerative and/or proliferative endohyphae (23-36(-65) µm x 5-7 µm. Conidiogenous cells integrated, terminal, cylindrical or slightly geniculate towards the apices, showing enteroblastic, pseudopercurrent proliferations which are commonly endohyphal (Figs 5.7, 5.8, 5.14), 12-32 µm long x 5-7 µm wide. Conidiogenous loci 0-3, narrow, pale and inconspicuous to moderately dark, thickened and protuberant, formed in association with 0-3 enteroblastic sympodial proliferations of the conidiogenous cell. Conidia dry, pale olivaceous brown, smooth, not attenuated at the septa, holoblastic, ellipsoid to obclavate, straight to slightly curved, 0-3(-7) septate, tapered gradually or more abruptly to an obtuse apex, and abruptly to a truncate base bearing a scar which is often slightly darkened, thickened and protuberant, (1.25-)1.7(-2.5) µm diam., (13-)54(-120) µm long x (3-)4(-5) µm wide at the widest point and 2.5-3 µm wide near the apex, except in very short conidia which may have swollen apices up to 4 µm wide.

Provisional type: _Amyema pendulum_ (Spreng.) Tiegh., growing on _Eucalyptus_ sp., Hastings Rd, Mornington Peninsular, Vic., V. & R. Beilharz, 8 Jan. 1988, VPRI 17407.

Slides of the type specimen show dark conidiophores containing very obvious endohyphae which could be regenerative or proliferative (Fig. 3.12). The old conidiophore walls have not broken off near the base like many of those seen in electron micrographs (Figs 5.14, 5.19), but have become wrinkled and rough with age. McAlpine found conidia as long as 150 µm, whereas the longest seen by Hansford in a South
Australian specimen was 60 µm. As in all obclavate cercosporoid fungi, conidium length is very variable and apparently influenced by environmental conditions. Petrak (1954) described *Cercospora loranthicola* (as *C. loranthincola*) from *Amyema pendulum*, distinguishing it from *Cercospora loranthi* on the basis of its shorter and narrower conidia and conidiophores. I have so far failed to locate this specimen.

In very young lesions, pale brown hyphae spread laterally beneath the epidermis, forming a subepidermal mycelial sheet. From there, much-branched or lobed hyphae extend deeper into the host tissue, where they are flattened against the surfaces of the host cells as they pass between them. Intercellular spaces are occupied by a mass of similar hyphae. Hyphae also extend into the subcuticular region, where a mycelial sheet develops, covering more or less the entire lesion (Fig. 3.31).

The fungus induces hyperplasia in the vicinity of each conidioma during the early stages of infection, and continues to act as biotroph during much of its development. Some host cells are squashed by the large stromatic masses of hyphae which build up beneath the cuticle and deeper in the leaf tissue, but this maybe an incidental, localised mechanical effect which is unrelated to the mode of survival of the fungus.

Specimens examined


On leaves of *Muellerina eucalyptoides* (DC) Barlow, on *Acacia* sp., Fraser National Park, Vic., V. Beilharz, VPRI 15511.
Fig. 3.12

A, B  The type specimen of *Cercospora loranthi* McAlpine

A, conidia; B, conidiophores. Many of the conidiophores in this specimen contained regenerative or proliferative endohyphae.

C-F *Pseudocercospora loranthi*

C, conidia of specimen VPRI 17417 from *Amyema preissii*; D, conidiophores of specimen VPRI 15513 from *A. pendulum*; E, external conidiophores of specimen VPRI 17502 from *A. pendulum*; F, attached and shed conidia from specimen VPRI 17408 from *A. pendulum*. Attached conidia with thickened abscission scars are rarely seen in this fungus, the conidia usually being shed as soon as thickening develops. Unpigmented thickening was seen in several instances.
Fig. 3.13  *Pseudocercospora loranthi*  A, a young conidioma which has not yet started sporulating. Note the large number of conidiogenous cells. B, an erumpent conidioma. Gaps in the stroma coincide with the section grazing or passing through host cells.
3.11 VERRUCISPOROTA DAVIESIAE (COOKE & MASSEE) COMB. NOV.

*Cercospora daviesiae* Cooke & Massee, 1889, *Grevillea* 18, 7.

*Lesions* hologenous, necrotic, angular, weakly vein-limited, light brown, often with a raised, darker margin, commonly up to 5 mm diam. but frequently coalescing and covering larger areas of the leaf, particularly around the margins. *Mycelium* immersed, mid-olivaceous brown, branched, regularly septate, intercellular, 1.5-3 µm wide, coated with a smooth or irregular ('bubbly') pale brown deposit ca 1 µm thick (Fig. 3.14, 3.50). *Stromata* (Fig. 3.48) amphigenous, substomatal, irregular, moderately dark brown, scattered, discrete, compact, composed of *textura angularis*. *Egress* stomatal without disruption of the stoma. *Conidiophores* (Figs 3.14, 3.48) caespitose, moderately dark brown, thick-walled, rigid, divergent, straight or slightly curved or bent, cylindrical, unbranched, smooth at the base becoming verruculose towards the apex which is often also paler, 0-3 septate. *Conidiogenous cells* integrated, terminal, cylindrical or becoming geniculate towards the apices, frequently showing enteroblastic sympodial or pseudopercurrent regenerative growth, mid-olivaceous brown, sometimes paler towards the apex. *Conidiogenous loci* broad, thickened, often protuberant, formed in association with 1-3 proliferations of the conidiogenous cell. *Conidia* (Fig. 3.14) holoblastic, light brown, verrucose, often paler and smoother towards the apex (less often towards the base or elsewhere), cylindrical to obclavate, straight or slightly curved, (0-3)-6 septate, sometimes constricted at the septa, tapered gradually or not at all to an obtuse (sometimes swollen) apex and slightly but usually abruptly to a truncate base (2)-3.5(-5) µm diam., (18)36(-56) µm x (4.5-)6(-7) µm.

Stomata are present on both surfaces of leaves of *Daviesia latifolia* and *D. mimosoides var. laxiflora*.

Spermogonia and ascomata were intermixed with conidiomata in some lesions, and the unusual, coated hyphae (Figs 3.14, 3.50) were associated with each of the three types of fruiting body. Spermogonia (Fig. 3.50) were substomatal, uniloculate, ca 55 µm deep (excluding the 15 µm long neck occupying the stoma) x 48 µm wide and contained rod-shaped spermatia. Ascomata (Fig. 3.49) were substomatal, uniloculate, ca 95 µm deep (excluding the 10 µm long neck occupying the stoma) x 87 µm wide, and contained immature asci with hyaline, unequally two-celled ascospores 15-19 µm long x 3.0-3.75 µm wide (Fig. 3.14). The walls of the ascomata and spermogonia were of the same colour as the darkest cells of the stroma of the anamorph. Neither spermogonia nor ascomata were described for the other two species of *Verrucisporota*. Examination of the holotype of *Cercospora daviesiae* revealed many lesions containing numerous asci with immature ascospores (Fig. 3.14). They differed from those in VPRI 17435 (Fig. 3.14) in being unrestricted at the septum. This may reflect a difference in maturity. The conidia of *C. daviesiae* were described as sub-hyaline to pale, and no mention was made of wall ornamentation. However, they proved to be pale brown and distinctly verrucose, as in the present collections. The verrucose nature of the conidia precludes the fungus from being placed in *Cercospora*, but it fits well in *Verrucisporota*, which was separated (under its earlier name *Verrucispora*) from *Cercospora* to accommodate a leaf-infecting fungus, *Verrucisporota proteacearum*, on *Finschia* and *Hakea* (Shaw & Alcorn, 1967). The
only other species of *Verrucisporota* currently recognised by Shaw & Alcorn (1993) is *V. brideliae* on *Bridelia retusa* Spreng, from India.

A third species, *Verrucispora indica* Kamal & Singh from *Smilax prolifera* Roxb. (Kamal & Singh, 1978), later thought to be conspecific with the earlier-described *Biharia smilacis* Agarwal from the same host and re-named *Verrucispora smilacis* (Agarwal) McKenzie (McKenzie, 1982), has since been recombined into *Stenella* because of its verrucose superficial mycelium (Shaw & Alcorn, 1993). Although *Verrucisporota* species can produce a small amount of external mycelium, this is always smooth-walled. Similarly, *Verrucispora luculiae* M. K. Khan, U. Budathoki & Kamal has been recombined into *Pseudocercospora* because its scars are unthickened, and *Verrucispora indica* Kamal & R. P. Singh has been recombined into *Sirisporium* because its conidia are occasionally longitudinally as well as transversely septate (Shaw & Alcorn, 1993).

*Verrucisporota daviesiae* appears therefore to be only the third valid species of *Verrucisporota*. It satisfies the criteria by which *Verrucisporota* is distinguished from *Stenella* and *Sirisporium* (Shaw & Alcorn, 1993) in that it has a stroma, its mycelium is immersed, its conidiophores are unbranched, and its conidia are solitary, verrucose and only transversely septate. The conidia of *V. daviesiae* are narrower and paler than those of *V. proteacearum* and more tapered towards the apex, and its conidiophores are shorter and narrower.

Specimens examined


Fig. 3.14 *Verrucisporota daviesiae*  A, conidia; B, conidiophores, showing a probable pseudoannellde, thickened scars, conidium initials and a conidiophore containing an endohypha; C, an ascus from specimen VPRI 17435; D, ascospores from the type specimen of *Cercospora daviesiae* Cooke & Massee; E, immersed hypha with a smooth or bubbly coating.
Species placed in *Pseudocercospora* show considerable variation in habit. Their conidiophores can be fasciculate (with or without a well-developed stroma) or sporodochial, or can occur on an external, secondary mycelium (Deighton, 1976). The species which produce conidiophores on external hyphae, even those which also form some fasciculate conidiophores, bear some resemblance to *Mycovellosiella* except that they lack the thickened scars typical of that genus (Deighton, 1976). Deighton (1976) suggested that species producing conidiophores on external hyphae resembled *Cercocladospora adinae* (the type of *Cercocladospora*) in this respect, but recognised that some of these species were too variable in habit to allow them to be transferred to *Cercocladospora* at present. While he thought it might be possible in the future to subdivide *Pseudocercospora* into sections, one of which might be based on *Cercocladospora*, he preferred in the meantime to regard *Cercocladospora* as a synonym of *Pseudocercospora*.

Sutton & Pascoe (1987) similarly considered the possibility of a generic reorganisation of *Pseudocercospora* based on differences in growth habit which they observed in *Pseudocercospora* infections of *Correa* spp. They noted, however, that Deighton (1976) described bridging species which sporulate on both leaf surfaces, their epigenous conidiophores being produced on pseudoparenchymatic stromata and hypogenous conidiophores either emerging through stomata or forming on a superficial secondary mycelium. As a result, Sutton and Pascoe, like Deighton, discarded the idea of a generic reorganisation of *Pseudocercospora* based on differences in habit, but distinguished *Pseudocercospora correicola* from *P. correae* primarily on this basis. A re-examination of specimens of these two species lodged at Herb. VPRI, however, indicates that the specimens represent only one species in which certain characters such as conidium scar diameter are variable, while habit is influenced by host anatomy.

The two leaf-spotting fungi in question, *Pseudocercospora correae* (Sutton *et al.*, 1987) and *P. correicola* (Sutton & Pascoe, 1988), were distinguished almost entirely on the basis of the symptoms they cause and differences in habit. *P. correae* was described as causing reddish lesions which are most evident on the upper leaf surface, where it forms large immersed stromata bearing cylindrical sporodochial conidiophores and cylindrical conidia, both pale brown, septate and with unthickened scars. It forms occasional hypogenous sporodochia and no superficial mycelium (Sutton *et al.*, 1987).

*P. correicola*, on the other hand, was described as causing less conspicuous, reddish epigenous lesions and conspicuous grey, hypogenous lesions, the grey colour being caused by sporulating hyphae ramifying among the leaf hairs. This superficial mycelium originates from small substomatal knots of hyphae and bears short, more or less cylindrical conidiogenous cells with inconspicuous scars. *P. correicola* was said not to form epigenous sporodochia. Its conidia closely resemble those of *P. correae*, but tend to be more abruptly tapered at the base. They tend also to be longer, wider and to have fewer septa (Sutton & Pascoe, 1988).
Several observations inconsistent with the published descriptions of these fungi led me to re-examine some of the herbarium specimens on which the original descriptions were based, as well as freshly collected samples of *P. correae*. Four specimens of *P. correae* were also examined ultrastructurally, three by TEM and one by SEM techniques. On some collections of *P. correae* with more or less glabrous leaves there was profuse hypogenous sporulation from external hyphae which emerged from the stomata and grew along the leaf surface, producing lateral and terminal conidiophores (Fig. 3.15). Hypogenous sporodochia were often also present. In the most advanced lesions of the holotype of *P. correae*, VPRI 14065, there was some hypogenous external sporulation of this type, as well as hypogenous sporodochia which were so numerous that they outnumbered epigenous sporodochia in some transverse sections.

Examination of numerous specimens of *P. correae* showed that some form of sporulation dependent on stomatal egress invariably developed at an early stage. Small knots of hyphae in the stomata or substomatal cavities produced either short stomatal conidiophores, external hyphae or both together (Fig. 3.15). Hypogenous stomatal sporulation preceded the necrosis of host tissue in young, chlorotic lesions, and occurred on apparently healthy tissue outside the margins of more mature lesions. Hypogenous sporodochia developed later, both in substomatal cavities (where their stromata probably developed from the small knots of hyphae seen at an earlier stage) and beneath the epidermis. Stroma formation in *P. correae* is described in detail in Chapter 6. Some *Correa* leaves infected with *P. correae* lack the densely hairy leaf undersurfaces typical of *C. reflexa* and *C. lawrenciana*, the hosts of *P. correicola*. In such cases, the superficial hyphae spread along the leaf surface (Fig. 3.15). Several specimens of *P. correae* on hosts with abundant leaf hairs also produced fertile 'surface hyphae' and immersed sporodochia which were exposed to view only after hairs had been scraped off.

The production of a fertile hypogenous external mycelium is therefore a regular feature of *P. correae* as well as *P. correicola*, although in the type specimen of *P. correae* the exposed hyphae were in contact with, or close to, the leaf surface whereas in that of *P. correicola* the hyphae climbed among the leaf hairs. The distinction is not absolute, however, as some collections of *P. correae* on leaves with densely hairy undersurfaces had fertile external mycelium ramifying among the hairs in the manner of *P. correicola*, as well as hyphae growing on the leaf surface (VPRI 13468, 14413, 13405 and 14826). The different habits displayed by the external hyphae do not seem sufficiently distinct to be taxonomically significant, and the conidiogenous cells are similar on the two types of growth.

Most collections of *P. correicola* include at least one leaf with one or more deep red epigenous lesions containing erumpent sporodochia (Fig. 3.16) resembling those regarded as typical of *P. correae* (Sutton *et al.*, 1987). The development of epigenous lesions was followed in sections through the holotype of *P. correicola*, VPRI 14478a. In some sections, the immersed intercellular mycelium was almost entirely restricted to the spongy mesophyll and stomatal cavities, although several hyphae had penetrated the palisade mesophyll. Palisade mesophyll cells adjacent to these hyphae had reddened contents. In more advanced lesions the palisade mesophyll was more heavily colonised, and all spongy and palisade mesophyll cells had reddened contents. Subepidermal stromata had formed in this region, and epigenous sporodochia had erupted through the upper leaf surface.
Three specimens (VPRI 14301, 14414 and 15394) exhibiting symptoms of both *P. correae* and *P. correicola* were diagnosed as being infected with both fungi. A piece of tissue was excised from the margin of an epigenous lesion of specimen VPRI 14301, and sections cut across the green and red tissue. In the healthy green tissue, hyphae ramified through the spongy mesophyll. Where the palisade mesophyll was penetrated, cells adjacent to the hyphae sometimes showed a slight orange-yellow discolouration but in other cases were scarcely affected. The immersed hyphae were mainly hyaline but developed pale brown pigmentation in the immediate vicinity of stomata. Hyphae emerged from the stomata and ramified among the leaf hairs where they bore the pale brown conidiogenous cells typical of *P. correicola* (Fig. 3.16). External hyphae also, although rarely, grew along the leaf surface (Fig. 3.16). The immersed mycelium in the green tissue was continuous with that in the red tissue, where the palisade mesophyll was more heavily colonised, erumpent stromata had formed, and the whole of the leaf tissue was discoloured. There is no doubt that the epigenous lesion diagnosed as resulting from infection with *P. correae* was the localised culmination of the same infection, attributed to *P. correicola*, which had resulted in hypogenous external sporulation over a wider area.

Sections through other specimens of *P. correicola* which exhibited sporulation on the upper leaf surfaces (VPRI 14533, 14826 and 15394) demonstrated similar mycelial links between the two types of conidiomata. Although hypogenous sporulation was generally diffuse, there was often a discernible correspondence in position between opposing lesions, as already noted by Sutton *et al.* (1987) for *P. correae* and Sutton & Pascoe (1988) for *P. correicola*. This was particularly evident in VPRI 15394, in which the unusually well-defined margin of a small, dense hypogenous colony corresponded exactly with the margin of an epigenous lesion.

It is shown that sporulation from epigenous sporodochia and hypogenous 'leaf hair' hyphae co-exist in many more specimens than was originally recognised (Table 3.1). Although the predominant form of sporulation varies markedly between specimens, *P. correicola* can not be distinguished from *P. correae* purely on the basis of habit because each of the species is able to sporulate in the manner supposed to be exclusive to the other. The epigenous lesions seen in specimens said to be infected with *P. correicola* are often fainter and less conspicuous than those said to be infected with *P. correae* only because they are not fully developed. In both groups of specimens, however, the spongy mesophyll and substomatal cavities are colonised first, then the palisade mesophyll and finally, but not necessarily, the upper epidermis.

Although *P. correae* and *P. correicola* were primarily distinguished on the basis of symptoms and habit, their conidia were also reported to differ in width, scar diameter (Table 3.2) and degree of attenuation to the base. According to Sutton & Pascoe (1988), conidia are 7-9 septate and 31-77 x 3.5-4.5 µm in *P. correae*, and 48 septate and 48-147 x 4.5-5.5 µm in *P. correicola*. In addition, conidium bases of *P. correae* were said to be 2.5-3.0 µm (Sutton *et al.*, 1987), but only 2.0-2.5 µm in *P. correicola* (Sutton & Pascoe, 1988). To test these findings, I compared the dimensions and septation of conidia produced on hypogenous external hyphae from specimens identified as *P. correicola* with those of conidia produced on epigenous conidiomata from specimens identified as *P. correae*. In so doing I was, in effect,
comparing the two populations of conidia whose characteristics had supported the erection of *P. correicola*. Conidium lengths are not presented here, partly because there is considerable overlap between species, and also because conidium length is a highly variable character in the cercosporoid fungi.

The two populations of conidia are indistinguishable on the basis of width (Fig. 3.17) and septation (Fig. 3.18), although each character shows variation between specimens. There exists, however, a real difference in scar diameters. Although there is considerable overlap between populations, *P. correicola* scar diameters peak in the 2.0-2.5 µm range while those of *P. correae* fall mainly in the 2.5-3.5 µm range (Fig. 3.19). A comparison of Tables 3.1 and 3.2 reveals gaps in the numerical data which result from the fact that conidia could not be obtained from some sporulation sites. Data are lacking particularly from specimens of *P. correae* which had only small amounts of hypogenous 'leaf hair' sporulation and specimens of *P. correicola* which had immature epigenous sporodochia or mature sporodochia with no attached conidia.

The general difference in scar diameters of conidia produced in typical *P. correae* and *P. correicola* conidiomata appears to support the concept of two distinct species which simply share more characteristics than was previously recognised. The validity of the two species depends, however, on samples of conidia from individual specimens being readily recognisable as belonging to one or the other, for species differentiation is clearly unsatisfactory if it depends on the analysis of the dimensions of populations of conidia from a number of specimens. It is instructive in this context to examine specimens individually with regard to the degree of attenuation towards the conidium bases and the diameter of conidium scars. The distinction between species is again not clearcut. For example, some specimens bear epigenous conidia resembling *P. correae* and hypogenous conidia typical of *P. correicola*. An example is specimen VPRI 14301, diagnosed as being infected with both fungi, but shown to sporulate on both leaf surfaces from the one immersed mycelium (Fig. 3.20). Conidia of specimen VPRI 17422 (diagnosed as *P. correae*) produced on hyphae growing on the leaf surface have scar diameters which fit *P. correicola*, while the epigenous conidia from the same specimen have the broader, more flaring scar bases of *P. correae* (Fig. 3.21). By contrast, however, the 'leaf surface' conidia of another sample of *P. correae* (VPRI 14065) are of the *P. correicola* type (Fig. 3.21). Some samples have such a mix of conidium base shapes and scar diameters that it is impossible to categorise them. Examples are the 'leaf hair' conidia from specimens VPRI 14533 (Fig. 3.22) and 14416 (Fig. 3.20), both diagnosed as *P. correicola*.

Although individual specimens do not always conform to the species descriptions there remains, nevertheless, an unmistakable trend towards narrower scars on hypogenous, 'leaf hair' conidia and broader scars on epigenous, sporodochial conidia (Table 3.2, Fig. 3.19). This means there are generally narrower abscission septa on the conidiogenous cells occurring on external hyphae, particularly those in the leaf hairs, in contrast with those formed on sporodochial conidiophores. Again, the distinction is not absolute. VPRI 14400 and VPRI 14216 ('leaf hair' conidia) and VPRI 14065 ('leaf surface' conidia) are relatively broad-based, and sporodochial conidia of VPRI 14826 are relatively narrow-based (Table 3.2). The variation can nevertheless be largely linked to conidiophore position. Because external and
sporodochial conidiophores differ in origin and morphology, different degrees of attenuation towards the abscission septum and variation in scar diameter, which is often linked to the degree of attenuation, could conceivably occur at times.

If it is accepted that the two species can not be separated on the basis of symptoms, habit or conidium morphology, *P. correicola* will need to be reduced to synonymy with *P. correae*.

Apart from the modifications needed to incorporate the characteristics of *P. correicola*, the description of *P. correae* requires several other amendments concerning the relationship the pathogen bears to the host, the precise location of epigenous conidiomata and the morphology of the fungus.

Firstly, the mycelium is strictly intercellular, not intracellular as stated in the original description. This is clearly seen in the chlorotic lesions which are the first symptom of infection, and the relationship persists as the lesions became necrotic. Resin-embedded sections studied by light and transmission electron microscopy were invaluable in providing accurate information on host-parasite relationships in *P. correae* and other fungi dealt with in this study.

Secondly, it was stated that the mycelium of *P. correae* is 'concentrated mainly in the epidermis but also present in the palisade tissue' (Sutton *et al.*, 1987). During the early, chlorotic stage the mycelium occupies the spongy mesophyll and substomatal cavities. Invasion of the palisade tissue follows, and the upper epidermis (which Sutton *et al.*, were referring to) is invaded only in the course of stroma development. This pattern of events, evident in specimens diagnosed as *P. correicola* as well as *P. correae*, strongly suggests that the pathogen infects through the stomata.

Thirdly, stromata of *P. correae* were said to be epidermal, and a published illustration of the holotype shows what appears to be an intraepidermal stroma (Sutton *et al.*, 1987). However, stromata are initiated beneath the epidermis, and by the time of emergence extend from well down in the palisade layer right through the epidermis into the subcuticular region. Re-examination of the slide deposited with the type specimen showed the illustrated stroma extending well below the epidermis in a different plane of focus. Similarly, the small knots of hyphae illustrated in several neighbouring epidermal cells were not intraepidermal hyphal coils or stroma initials as might be supposed, but the extreme edges of other large stromata visible in a different focal plane. The application of the terms 'epidermal' and 'intraepidermal' to stromata is discussed in Chapter 6. In brief, however, I suggest that epigenous stromata of *P. correae* should be described as being initiated beneath the epidermis, further stroma development occurring beneath the epidermis, within or between the epidermal cells and beneath the cuticle, which is eventually ruptured. It is relevant to mention here that epigenous stromata frequently invade, and emerge from, the basal cells of the club-shaped trichomes which occur on the adaxial leaf surfaces of *C. reflexa*.

Lastly, conidiophores and conidia were said to be verruculose. Verrucose is defined as 'having small rounded processes or "warts"', and verruculose as 'delicately verrucose' (Hawksworth *et al.*, 1983). *P. correae* conidia appear smooth-walled in a scanning electron micrograph (Fig. 3.53), while the wall of a conidiogenous cell appeared a little rough in a transmission electron micrograph (Fig. 4.37). This
roughness may be an artefact, however, as it is at least partly due to the blistering of the external membrane and its separation from the conidiophore wall. This is a recognised effect of preparation for ultrastructural examination (Roberts & Swart, 1980). One conidium of VPRI 14533 was distinctly verruculose towards the base, however, and several conidiophores were rough-walled towards the apex.

It should be mentioned, also, that loosely branched conidiophores were seen in epigenous stromata of VPRI 14553 (*P. correicola*) and several specimens of *P. correae*. They did not bear apical abscission scars, and could have been exhibiting mycelial growth.

Cultures of *P. correae* (VPRI 16410) were prepared by mass inoculation of conidia picked off epigenous and hypogenous fructifications, the latter consisting of heavily sporulating superficial colonies. The cultures were indistinguishable. It would be interesting to culture hypogenous conidia from a *P. correicola* type of infection when a fresh specimen becomes available.

*Pseudocercospora correae*, emended description.

*Immersed mycelium* hyaline to pale olivaceous brown, branched, septate, intercellular, initially in the spongy mesophyll and substomatal cavities and later colonising the palisade mesophyll, 2-4(-5) µm diam; *secondary mycelium*, when present, emerging through the stomata, pale brown, irregularly branched and septate, smooth to verruculose, lax, anastomosing and growing along the leaf surface or ramifying among the leaf hairs but not penetrating them. *Stromata* epigenous, hypogenous, amphigenous or lacking. *Epigenous stromata* usually somewhat zonate, less obviously so when only a few are produced, initiated beneath the epidermis and extending at maturity from deep in the palisade mesophyll to beneath the cuticle, erumpent, 40-80(-180) µm diam. x 40-80 µm deep, discrete or confluent, composed of compact, pale brown textura angularis. *Hypogenous stromata* substomatal or subepidermal, resembling the epigenous stromata when subepidermal and erumpent, often smaller when substomatal. *Sporodochial conidiophores* olivaceous brown towards the base, paler towards the apex, smooth to verruculose, 0-6(-10) septate, (8)-18-44(-77) µm long x (2-)3.5-4.5(-5.5) µm wide, formed from the upper cells of the stroma. *External conidiophores* mononematous, pale to medium brown, smooth to verruculose, irregularly septate, branched and anastomosing, 10-120 µm 3.0-4.5 µm. *Conidiogenous loci* 1-3, unthickened, usually non-protruberant, flattened to slightly convex, 1.75-3.25(-4.5) µm diam., formed in association with holoblastic sympodial, enteroblastic percurr ent or enteroblastic sympodial proliferations of the conidiogenous cell. *Conidia* dry, olivaceous brown in mass, holoblastic, pale brown, smooth or less commonly verruculose, cylindrical, straight or very slightly curved, (0-)7-9(-11) euseptate, rarely restricted at the septa, irregularly guttulate, tapering gradually to an obtuse or subacute apex and more abruptly to a truncate base (1.5-)2-3.5(-4) µm diam., (22-)31-77(-105) x (2.5-)3.5-4.5(-5.5) µm.

Specimens examined

*C. backhousiana* Hook., National Botanic Gardens, Black Mountain, Canberra, A.C.T., I. K. Sharma, May 1984, DAR 49508; *C. lawrenciana*, Hook., in bush, Great Ocean Rd, several km west of Apollo Bay, V. & R. Beilharz, 24 Nov. 1988, VPRI 17475; *C. reflexa* (Labill.) Vent., Jawbone Track, Cathedral Range
Fig. 3.15 *Pseudocercospora correae*, hypogenous sporulation
A-C VPRI 17422 Conidiophores, and external hyphae on which conidiogenous cells have formed, emerging from stomata. A conidium initial has formed on one conidiogenous cell in C.
D VPRI 16410 Conidiophores on external hyphae growing on the leaf surface.
E VPRI 14065, holotype External conidiophores with attached, septate conidia.

Fig. 3.16 *Pseudocercospora correicola*
A VPRI 14826 Epigenous sporodochium.
B VPRI 14478a, holotype External conidiophore with attached, septate conidium (compare with Fig. 3.15(E)).
C VPRI VPRI 14826 External conidiophore on a hypha growing on the leaf surface.
D VPRI 14301 External conidiophores on a hypha growing among leaf hairs.
E VPRI 14414 External conidiophores on a hypha growing among leaf hairs.
Table 3.1 Occurrence of conidiomata of *Pseudocercospora* on specimens of *Correa* diagnosed as being infected with *P. correiae*, *P. correicola* or both.

<table>
<thead>
<tr>
<th>Accession #</th>
<th>Diagnosis</th>
<th>Det†</th>
<th>Host</th>
<th>Locality</th>
<th>Hypogenous sporulation</th>
<th>Epigenous spor'n Stromatal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>'Leaf hair'</td>
<td>'Leaf surface'</td>
</tr>
<tr>
<td>VPRI 14478a</td>
<td><em>P. correicola</em></td>
<td>BCS</td>
<td><em>C. reflexa</em></td>
<td>Grampians</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>VPRI 14826</td>
<td><em>P. correicola</em></td>
<td>BCS</td>
<td><em>C. reflexa</em></td>
<td>N.S.W.</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>VPRI 14416</td>
<td><em>P. correicola</em></td>
<td>BCS</td>
<td><em>C. reflexa</em></td>
<td>Grampians</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>VPRI 14533</td>
<td><em>P. correicola</em></td>
<td>BCS</td>
<td><em>C. reflexa</em></td>
<td>Grampians</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>VPRI 14400</td>
<td><em>P. correicola</em></td>
<td>BCS</td>
<td><em>C. reflexa</em></td>
<td>Grampians</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>VPRI 14301</td>
<td>Both*</td>
<td>BCS</td>
<td><em>C. reflexa</em></td>
<td>Grampians</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>VPRI 14414</td>
<td>Both*</td>
<td>BCS</td>
<td><em>C. reflexa</em></td>
<td>Grampians</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>VPRI 15394</td>
<td>Both*</td>
<td>H-YY</td>
<td><em>C. lawrenciana</em></td>
<td>Wallaby Creek</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>VPRI 14065</td>
<td><em>P. correae</em></td>
<td>BCS</td>
<td><em>C. 'mannii'</em></td>
<td>Cheltenham</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>VPRI 13468</td>
<td><em>P. correae</em></td>
<td>BCS</td>
<td><em>C. reflexa</em></td>
<td>Montrose</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>VPRI 14413</td>
<td><em>P. correae</em></td>
<td>BCS</td>
<td><em>C. reflexa</em></td>
<td>Grampians</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>VPRI 13405</td>
<td><em>P. correae</em></td>
<td>BCS</td>
<td><em>C. reflexa</em></td>
<td>Cathedral Ranges</td>
<td>Present</td>
<td>Present</td>
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<tr>
<td>VPRI 14216</td>
<td><em>P. correae</em></td>
<td>BCS</td>
<td><em>C. alba</em></td>
<td>Canberra</td>
<td>Present</td>
<td>Present</td>
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<tr>
<td>VPRI 15917</td>
<td><em>P. correae</em></td>
<td>VB</td>
<td><em>Correa sp.</em></td>
<td>Cockatoo</td>
<td>Present</td>
<td>Present</td>
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<tr>
<td>VPRI 16410</td>
<td><em>P. correae</em></td>
<td>VB</td>
<td><em>Correa sp.</em></td>
<td>Doncaster</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>VPRI 17425</td>
<td><em>P. correae</em></td>
<td>VB</td>
<td><em>C. aemula</em></td>
<td>Kinglake</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>DAR 49508</td>
<td><em>P. correae</em></td>
<td>BCS</td>
<td><em>C. backhousiana</em></td>
<td>Canberra</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>VPRI 17422</td>
<td><em>P. correae</em></td>
<td>VB</td>
<td><em>Correa sp.</em></td>
<td>Doncaster</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>VPRI 16411</td>
<td>? <em>P. correae</em></td>
<td>VB</td>
<td><em>Correa sp.</em></td>
<td>Doncaster</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>VPRI 14214</td>
<td><em>P. correae</em></td>
<td>BCS</td>
<td><em>C. pulchella</em></td>
<td>Camberwell</td>
<td>Present</td>
<td>Present</td>
</tr>
</tbody>
</table>

*On these specimens epigenous sporulation was diagnosed as *P. correae* and hypogenous sporulation as *P. correicola*.
†Diagnoses were determined by Dr B. C. Sutton (BCS), Dr H-Y. Yip (H-YY) and the present author (VB).
Table 3.2. Means and ranges of scar diameters (μm) of populations of conidia from specimens of *Pseudocercospora* infecting species of *Correa* and diagnosed as *P. correae* or *P. correicola*. Conidia were collected from conidiomata of different types occurring in different locations on the leaf.

<table>
<thead>
<tr>
<th>Accession #</th>
<th>Diagnosis</th>
<th>'Leaf hair' sporulation</th>
<th>Sporulation on surface hyphae</th>
<th>Hypogenous stromata</th>
<th>Epigenous stromata</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>Range</td>
<td>No. of conidia</td>
<td>Mean</td>
</tr>
<tr>
<td>VPRI 14478a</td>
<td><em>P. correicola</em></td>
<td>2.2</td>
<td>1.75 - 2.75</td>
<td>10</td>
<td></td>
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<tr>
<td>VPRI 14826</td>
<td><em>P. correicola</em></td>
<td>2.1</td>
<td>1.75 - 2.75</td>
<td>20</td>
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<td>VPRI 14416</td>
<td><em>P. correicola</em></td>
<td>2.4</td>
<td>2.0 - 2.75</td>
<td>17</td>
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<td>VPRI 14533</td>
<td><em>P. correicola</em></td>
<td>2.5</td>
<td>2.25 - 2.75</td>
<td>6</td>
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</tr>
<tr>
<td>VPRI 14400</td>
<td><em>P. correicola</em></td>
<td>2.6</td>
<td>2.25 - 3.0</td>
<td>5</td>
<td></td>
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<tr>
<td>VPRI 14301</td>
<td>Both*</td>
<td>2.6</td>
<td>2.0 - 3.0</td>
<td>10</td>
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<tr>
<td>VPRI 14414</td>
<td>Both*</td>
<td>2.7</td>
<td>2.0 - 3.5</td>
<td>8</td>
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<tr>
<td>VPRI 15394</td>
<td>Both*</td>
<td>2.6</td>
<td>2.0 - 3.0</td>
<td>12</td>
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<tr>
<td>VPRI 14065</td>
<td><em>P. correae</em></td>
<td>2.8</td>
<td>2.0 - 3.0</td>
<td>28</td>
<td></td>
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<tr>
<td>VPRI 13468</td>
<td><em>P. correae</em></td>
<td>2.8</td>
<td>2.0 - 3.0</td>
<td>28</td>
<td></td>
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<tr>
<td>VPRI 14413</td>
<td><em>P. correae</em></td>
<td>2.8</td>
<td>2.0 - 3.0</td>
<td>28</td>
<td></td>
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<tr>
<td>VPRI 13405</td>
<td><em>P. correae</em></td>
<td>2.7</td>
<td>2.0 - 3.0</td>
<td>5</td>
<td>2.7</td>
</tr>
<tr>
<td>VPRI 14216</td>
<td><em>P. correae</em></td>
<td>2.8</td>
<td>2.25 - 3.25</td>
<td>10</td>
<td></td>
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<tr>
<td>VPRI 15917</td>
<td><em>P. correae</em></td>
<td>2.9</td>
<td>2.5 - 3.25</td>
<td>15</td>
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<tr>
<td>VPRI 16410</td>
<td><em>P. correae</em></td>
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<td>3.0 - 4.0</td>
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<tr>
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<td><em>P. correae</em></td>
<td>3.3</td>
<td>2.75 - 4.0</td>
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<tr>
<td>DAR 49508</td>
<td><em>P. correae</em></td>
<td>2.9</td>
<td>2.5 - 3.25</td>
<td>10</td>
<td></td>
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</tbody>
</table>

*Specimens for which a diagnosis of *P. correae* was made for epigenous sporulation and *P. correicola* for hypogenous sporulation.
Fig. 3.17 Proportion of conidia (%) with maximum widths of 3, 3.25...6 μm from epigenous sporodochia of specimens identified as *P. correae* (77 conidia) and from hypogenous 'leaf hair' sporulation of specimens identified as *P. correicola* (65 conidia).

Fig. 3.18 Proportion of conidia (%) with 0, 1...11 septa from epigenous (sporodochial) and hypogenous ('leaf hair') conidiomata of specimens diagnosed as *P. correae* (76 conidia) and *P. correicola* (58 conidia) respectively.

Fig. 3.19 Proportion of conidia (%) with scar diameters of 1.75, 2.0...4.5 μm from epigenous sporodochia of specimens identified as *P. correae* (82 conidia) and from hypogenous 'leaf hair' sporulation of specimens identified as *P. correicola* (87 conidia).
Fig. 3.20 Conidia from specimens diagnosed as bearing *Pseudocercospora correae* on the upper leaf surface and *P. correicola* on the lower surface (A-D), and from specimens diagnosed as *P. correicola* (E, F).

Hypogenous conidia are all from 'leaf hair' hyphae.

A, VPRI 14414 epigenous
B, VPRI 14414, hypogenous
C, VPRI 14301, epigenous
D, VPRI 14301, hypogenous
E, VPRI 14416, hypogenous
F, VPRI 14400, hypogenous
Fig. 3.21 Conidia from specimens diagnosed as *Pseudocercospora correae*

A, VPRI 14065, hypogenous on 'leaf surface' hyphae
B, VPRI 14065, epigenous
C, VPRI 17422, hypogenous on 'leaf surface' hyphae
D, VPRI 17422, epigenous
E, VPRI 14216, epigenous
F, VPRI 14216, hypogenous on 'leaf hair' hyphae
G, VPRI 15917, epigenous
Fig. 3.22 Conidia from specimens diagnosed as *Pseudocercospora correicola*

The hypogenous conidia are all from 'leaf hair' hyphae

A, VPRI 14826, hypogenous
B, VPRI 14826 epigenous
C, VPRI 14533, epigenous
D, VPRI 15394, hypogenous
E, VPRI 14478, hypogenous
F, VPRI 14533, hypogenous.
3.13 *PSEUDOCERCOSPORA PHEBALII* SP. NOV.

Lesions epigenous, 2-6 mm diam., each comprising a cluster of 1-8(-13) raised, subcircular, yellowish mesas which may have a distinct raised, dark margin and which become red on senescent leaves. 1-6 pale grey-brown erumpent pustules occur in the centre of most mesas. Mycelium immerse, hyaline to light golden yellow, septate, branching, 23 µm diam. Stromata (Figs 6.14-6.16) epigenous, initiated beneath the epidermis but developing mainly within the epidermis, spreading laterally between cells, composed of textura prismatica, reddish to golden brown. Egress erumpent. Conidiophores (Fig. 3.23) sporodochial, golden brown, erect, straight or slightly curved, cylindrical on a swollen basal cell, and often with some swollen cells, unbranched, smooth, 04 septate, 14-55 µm long x 46 µm wide. Conidiogenous cells integrated, terminal, cylindrical or slightly bent, sometimes becoming geniculate towards the apices, terminating in a thin, broad, unthickened scar 2.5-3.5 µm diam. Conidiogenous loci broad, unthickened. Conidia (Fig. 3.23) holoblastic, pale golden olivaceous, smooth, cylindrical but often with one or more swollen cells, straight or slightly curved, 1-3(-6) faintly septate, frequently constricted at the septa, aguttulate, tapering gradually if at all to an obtuse apex and gradually or more abruptly to a truncate base which is often slightly flared with a visible frill, and which bears a broad unthickened scar (2-) 2.5 (-2.75) µm diam., (12-)29(-36) x (4-)4.9(-6) µm.

Provisional type: On living leaves of *Phebalium woombye* Domin., Peregian Beach, Qld., J. L. Alcorn, 16 Aug. 1972, BRIP 8764.

Dr J. Alcorn, who collected the two specimens of *P. phebalii* upon which this description was based, has been unable to find more specimens of this fungus since his second collection in 1973. I am unaware of any other collection. The fungus bears some similarity to *Pseudocercospora correae*, a foliar pathogen of *Correa* which, like *Phebalium*, is a member of the Rutaceae. A large number of specimens of *P. correae* has been examined in detail, but only about 16 conidia and fewer conidiophores of *P. phebalii* have been drawn, and these all came from the same specimen. It is not entirely satisfactory to compare specimens on this basis, but *P. phebalii* appeared sufficiently distinctive to warrant new species status.

Specimens examined

Fig. 3.23 *Pseudocercospora phebalii*, BRIP 8764 Conidia A), conidiophores and conidiophore apices (B); conidiophores with 'floppy' conidium initials (C).
The species *Pseudocercospora eucalyptorum* was erected for a *Cercospora*-like fungus collected from the diseased leaves of eight species of *Eucalyptus* in South Africa (Crous et al., 1989). *Cercospora eucalypti* Cke. & Mass. (Cooke & Massee, 1889) and *C. epicoccoides* Cke. & Mass. (Cooke & Massee, 1891) had been described much earlier from Australian material. However, the type specimens of these two species were found by Sutton to represent two different pycnidial fungi, one considered to be conspecific with *Phaeoseptoria eucalypti* Hansf. emend Walker and the other probably congeneric with it (Crous et al., 1989). Crous et al. (1989) concluded that collections misidentified as *C. epicoccoides* or *C. eucalypti* in South African and other collections (including Australian) belonged in *Pseudocercospora* Speg., and they erected the species *P. eucalyptorum* to accommodate these fungi. In the same year, Guo & Liu (1989) transferred the name *C. eucalypti* to *Pseudocercospora*, and Ray (1989) transferred the name *C. epicoccoides* to *Cercoseptoria* Petrak, in both cases without comment and apparently without having examined the type specimens. Both *Cercospora epicoccoides* and *Phaeoseptoria eucalypti* Hansf. have since been reduced to synonymy with *Kirramyces epicoccoides* (Cooke & Massee) J. Walker, B. Sutton & I. Pascoe (Walker, Sutton & Pascoe, 1992). A number of South African and other collections of *C. eucalypti* and *C. epicoccoides* lodged at CUP bore a cercosporoid hyphomycete which Deighton agreed was closer to *Pseudocercospora* than to *Cercospora*, because both conidiophores and conidia were pigmented and the abscission scars were unthickened (Crous et al., 1989). The latter authors examined 26 specimens from various eucalypt species in Africa, Asia, Australia, Europe, New Zealand, Papua New Guinea and South America, and concluded that they all represented one species which they named *P. eucalyptorum*. They noted that the dimensions of material of mis-determined *C. eucalypti* and mis-determined *C. epicoccoides* overlapped, and that all this material also fitted in the newly erected *P. eucalyptorum*. They reported that the fungus affects a wide range of eucalypts, including 45 species in Italy, and that it is a potentially serious pathogen of cultured *Eucalyptus nitens* in South Africa.

The present study was of 31 specimens of *Pseudocercospora* causing leaf spots on a variety of eucalypts growing in Victoria, Queensland, NSW and New Zealand. Type material of *P. eucalyptorum* (PREM 49112) and another South African specimen (PREM 49111) were also examined. Of the Queensland specimens, BRIP 4578 (IMI 161742) and BRIP 4579 (IMI 151656) consisted of diseased leaves of *E. morrisbyi* Brett. Both were listed (mistakenly as *E. morrisii* R.T. Bak.) by Crous et al. (1989) as examples of *P. eucalyptorum*.

The holotype of *P. eucalyptorum* (PREM 49112) was on elongate, juvenile leaves of *Eucalyptus nitens* Deane & Maid. collected from the Stellenbosch area of South Africa. The leaves were isobilateral, with a double palisade layer and abundant stomata on both sides of the leaf. Sporulation was amphigenous on hologenous, necrotic lesions. The dominant conidiomata were large, sporodochial and erumpent (Crous et al., 1989, Fig. 3). They originated as small, subepidermal hyphal aggregates which extended between the anticlinal walls of the epidermal cells (Fig. 3.51). Lenticular stromata subsequently developed beneath the cuticle which was eventually ruptured (Fig. 3.51), exposing sporodochial conidiomata densely covered with a mat of short conidiophores, many with attached conidia. Stromata were almost colourless when
immature but pigmented at maturity. Erumpent sporodochia were more abundant on one surface of each leaf than the other, in the three leaves examined. As the leaves are pendulous and isobilateral (that is, without morphologically distinct upper and lower surfaces), it seems likely that the sporodochia have formed mainly on the more exposed leaf surfaces.

As well, stomatal sporulation was common on both leaf surfaces. A hyphal knot or small subspherical stroma in the substomatal cavity subtended a narrow column of hyphae which passed between the guard cells without disrupting them and gave rise to very small clusters of short, caespitose conidiophores.

Although a published scanning electron micrograph of the type of *P. eucalyptorum* showed apparently smooth conidia, drawings of the fungus indicated verruculose walls (Crous *et al*., 1989). Conidia were described as smooth in the English description of *P. eucalyptorum*, but conidium wall characteristics were not mentioned in the Latin description. The conidia of *P. eucalyptorum* were, however, referred to in a later publication by Crous & Wingfield (1991) as ‘slightly roughened’. The intent of the authors is thus unclear. In my opinion the conidia and conidiophores of the South African specimens are smooth-walled. I have observed verruculose conidia and conidiophores very infrequently in Pseudocercosporas from Australian eucalypts, and then only in specimens with obclavate conidia.

Crous *et al.* (1989) commented that collections of *P. eucalyptorum* from different parts of South Africa showed considerable variation in symptoms and in conidiophage and conidium morphology. They compared the holotype with a specimen from Knysna (PREM 49111) whose conidiomata, they said, were black and compact on some leaves but grey and less dense on others. PREM 49111 comprised rounded leaves misidentified as a specimen of *E. nitens*, and apparently from a type of box (Prof. P. Ladiges, Botany School, University of Melbourne, pers. comm.). The leaves had a single upper palisade layer, and stomata only on the abaxial surface. Examination of three leaves of this specimen revealed erumpent sporodochial ('black, compact?') conidiomata on both surfaces, similar to those in the holotype of *P. eucalyptorum* but less mature. They developed as in the holotype on the upper leaf surface and possibly by rupture of the epidermis and cuticle on the lower surface. Stomatal egress in the Knysna specimen was necessarily restricted to the abaxial leaf surface. Caespitose stomatal conidiomata were more developed in this specimen than in the holotype, and because the numerous conidiophores often had conidia attached they lent a velutinous appearance to some lesions. The fungi on specimens PREM 49111 and PREM 49112 were conspecific.

Although the erumpent sporodochia were the more conspicuous, and probably the more productive, fruiting bodies present in the holotype, and were illustrated in a scanning electron micrograph, conidiophores of *P. eucalyptorum* were described as ‘aggregated in fascicles emerging through stomata’ (Crous *et al*., 1989). The only Australian specimen exhibiting erumpent, non-stomatal egress was VPRI 15131a, a *Pseudocercospora* quite unlike PREM 49112. In this specimen, numerous small, pigmented, epigenous erumpent stromata developed in the same way as those of PREM 49111, causing long fissures to form in the upper leaf surface.
The Australian specimens vary sufficiently in conidium and conidiophore morphology that it is difficult to see them all as representatives of the already broadly circumscribed genus *P. eucalyptorum*, as intimated by Crous *et al.* (1989). Several Australian specimens resembled the type of *P. eucalyptorum* in producing more or less cylindrical, persistent conidia on more or less cylindrical conidiophores. Broad, unthickened scars were left on prominent pegs following enteroblastic sympodial proliferation of the conidiogenous cell. The persistent conidia were delimited by a conspicuous, broad abscission septum and could become faintly 2-3 septate prior to being shed. Specimens from *Eucalyptus ?globulus* growing in a field trial in Gippsland (VPRI 17498), from *E. ?globulus* growing at Healesville (VPRI 16265) and from two unidentified adjacent eucalypts planted as street trees in the Dandenong Mountains (specimens VPRI 17433, 17441, 17442, 17443, 17445, 17446) were morphologically similar to PREM 49112 but significantly larger in every dimension (Fig. 3.24). For example, scars on conidia from PREM 49112 (mean 2.3 µm) were significantly narrower (p<0.001) than scars on conidia from specimen VPRI 17498 (mean 2.9 µm) (10 conidia in each sample). The variation among a large number of Australian specimens of this morphological type needs to be established before their relationship with PREM 49112 can be discussed.

The other Australian collections examined in this study differed from PREM 49112 in having conidia which are more or less obclavate and in which the basal cell is attenuated towards the scar. One type which stood out as distinctively different from the rest was found in seven collections from at least three species of eucalypt growing in the Brisbane region. These were from *Eucalyptus alba* (BRIP 4584, Fig.3.26), *E. exserta* (BRIP 4578, Figs 3.25, 3.52, and BRIP 8742), *E. morrisbyi* (BRIP 4579 and 4580, Fig. 3.25); and *Eucalyptus sp.* (VPRI 15131a, Fig. 3.26). Another specimen collected north of Townsville (on *E. nicholii*, BRIP 8772) was of the same type. In all these specimens, conidiogenous cells and conidia tapered gradually to very narrow scars (1-1.6(-2) µm in diameter which were often thickened but not conspicuously so. Conidiophores were commonly 3-4 septate, and as much as 7-septate (conidiophores are 1-2-septate in *P. eucalyptorum*). Conidia were pale brown with well-defined septa, thin-walled, fragile, usually short and wide (up to 6 µm), but sometimes longer and narrower. Attached conidia were rarely found, but several were distinctly 1-3-septate although they were not yet delimited by an abscission septum. This order of septum formation, if confirmed in future studies in these and some other *Pseudocercosporas* from eucalypts, differs from that of the type of *P. eucalyptorum* in which a conspicuous conidium-delimiting septum forms prior to conidium septation.

Culture extracts of certain isolates of *Pseudocercospora* from eucalypts were subjected to analysis for alkaline phosphatase isozyme activity at the Victorian Plant Research Institute, Burnley, under the supervision of Dr J. Woodwood (for methods, see Appendix G). Also included were extracts from a *Pseudocercospora* from *Lophostemon confertus* (Myrtaceae) and from *Cercospora beticola*, which was run as a standard in this and several other gels. The specimens were run in the following lanes:

1 *Cercospora beticola*.

2 *E. macrorhyncha*, VPRI 15509 (Type 4*)

3 *E. ?obliqua*, VPRI 17439 (Type 3)
4 *E. morrisbyi*, BRIP 4580 (Type 2)

5 *E. ?obliqua*, VPRI 17447 (Type 3)

6 *Lophostemon confertus*, VPRI 17492.

7: *E. ?globulus*, VPRI 16265 (Type 1).

8: *Eucalyptus ?globulus*, VPRI 17445 (Type 1).

*The eucalypt specimens were typed, for the purpose of this exercise, according to the shape of the conidia (particularly their bases) and the characteristics of their hila, features which are reflected exactly in the conidiogenous cells of the same fungus. These represent only some of the types of *Pseudocercospora* found on eucalypts.*

Type 1 specimens (Figs 3.24, 4.19) have broad, thin scars on more or less cylindrical conidia which are often flared at the base. These specimens resemble the type of *P. eucalyptorum* in shape but not in dimensions.

Type 2 conidia (Figs. 3.25, 4.12, 4.13) are short and wide, with extreme attenuation towards a narrow, slightly thickened scar.

Type 3 conidia (Fig. 3.27A) are often obclavate, but vary somewhat in the shape of their basal cell. Most conidia are barely or gradually attenuated towards the scar, but a small proportion has swollen basal cells which are very abruptly attenuated to a narrow, short, sometimes rostrate base. This precise variation in shape has been found in several other *Pseudocercospora* specimens from eucalypts, including one of *E. regnans* from New Zealand (VPRI 15450).

Type 4 conidia (Fig. 3.26A) are long, obclavate and persistent and are attenuated towards narrow scars which occasionally show external thickening.

The resultant gel is shown in Fig. 3.55. The Type 1 specimens in lanes 7 and 8 produced almost identical profiles, and some bands were unique to those fungi. The Type 3 fungi in Lanes 3 and 5 were also very similar to each other. The Type 4 fungus in Lane 2 produced only 3 bands, 2 of which appear to be unique. Finally, none of the bands produced by the Type 2 fungus in lane 4 were produced by any other of the fungi tested.

The results of this preliminary experiment show that differences in morphology of a small number of eucalypt fungi are reflected in differences in their isoenzyme activity. More specifically, they support the notion that the *Pseudocercospora* from *E. morrisbyi* (Lane 4) is distinctly different from the others. The extract from *Lophostemon confertus* produced faint bands which nearly all differed from those of the various eucalypt isolates.
The same group of fungi was tested for aryl esterase activity, with the *Pseudocercospora* from *E. morrisbyi* again proving distinctly different from the others. Because some enzyme activity had been lost during thawing of the extract, the bands were faint and are not reproduced here. A starch gel run earlier at Melbourne University and stained for acid phosphatase activity in 2- to 3-month, non-sporing cultures of *Pseudocercosporas* from a variety of host genera showed intense bands that differed between isolates.

These results suggest that the application of gel electrophoretic techniques may be useful in at least resolving the status of the various types of *Pseudocercospora* found on eucalypts, which are at present all included in *P. eucalyptorum*. Future success depends on accurate host species identification (not always easy with eucalypts), and on obtaining a culture of the type of *P. eucalyptorum*, or of a validated specimen of the species, from South Africa. We need to determine the host-specificity, if any, of the different morphological types, and their geographical distribution. The fungus on *E. morrisbyi*, for example, which was found on at least 4 species of eucalypt, may be peculiar to Queensland, although not all Queensland specimens were of this type.

Specimens examined

Fig. 3.24 Hypogenous conidia and conidiophores of specimens of

*Pseudocercospora* from eucalypts

These specimens have ± cylindrical conidia with broad, unthickened scars. Similar scars are left on broad pegs after proliferation of the conidiogenous cell.

A VPRI 17498 from *E. ?globulus*, Gippsland
B VPRI 17449 from *E. ?crenulata*, Healesville Sanctuary
C VPRI 17443 from *E. ?globulus*, Dandenong Ranges
D VPRI 16265 from *Eucalyptus* sp., Healesville Sanctuary
E PREM 49112, the type specimen of *Pseudocercospora eucalyptorum*
Fig. 3.25 Hypogenous conidia and conidiophores of specimens of *Pseudocercospora* from eucalypts

Specimens A and B have short, broad conidia which are markedly attenuated towards a narrow scar. Similar narrow scars are left on attenuated pegs after proliferation of the conidiogenous cell. An illustration of conidia of the type specimen of *Pseudocercospora eucalyptorum* (PREM 49112) is included for comparison.

A BRIP 4580 from *Eucalyptus morrisbyi*, Brisbane. Note faint abscission septum (fine arrow) delimiting a conidium which is already strongly 2-septate, and a 1-septate conidium initial which is not yet delimited (broad arrow). Two conidia and two conidiophores (a) are from a culture on V8 agar.

B BRIP 4578 from *Eucalyptus exserta*, Brisbane. Note anastomosing conidia.

C PREM 49112
Fig. 3.26 Hypogenous conidia and conidiophores of specimens of *Pseudocercospora*
from eucalypts. Scar thickening has been observed in all these specimens.

A VPRI 15509 from *Eucalyptus macrorhyncha*, Fraser National Park. Conidia are
long, obclavate and persistent. The abscission septum is sometimes, but not
always, laid down after the onset of conidium septation (arrow indicates the
isthmus where the abscission septum can be expected to develop).

B BRIP 8907 from *Eucalyptus maculata*, Brisbane. The conidia resemble those of the
specimens on *E. morrisbyi* and *E. exserta*, but the conidiophores are often
plumper.

C VPRI 15131a from *Eucalyptus* sp., Brisbane area. Conidia and conidiophores (not
shown) are very like those on the specimens on *E. morrisbyi* and *E. exserta.*
even though the conidia are not as wide.

D VPRI 8741 from *Eucalyptus alba*, Rocklea, Queensland. This fungus, also, is very
like that found on the specimens of *E. morrisbyi* and *E. exserta.*
Fig. 3.27 Hypogenous conidia and conidiophores of specimens of *Pseudocercospora* from eucalypts.

A VPRI 13778 on *Eucalyptus ?regnans*, near Cape Otway. This fungus has long, multiseptate conidia which can be cylindrical with a broad, truncate base or obclavate with a small, swollen basal cell and narrowing to an obconical or even rostrate base. This precise mix of types was see in several other collections.

B DAR 28129 on *Eucalyptus delegatensis*, East Gippsland.
Fig. 3.28 Conidia of *Pseudocercospora loranthi*. SEM Bar, 40 μm

Fig. 3.29 External conidiophores of *Pseudocercospora kennediacola*. Two conidiophores display enteroblastic pseudopercurrent proliferations (arrowed), and a probable displaced scar is indicated by the arrow in the upper one. SEM Bar, 5 μm.

Fig. 3.30 Angular lesions on the abaxial surface of a leaf of *Kennedia* sp. (VPRI 17461) infected with *Pseudocercospora kennediacola*.

Fig. 3.31 A subcuticular sheet of hyphae in a leaf of *Amyema pendulum* infected with *Pseudocercospora loranthi*. The hyphae are out of the focal plane at the boundaries of some epidermal cells because the cuticle extends down between the cells. NIC
Figs. 3.32, 3.33 *Pseudocercospora platylobii*

3.32 Dense clusters of conidiophores emerging from the stomata, many with attached conidia. A fringe of torn epidermis and cuticle surrounds one conidioma (arrowed). TEM Bar, 100 μm

3.33 Portion of a hypogenous conidioma, showing a group of persistent, acicular conidia (white arrow), a conidiogenous cell from which a conidium has been shed leaving a broad scar (fine black arrow) and the constriction at the base of a small conidium initial (medium black arrow). SEM Bar, 20 μm
Figs. 3.34-3.39 *Pseudocercospora platylobii*. Bars, 20 μm

3.34 A protuberant, erumpent epigenous conidioma, with persistent conidia. BF

3.35 An emerging erumpent epigenous conidioma with a piece of broken cuticle lying on top. BF

3.36 A young hypogenous stomatal conidioma. BF

3.37 A mature hypogenous stomatal conidioma, showing the truncate ends of conidiogenous cells after conidium abscission. PC

3.38 Two hypogenous conidiomata whose walls have merged. They have originated as separate fruiting bodies, and their cavities are separated by a normal thick, dark wall. Conidiophore bases can be seen at the top of the conidiomata (arrowed). BF

3.39 A hypogenous, multiloculate spermogonium surrounded by a thick, dark wall and bisected by a straight, vertical wall. Conidiophore bases can be seen at the top of the spermogonium (arrowed). BF
Figs 3.40-3.42 Hypogenous conidiomata of *Pseudocercospora pultenaeae*

3.40 Emergence of conidiophores through stomata and by rupture of the leaf surface. SEM Bar, 50 µm

3.41 Caespitose conidiophores emerging from a stoma. Note the lateral conidiophores (arrowed) on several external hyphae. SEM Bar, 50 µm

3.42 Conidiophores emerging from a fissure in the leaf surface. Note the constrictions at the bases of numerous conidium initials (arrows). SEM Bar, 20 µm
Figs 3.43-3.47  Hypogenous conidiomata of *Pseudocercospora pultenaeae*

3.43  Conidiophores and conidia from an erumpent, hypogenous fructification. One conidium has just seceded (arrowed). SEM Bar, 10 μm

3.44  A conidiophore produced laterally on an external hypha, bearing a conidium initial which has been delimited by a septum. SEM Bar, 10 μm

3.45  Conidium initials on conidiophores emerging through a stoma. SEM Bar, 10 μm.

3.46  Erumpent emergence of an epigenous stroma. BF Bar, 40 μm

3.47  A fascicle of conidiophores emerging from a stoma. BF Bar, 40 μm
Figs 3.48-3.50 *Verrucisporota daviesiae*. BF Bars, 20 μm

3.48 A young conidioma exhibiting stomatal egress.

3.49 An asccarp.

3.50 A young conidioma and a spermogonium occupying adjacent stomata. A hypha (arrowed) with an irregular (bubbly') coating is seen near the spermogonium.
Fig. 3.51 A developing erumpent conidioma in the type specimen of *Pseudocercospora eucalyptorum*, PREM 49112. Stroma initiation is subepidermal, but most of the stroma development is subcuticular. BF Bar, 20 μm

Fig. 3.52 Conidiophores of the *Pseudocercospora* on *Eucalyptus exserta*.

This is a negative image printed directly from a colour transparency. NIC Bar, 10 μm

Fig. 3.53 Conidia of *Pseudocercospora correae* produced on the prolific hypogenous external mycelium of specimen VPRI 17421. SEM Bar, 10 μm

Fig. 3.54 External conidiophores of the *Pseudocercospora* on *Eucalyptus* sp., specimen VPRI 17444. The conidiophore on the right has developed enteroblastically and shows one enteroblastic pseudopercurrent proliferation. The other conidiophore may have developed holoblastically. SEM Bar, 2 μm
3.55 Alkaline phosphatase activity in cultures of 6 specimens of *Pseudocercospora*
from eucalypts and one from *Laphostemon confertus*, and in a culture of
*Cercospora beticola*. (See pp. 94-95 for details)
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Chapter 3

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