Chapter 6

Stroma formation and mode of egress

6.1 INTRODUCTION

Barriers to the emergence of leaf pathogens include the cuticle-coated epidermis, which comprises a continuous sheet of cells interrupted only by specialised structures such as leaf hairs and naturally occurring openings such as stomata. It is not surprising that the conidiophores of many cercosporoid and other leaf pathogens utilise stomata as sites of egress, as emergence through natural openings probably requires the least energy (Aist, 1981). Stomatal egress was at one time regarded as a characteristic of the genus *Pseudocercospora* (Deighton, 1976), but most other recent authors omitted the character 'mode of egress' from their generic descriptions (Ellis, 1971; Carmichael *et al*., 1980; Pons & Sutton, 1988), either in recognition of its diversity or else because they regarded it as a host-dependent character. Published descriptions of cercosporoid fungi often fail to include details of stroma position (other than Deighton's traditional 'substomatal') or mode of egress (other than stomatal). Furthermore, the pattern of development of conidiomata in amphigenously sporulating species of *Pseudocercospora* has rarely been described separately for upper and lower leaf surfaces, even though the abaxial surface is more likely to be stomatophorous (stoma-bearing) and hairy, and the adaxial surface often has the thicker cuticle.

Of the 55 *Pseudocercospora* species described by Deighton (1976) in his major publication on that genus, sporulation was said to be amphigenous in 38 species, hypogenous in 16 species and epigenous in one. This means that 54 of the 55 species exhibited some hypogenous sporulation and epigenous sporulation occurred in 39 species. One must wonder whether the epigenous sporulation on all of these 39 hosts was actually stomatal, given that many plants have only abaxial stomata (Esau, 1977). It is possible that alternative strategies for egress were available to some of these pathogens, enabling their conidiophores to emerge other than through stomata. Deighton referred to *Pseudocercospora guazamae* which was originally given two names by Sydow, the densely fasciculate, epiphyllous caespituli being described under the name *Cercospora guazamae* and the hypophyllous, effuse, non-fasciculate caespituli being described under the name *C. flocculosa*. Deighton mentioned several other species of *Pseudocercospora* with a different type of growth on the opposing leaf surfaces, but did not seek an explanation for this variation in habit.

In contrast, Evans (1984) was well aware of the two types of conidiomata formed by *Cercoseptoria pini-densiflorae* (syn. *Cercospora pini-densiflorae*) on *Pinus oocarpa*. He described one type as substomatal and emerging through the stomata, the other as deep-seated and rupturing the epidermis. In other recent publications the amphigenous stromata of the type species of *Pseudocercospora, P. vitis*, were described as subepidermal (Pons & Sutton, 1988) and those of *P. correae* as epidermal (Sutton *et al*., 1987). Similarly, Verma *et al*., (1989) described the amphigenous stromata of *Pseudocercospora caryae* (T. S. & K. Ramakrishnan) Verma, Khan & Kamal and *P. samuhabeeja* Verma, Khan & Kamal as subepidermal. The
amphigenous stromata of *P. symphyti* Goh & Hsieh were said to be substomatal or erumpent (Goh & Hsieh, 1989).

Such details regarding the location of stromata are, unfortunately, all too rare in the literature on cercosporoid fungi, and one notes even in the above examples the unstated assumption that mode of egress is the same from both leaf surfaces. While this may be the case in some instances it is almost certainly not in others. To further complicate matters, illustrations of conidiomata are commonly not labelled according to their position in the leaf (epigenous or hypogenous). Other inconsistencies also creep in. For example, the value of the admirably detailed description of *P. caryae* (Verma, Khan & Kamal, 1989) is diminished by the accompanying illustration being labelled as showing 'emergence of conidiophores through a stoma', a statement seemingly in conflict with the text ('stromata subepidermal'). The reader is left unsure of both the position of the stroma and the mode of egress of the pathogen. Similarly, the reader has no way of knowing in which surfaces the erumpent and substomatal stromata of *P. symphyti* occur (Goh & Hsieh, 1989), because the host surfaces were not labelled and the cellular structure of the leaf was not indicated in the accompanying illustration. These few examples show that even where reasonably detailed descriptions have been provided, stroma position and mode of egress have often been inadequately described.

Preliminary investigations of some Australian specimens revealed considerable variation in how and where stromata develop and how egress occurs. The results of an investigation into these characteristics in a range of *Pseudocercospora* species is presented in this chapter.

In some of the following descriptions a judgement is made on whether a pathogen is breaching a host wall or cuticle principally by mechanical or chemical (enzymatic) means. In discussing possible ways in which the cuticle can be penetrated during infection by pathogenic fungi, Kolattukudy (1985) referred to the interpretation of evidence from ultrastructural studies: 'If the cuticle and/or cell wall at the point of penetration is depressed inward, the penetration is considered accomplished by physical force. Lack of such depression and the appearance of digestion on the cuticle are taken as evidence to support an enzymatic penetration. Obviously this type of evidence is indirect and cannot be conclusive'. The same reasoning is applied here to the ultrastructural appearance of host cell walls and cuticle during egress. Sections viewed with the light microscope also provided useful information in this regard.

### 6.2 MATERIALS AND METHODS

Fresh and dried specimens were sectioned by hand, mounted in lactic acid and warmed to dispel air bubbles prior to examination under Nomarski interference contrast and bright field optics. Some freshly collected specimens were prepared for examination by ultrastructural means. The specimens examined by transmission electron microscopy and the method of their preparation are given in Appendices C and E, respectively. Specimens examined by scanning electron microscopy, and the method of their preparation, are listed in Appendices D and F, respectively.
6.3 OBSERVATIONS

An impression of the range of strategies which has evolved in species of *Pseudocercospora* to facilitate sporulation on host surfaces is gained from the descriptions of the following nine pathogens, each of which occurs on a different host genus. The presence or absence of stomata on the leaf surfaces of each host is recorded.

### 6.3.1 *Pseudocercospora clematidis* on *Clematis aristata*

Sporulation of *P. clematidis* on leaves of *Clematis aristata* was entirely stomatal. Stromata were initiated in, and developed entirely within, substomatal cavities, fascicles of conidiophores emerging through the stomata without disrupting the guard cells (Fig. 6.18). Stomata were abundant on the abaxial leaf surfaces of all specimens examined but lacking on abaxial surfaces. Intercellular hyphae occupied the spongy and palisade mesophylls of mature lesions but failed to penetrate the upper epidermis.

### 6.3.2 *Pseudocercospora* sp. on *Solanum* spp.

The *Pseudocercospora* on *Solanum* spp. is, like *P. clematidis*, entirely dependent on stomatal egress, and sporulation is prolific on the abaxial leaf surface. However, some *Solanum* specimens have a small number of adaxial stomata, which leads to sporulation being almost entirely hypogenous but occasionally amphigenous. Again as in *P. clematidis*, the spongy mesophyll and palisade mesophyll were colonised by hyphae but the upper epidermis was not breached. Several small aggregations of hyphae resembling stroma initials were seen abutting the upper epidermis in a densely sporulating lesion in specimen VPRI 17499, but the epidermis was unaffected and epigenous sporulation in this specimen was limited to a few caespituli emerging from stomata overlying the mid-vein.

### 6.3.3 *Pseudocercospora platylobii* on *Platylobium formosum* and *P. obtusangulum*

Sporulation of *Pseudocercospora platylobii* on *Platylobium* was amphigenous on hologenous lesions, the distribution of conidiomata varying from largely hypogenous to largely epigenous. *Platylobium* leaves had abaxial stomata only.

#### 6.3.3.1 Hypogenous sporulation

Because the stomata were obliterated by the expanding hypogenous stromata of *P. platylobii*, stroma position was determined by the examination of immature fructifications.

Stroma initials formed consistently in substomatal cavities (Figs 6.17), but not all cavities were occupied. The expanding, immersed stromata initially resembled leaden grey blisters on the leaf surface. In transverse section, immature stromata appeared irregular because they extended between neighbouring mesophyll cells which were eventually engulfed and incorporated into the mature stromata (perhaps more correctly termed 'pseudostromata') (Fig. 6.1). Guard cells were forced apart by the expanding stromata, and the stomata were eventually ruptured, leaving a fringe of peeled-back epidermis and cuticle around each
conidioma (Fig. 3.32). At this stage, egress could hardly be termed stomatal (as in *P. clematidis* and the *Pseudocercospora* on *Solanum*), as the stomata were completely disrupted and the conidiophores were borne on largely protuberant, erumpent conidiomata. However, the term 'stomatal egress' comes closer than any other to categorising the close spatial relationship between stroma and stoma in this fungus.

6.3.3.2 Epigenous sporulation

Epigenous stromata were initiated directly below the upper epidermis, between the palisade mesophyll cells (Fig. 6.1). Hyphae grew between the anticlinal walls of the epidermal cells until they reached the cuticle. The pattern of events then varied, but always involved the development of substantial stromata. If the cuticle remained attached to the epidermis, the bulk of the stroma developed between the palisade mesophyll and the epidermis. In other instances, a wedge of hyphae grew beneath the cuticle, separating it from the epidermis which was squashed down onto the palisade mesophyll (both types of development are evident in one stroma in Fig. 6.1). In the course of both epigenous and hypogenous stroma development the epidermis was often separated from both the palisade mesophyll and the cuticle. The individual epidermal cells then became completely surrounded by hyphae (Fig. 6.1) and incorporated into a pseudostroma which extended from the palisade mesophyll to the cuticle. These trapped host cells stained a distinctive pink in toluidine blue, in thick (2 µm) sections through mature resin-embedded conidiomata.

Epigenous egress was finally effected by rupture of the cuticle, fragments of which remained on the surfaces of some exposed stromata (Fig. 3.35).

6.3.4 *Pseudocercospora kennediicola* on *Kennedia* spp.

Sporulation of *P. kennediicola* was amphigenous on hologenous lesions; *Kennedia* leaves had stomata only on their abaxial surfaces.

6.3.4.1 Hypogenous sporulation

*P. kennediicola* proved to be far less dependent on stomata for hypogenous sporulation than were *P. platylobii*, *P. clematidis* and the *Pseudocercospora* on *Solanum*. Although small aggregations of hyphae formed in the substomatal cavities of immature lesions, and caespitose conidiophores (Fig. 6.2A) protruded from many stomata, transverse sections demonstrated beyond doubt that stromata were initiated more or less equally in the substomatal cavities and beneath the epidermis. Substomatal stromata gave rise to sporodochial conidiophores which soon ruptured the stomata through which they emerged, while subepidermal stromata ruptured the epidermis and cuticle and developed into sporodochial conidiomata (Fig. 3.3) which were soon indistinguishable from those originating in the stomata. Fig. 6.2B shows egress occurring adjacent to a stoma. The fragmented host cells visible among the fungal cells, the guard cell trapped between the two groups of emerging conidiophores, and the ruptured epidermis and cuticle on the side of the stroma away from the stoma, demonstrate that egress was erumpent. Yet another section (Fig. 6.2C) shows a stroma which was partly subepidermal and partly substomatal. Several
conidiophores had penetrated the host cuticle (and possibly also the epidermis) next to the stoma, while others had emerged through the stoma.

*P. hardenbergiae* and the *Pseudocercospora* on *Hakea* behave similarly to *P. kennediicola* at the stomatophorous surfaces of their hosts. The location of hypogenous stroma initials of *P. hardenbergiae* in relation to host stomata is shown in Fig. 6.3. The various modes of egress exhibited by the *Pseudocercospora* on *Hakea* illustrate a high level of adaptability and lack of specialisation with regard to egress (Figs 6.7-6.11).

6.3.4.2 Epigenous sporulation

Stromata were initiated beneath the upper epidermis. From there, hyphae grew between the anticlinal walls of adjacent epidermal cells (Fig 6.2D). There they aggregated into wedge-shaped intercellular stromata extending from relatively deep in the palisade mesophyll right through to the cuticle, which became stretched over a compact, domed stroma prior to its eventual rupture. There was also some evidence of stroma development beneath the epidermis and subsequent erumpent egress by simultaneous rupture of the epidermis and cuticle.

The pattern of development of *P. hardenbergiae* stromata was similar to that of *P. kennediicola*.

6.3.5 *Pseudocercospora loranthi* on *Amyema pendulum*

Leaves of the mistletoe *A. pendulum* are isobilateral and stomata occur equally on both surfaces. While the pathogen utilised stomata for sporulation, conidioma development in *P. loranthi* was found to vary more than in the fungi described above. Sections through actively sporulating, mature lesions revealed large, erumpent sporodochia bursting through the leaf surface but few clues as to how the stromata had developed. Serial 2 µm thick sections of resin-embedded, very young lesions and tangential sections of fresh immature lesions provided the necessary information.

During the earliest stages of host colonisation, a sheet of pigmented hyphae spread beneath the epidermis. From there, hyaline, frequently lobed, intercellular hyphae ramified through a localised area in the spongy mesophyll, inducing hyperplasia in the host cells. By the time the mycelium had extended two or three cells deep into the leaf tissue, and a similar distance to each side, intercellular hyphae began to penetrate the epidermis by growing between the periclinal walls (Fig. 6.4A, B) and started to spread beneath the cuticle. Substantial sheets of much-branched, septate hyphae soon developed beneath the epidermis and between the epidermis and cuticle, and hyphae also aggregated in many substomatal chambers. Fructifications were initiated in all three locations, and egress occurred as described below.

6.3.5.1 Stomatal egress

Small, pigmented knots of hyphae in the substomatal cavities gave rise to caespitose conidiophores which emerged through the stomata (Fig. 3.13). These were the first fructifications to emerge. The stromata grew to a moderate size within the substomatal cavities, and the conidiophores became very numerous.
6.3.5.2 Egress from subepidermal stromata

In the very young lesion, subepidermal stromata emerged by rupture of the epidermis and cuticle and developed into substantial sporodochial conidiomata. Adjacent sporodochia readily merged.

6.3.5.3 Egress from erumpent subcuticular stromata

In places, the single-layered, flattened sheet of subcuticular hyphae which spread over much of each lesion (Fig. 3.28) developed into a stroma two or three cells thick. Conidiophores developed from the upper cells, and the expanding stroma burst the overlying cuticle whose thin, stretched nature (Fig. 6.4C) suggests that it may have been weakened enzymatically.

By interrupting the spread of the subcuticular mycelium, stomata occasionally stimulated the development of subcuticular stromata over subsidiary cells. An inconspicuous fringe of conidiophores then emerged where the guard cells bordered the subsidiary cells.

Over time, the subepidermal and subcuticular hyphal aggregations substantially increased in mass, became confluent, and contributed to the raised nature of the lesions by their sheer bulk (the hyperplasia induced in the host tissues was the other contributing factor). Large masses of subepidermal hyphae caused the epidermis and cuticle to rupture, resulting in the production of confluent sporodochia which emerged from substantial rifts in the leaf surface (Fig. 3.13).

In addition, occasional individual conidiophores were produced on exposed, previously immersed hyphae after pieces of epidermis flaked off old, weathered lesions.

6.3.6. *Pseudocercospora correae* on *Correa* spp.

Sporulation was amphigenous on hologenous lesions, but varied from mostly epigenous to mostly hypogenous. All *Correa* specimens had abaxial stomata only. Leaves were sometimes glabrous, but more commonly the undersurfaces were covered with a sparse to dense layer of branched hairs.
6.3.6.1 Hypogenous sporulation

Three types of hypogenous sporulation commonly occurred, often concurrently. Sporulation was initially stomatal, with conidiophores, external hyphae or both emerging through the stomata (Fig. 3.15).

(a) Direct stomatal sporulation Small fascicles of conidiophores borne on small substomatal knots of hyphae emerged from many or most stomata of certain specimens (Fig. 6.5).

(b) Sporulation on external hyphae In other specimens, the small substomatal knots of hyphae each produced one or several hyphae which emerged from the stomata and then grew across the leaf surface or climbed in the leaf hairs, bearing simple short, erect conidiophores at intervals. A mixture of caespitose conidiophores and external hyphae could develop from the same substomatal hyphal knot. External sporulation was present to a variable extent. Specimens earlier diagnosed as *P. correicola* (see Chapter 3.12) exhibited profuse sporulation among the leaf hairs, and the Doncaster Gardens specimens (VPRI 16410, VPRI 17411, VPRI 17421, VPRI 17422, VPRI 17423 and VPRI 17504) exhibited similarly prolific external growth on the leaf surface. External hypogenous sporulation ranged from sparse to relatively prolific on other *Correa* specimens.

(c) Sporodochia Many specimens on *Correa*, including the holotype of *P. correae* (VPRI 14065), formed numerous large hypogenous sporodochia. Stromata were variously substomatal or subepidermal in origin. As already mentioned, substomatal stromata initially produced small fascicles of conidiophores which emerged through the stomata. These stromata often increased in bulk and broadened, extending beneath the epidermis to some extent and finally bursting through the epidermis and cuticle near the stoma, where they developed into sporodochial conidiomata (Fig. 6.13). Other stromata were initiated beneath the epidermis. These were necessarily erumpent and the resulting conidiomata were again sporodochial. The precise modes of development and egress of hypogenous subepidermal stromata were difficult to determine, but there was some evidence of chemical wall penetration resembling that described below for the epigenous stromata of the same fungus.

In summary, hypogenous stromata formed both in the substomatal cavities and beneath the epidermis, and egress was either stomatal, erumpent or both at once from the same stroma.

6.3.6.2 Epigenous sporulation

The assimilative hyphae of *P. correae* were at first restricted to the lower epidermal region and the spongy mesophyll. At a later stage, hyphae worked their way between the cells of the palisade mesophyll and spread between the palisade layer and the epidermis, where they formed compact aggregations. From each of these, a front of hyphae usually passed directly through the lower epidermal wall into an epidermal cell, aided by chemical dissolution of the cell wall.

Once within the epidermal cell, the hyphae multiplied to form a pseudoparenchymatous mass. Even before the lumen was filled, however, hyphae began dissolving a passage through the outer epidermal wall (Fig.
6.19), emerging beneath the cuticle. There the hyphae multiplied further (Fig. 6.20) causing the cuticle to bulge and eventually rupture. Mature stromata of *P. correae* extended from about a third of the way down in the palisade mesophyll to beneath the cuticle. Although each epigenous stroma resulted from the invasion of a single epidermal cell, adjacent stromata often eventually merged to form large erumpent stromatic masses.

Some epigenous stromata may have breached the epidermis by means of intercellular hyphae. The mature stromata again extended from beneath the epidermis to the subcuticular region. Whether they remained intercellular or extended into the epidermal cells was not determined but again the thick outer epidermal wall was penetrated by an advancing front of hyphae which dissolved their way through.

The thick, outer epidermal wall, and perhaps also the inner epidermal wall, was actually colonised by *P. correae*, and a substantial part of the mature stroma could be described as intramural.

**6.3.7 Pseudocercospora phebalii on *Phebalium woombye***

The genus *Phebalium* belongs with *Correa* in the family Rutaceae, and leaves of *P. woombye*, like those of some *Correa* species, have densely hairy undersurfaces. Again as in *Correa*, stomata are restricted to the abaxial surfaces. Lesions were hologenous but only epigenous sporulation was observed in the two available herbarium specimens.

**6.3.7.1 Epigenous sporulation**

The striking feature of the stroma of *P. phebalii* was its radial spread within the upper epidermis. The invasion of probably a single epidermal cell resulted in the formation of a large stroma occupying a group of cells. Figures 6.6 and 6.14-6.16 illustrate the lateral spread of *P. phebalii* between epidermal cells and the development of intraepidermal stromata which eventually packed the infected cells. The subepidermal aggregation of hyphae, which can be regarded as the stroma initial and which enables the initial invasion of the epidermis, is seen only in sections through the centre of the conidioma. Expansion of the numerous intraepidermal stromata which constitute the single conidioma eventually causes concurrent rupture of the outer epidermal cell wall and cuticle. The shape of the exposed stromata reflects their intra-cellular origin (Fig. 6.16).

**6.3.8 Pseudocercospora pultenaeae on *Pultenaea daphnoides***

Sporulation of *P. pultenaeae* was amphigenous on hologenous lesions. Stomata were restricted to the abaxial surfaces of the host leaves.

**6.3.8.1 Hypogenous sporulation**

Initially, sporulation was confined to the abaxial leaf surface. In the early stages of infection, the emergence of fascicles of branched conidiophores from most stomata (Fig 3.10, 3.47) in the infection court resulted in the formation of a velutinous mat of conidiophores. Intercellular mycelium was sparse at this
stage, and the leaf was still green or only mildly chlorotic. The first-formed conidiophores arose directly from a few pale substomatal hyphae which soon developed into small pigmented substomatal stromata (Fig. 3.47). Later, small external stromata developed immediately outside the stomata, and these bore the conidiophores of the mature conidiomata. In addition, occasional conidiophore branches, or hyphae emanating from the base of stromata, developed into lax, external hyphae bearing short conidiophores along their lengths (Fig. 3.44).

Not all hypogenous sporulation was stomatal, however. Tangential sections of some specimens showed stroma initials which were partly or wholly subepidermal, and erumpent conidiomata whose egress was by rupture of the epidermis and cuticle. There was some evidence that intraepidermal and even subcuticular stromata also developed. Erumpent conidiomata formed later than stomatal conidiomata, after the internal mycelium had increased considerably in mass. Hypogenous sporulation was predominantly stomatal in some leaves, while in others it was predominantly erumpent or a combination of the two.

Massive eruptions of conidiophores from fissures in the leaf undersurface were also occasionally seen. An example of this and of stomatal egress is seen in Fig. 6.12.

6.3.8.2 Epigenous sporulation

Once the spongy mesophyll was heavily colonised, intercellular hyphae gradually penetrated the palisade mesophyll and hyphal aggregations became evident beneath the epidermis. These represented the sites of penetration of epidermal cells which then became packed with hyphae (Fig. 3.46). Erumpent egress occurred when the epidermal cells split and the outer epidermal cell wall and cuticle were lifted by the expanding stroma. It is not certain whether the stroma spread from cell to cell within the epidermis as in *Pseudocercospora phebalii* on *Phebalium woombye*, or whether each colonisation of an epidermal cell derived from a separate invasion. Observations suggested that the behaviour of the fungus could vary in this respect.

Some hyphae passed between, rather than into, the upper epidermal cells, and subcuticular sheets of hyphae were observed in several specimens. It is possible that subcuticular stromata also develop, as they do in *Pseudocercospora loranthi*.

Erumpent masses of conidiophores arising from large or merged stromata commonly emerged from long fissures in the upper as well as the lower leaf surface.

6.3.9 *Pseudocercospora uluruensis* on *Santalum lanceolatum*

Sporulation was amphigenous on amphigenous lesions. The leaves of *S. lanceolatum* are well endowed with stomata on both surfaces, although the stomata are more plentiful on the abaxial surfaces. The leaves are notable for the small volume of their intercellular spaces which, along with the sunken nature of their stomata, is typical of plants growing in arid habitats (Esau, 1977).
The pathogen behaved similarly on the two leaf surfaces. The leaf tissue was occupied by an extensively branched intercellular mycelium, and in immature lesions, small, hyaline stroma initials were present in many substomatal cavities. Each stomatal cavity was later completely filled with a hyaline to pigmented stroma from which a small number of pale conidiophores passed between the guard cells without disrupting them (Fig. 3.11).

The stomatal conidiomata were still present and unchanged in mature, heavily sporulating lesions, but they were relatively insignificant compared with the larger, erumpent, irregularly pigmented sporodochial fructifications which developed later. The sporodochial conidiomata (Fig. 3.11) were initiated as hyphal aggregations beneath the epidermis, from where hyphae penetrated the epidermal cells. Darkly pigmented pseudoparenchymatous stromata often filled a cluster of contiguous epidermal cells, either through separate infections or by lateral spread. Erumpent egress was by rupture of the outer epidermal cell wall and cuticle. The erumpent conidiomata and their conidiophores were usually more heavily pigmented than their stomatal counterparts. In addition, the stomatal conidiophores very often bore persistent conidia, making abscission scars hard to find, whereas sporodochial conidiophores often terminated in a scar.

6.4 DISCUSSION

In redescribing the genus *Pseudocercospora*, Deighton (1976) stated that conidiophores were 'aggregated in fascicles emerging through the stomata and/or produced terminally and laterally on extended mycelial hyphae.' Later authors (Ellis, 1971; Carmichael et al., 1980; Pons & Sutton, 1988) omitted details of egress from their descriptions of *Pseudocercospora*, and still others (e.g. Sutton et al., 1987; Sutton & Pascoe, 1988; Verma et al., 1989) published descriptions of *Pseudocercospora* species in which they described egress which was other than stomatal.

My investigations show that in some species of *Pseudocercospora* egress can be regularly non-stomatal even on stomatophorous leaf surfaces. Modes of egress displayed within even this small group of Pseudocercosporas are more diverse and complex than previously recognised. All modes of egress mentioned by Aist (1981) as occurring in the closely allied acervular fungi, other than the formation of egress pegs by intraepidermal hyphae, occurred in at least one of the species under study.

*Pseudocercospora* species adopt both stomatal and erumpent egress, and both modes of egress are often employed by the one fungus. For example, conidiomata of the type species, *P. vitis*, are stomatal on the lower leaf surface and erumpent on the upper surface, which lacks stomata. Furthermore, there are subtle variations in the mode of development of conidiomata in these fungi which have not been recognised by previous workers.

Stomatal egress can, by definition, occur only from stomatophorous leaf surfaces. In the hosts under study, abaxial leaf surfaces were always stomatophorous, while adaxial stomata were absent, rare or common depending on the host species or, as in *Solanum*, on the individual specimen. Erumpent egress can occur from both sides of the leaf.
Stomatal egress from a relatively small substomatal stroma or knot of hyphae was common in the species of *Pseudocercospora* examined in this study. Its occurrence, however, did not preclude the possibility of the development of erumpent conidiomata by the same fungus on the same surface, either at the same time or later, or on the non-stomatophorous surface.

The degree of dependence on stomatal egress varies between species of *Pseudocercospora*. At one end of the spectrum are *P. clematidis* and the *Pseudocercospora* species on *Solanum* spp. and *Acacia genistifolia*. *Cercosporidium duboisiae* also falls into this category. For these fungi, stomata provide both the stimulus for conidioma initiation and the sole means of egress for conidiophores, without disruption of the guard cells.

Sporulation such as this, which is completely dependent on the presence of stomata, can be termed obligate stomatal sporulation, and the emergence of the conidiophores can be regarded as obligate stomatal egress.

At the other end of the spectrum are *Pseudocercospora hardenbergiae*, *P. kennediicola* and the *Pseudocercospora* on *Hakea*, in which hypogenous stromata are apparently initiated more or less randomly beneath the epidermis, including within substomatal cavities. Emergence of these stromata can be erumpent, stomatal or both. Egress from subepidermal stromata which are not in close proximity to any stoma is necessarily erumpent. A subepidermal stroma in close proximity to a stoma may extend into the substomatal cavity and produce a few stomatal conidiophores, but the bulk of the stroma usually becomes erumpent. Similarly, although the first conidiophores produced on substomatal stromata usually emerge through the stomata, these stromata frequently expand beyond the substomatal cavity and eventually rupture the leaf surface nearby. It appears, therefore, that stomata are essential to neither stroma initiation nor conidiophore egress in these fungi, which can be described as exhibiting erumpent egress combined with casual stomatal egress. Hypogenous conidioma initiation in these fungi is essentially subepidermal, with some substomatal initiation occurring either by chance or as a result of weak stomatal influence. Although stomata are utilised when available, there is a strong tendency towards stroma expansion and subsequent erumpent egress.

In these three species, and in *P. correae*, erumpent and stomatal egress often occur concurrently from the same hypogenous stroma. In such cases, relatively few conidiophores emerge from the stoma compared with those forming on the erumpent portion of the stroma. The sporodochial conidiophores on the erumpent stromata have the capacity to produce many more conidia than do the stomatal conidiophores, and consequently they contribute more to the spread and ultimate survival of the pathogen.

The behaviour of the fungi described above should not be confused with that of others in which egress is apparently of the obligate stomatal type, yet in which the mature stroma can be slightly off-set relative to the associated stomata because of some lateral subepidermal development. In such instances stroma initiation is substomatal and egress appears to remain entirely stomatal. Examples are seen in *Pseudocercospora* spp. on *Dioscorea transversa* (DAR 16984) and *Solanum vescum* (DAR 16979) and,
judging from published micrographs, probably also *Pseudocercospora montantiana* Mehrotra on *Pinus kesiya* Royle ex Gord (Mehrotra, 1987).

The hypogenous stromata of *Pseudocercospora platylobii* are unusual in being both large and substomatal in origin, as are those produced by *Verrucisporota daviesiae*. The first-formed conidiophores in both species emerge through intact stomata, but whereas the well-defined stromata of *V. daviesiae* remain fully immersed beneath intact stomata, the enlarging stroma of *P. platylobii* ruptures the stoma and lifts and splits the epidermis and cuticle. The hypogenous sporodochial conidiophores of *P. platylobii* are subsequently borne on the upper cells of a large, protuberant, exposed stroma. Hypogenous egress in *P. platylobii* is, therefore, initially stomatal, and in a sense it continues to be strongly stoma-oriented in that the stoma becomes the weak point facilitating erumpent emergence.

Where hypogenous sporulation is of the obligate stomatal, velutinous type, conidiophores initially emerge from green leaf tissue, and the internal mycelium is relatively sparse. *P. clematidis* and the *Pseudocercospora* from *Solanum* are examples of this type of development. *P. pultenaeae* can also exhibit a well-developed stomatal sporulation phase, given favourable environmental conditions, and if erumpent conidiomata form, they do so later on chlorotic or necrotic tissue in which the immersed mycelium is more developed. The environment probably influences this sequence of events. For example, some *Pultenaea daphnoides* leaves infected with *P. pultenaeae* have a dense velutinous mat of stomatal conidiophores on their undersurfaces, while others have mainly erumpent stromata. An example of the first type was collected in a wet rainforest, while examples of the second type came from drier, more exposed habitats. This suggests that high humidity favours the production of stomatal conidiophores.

In species exhibiting casual stomatal egress (for example, *P. hardenbergiae*, *P. loranthi* and *P. kennisicola*), subepidermal stromata are initiated at about the same time as the substomatal conidiomata. Substomatal stroma formation precedes that of subepidermal stroma in *P. uluruensis*.

The correct interpretation of stomatal egress depends upon careful examination. Many infected leaves exhibit more or less evenly scattered conidiomata on their lower surfaces which can give the impression that sporulation is stomatal, especially as stomatal emergence is often evident in sections through these specimens. It is easy to conclude that in such specimens egress is stomatal and to infer that stomata are essential to egress. While this may often be the case, it must be remembered that subepidermal stromata which will eventually emerge by means of erumpent egress will, in the meantime, form a few stomatal conidiophores if their direction of growth brings them close to a stoma. In species such as *P. hardenbergiae*, *P. kennisicola*, *P. hakeae* and *P. uluruensis*, stomatal emergence appears to be of minor importance and may account for only a small proportion of their sporing capacity.

When mature stromata are small, relatively symmetrical and consistently confined to the substomatal cavity, and when egress is always stomatal, it is likely that stroma initiation has been substomatal and that stomatal egress is obligatory on that leaf surface (another method of egress could operate on the non-stomatophorous leaf surface or later on the same surface). When mature stromata are large, often asymmetrical because of uneven lateral spread, frequently not positioned under stomata, and frequently
erumpent, it is likely that some, at least, were initiated beneath the epidermis. In this case, any association of stroma initiation and egress with stomata is casual, and erumpent egress is likely to be of considerable importance.

While these guidelines are useful, they do not substitute for establishing the positions of stroma initials and their subsequent development in sections of appropriate material. The entire range of developmental stages is often available in different parts of one lesion.

So far, the discussion has dealt with stomatal and erumpent egress from the stomatophorous leaf surface. Some fungi described in this chapter have the ability to form erumpent conidiomata in the non-stomatophorous (adaxial) leaf surface. The way in which they breach the epidermis and cuticle varies between species depending on where stromata are initiated, which host tissues they occupy as they develop, and to what extent mechanical pressure and chemical activity contribute to the passage of the fungus through the host tissues.

Consider the essential features of some of the fungi described earlier. *Pseudocercospora correae* is typified by the ability of its hyphae to dissolve a passage through the anticlinal walls of adaxial epidermal cells. Each stroma extends from beneath the epidermis to beneath the cuticle, and the portion within the epidermal layer is usually, if not always, intracellular.

*Pseudocercospora phebalii* also enters the epidermal cells of its host, but in this case penetration appears to be by physical rather than chemical means. The stroma then spreads laterally from cell to cell within the upper epidermis until a group of contiguous cells is occupied by stromata. Emergence of the conidioma involves the concomitant rupture of the outer epidermal cell walls and cuticle by pressure from the expanding intraepidermal stromata.

Whereas the penetration of an epidermal cell by hyphae of *P. correae* results in the occupation of that cell alone, the same event in *P. phebalii* results in the formation of stromata in a number of epidermal cells. In both these fungi, stroma production was preceded by the aggregation of hyphae beneath the epidermis.

In *P. kennedicola* and *P. hardenbergiae*, epigenous stromata were again initiated below the epidermis. However, these fungi breached the epidermis by means of intercellular hyphae, and the fully developed stroma was intercellular. Nevertheless, the mature stroma superficially resembled that of *P. correae* in that it extended from beneath the epidermis to beneath the cuticle.

The fungi described above all exhibited relatively consistent and well-defined patterns of stroma development and egress. The massive erumpent intercellular stromata of *Pseudocercospora loranthi*, by contrast, appeared to develop more or less indiscriminately within the occupied host tissues. This fungus was the only one found to develop a subcuticular sheet of mycelium over more or less the entire lesion. In places, these subcuticular hyphae multiplied and developed into localised stromata which emerged by rupturing the cuticle, as described earlier for the anamorph of *Venturia inaequalis* (Corlett et al., 1976). This was the only example of subcuticular stroma initiation seen in the fungi under study. It is possible that the primary role of the subcuticular hyphae is nutrient assimilation, and that its reproductive capacity...
is almost incidental. Subcuticular sheets of hyphae were occasionally detected in *P. pulleniaeae*, but stromata were not observed.

The epigenous stromata of *Pseudocercospora platylobii* also vary in behaviour relative to the surrounding host tissues. Depending on where each stroma develops, erumpent egress can involve the rupture of the epidermis and cuticle together or separately, or the rupture of the cuticle alone. The latter occurs when the epidermis has been squashed down onto the palisade mesophyll by a subcuticular stroma, or when individual epidermal cells have been surrounded by intercellular hyphae.

Although the character ‘position of stroma’ is included in many descriptions of cercosporoid fungi, the pattern of stroma development and subsequent mode of egress have been largely ignored. In the present study, problems were encountered when the epigenous stromata of *P. correae*, described by Sutton *et al.* (1987) as epidermal, were compared with those of *P. phebalii*, which appear to be epidermal (more specifically, intraepidermal), but which are distinctly different. Mature epigenous stromata of *P. correae* extend from some distance down in the palisade layer right through the epidermal cell to beneath the cuticle. In the course of their development, the stromata expand more within the epidermal cell lumen than elsewhere, although the epidermal cells are not always completely filled.

The original description of *P. correae* was accompanied by an illustration of what appeared to be a purely intracellular stroma (Sutton *et al.*, 1987). However, re-examination of the original microscope slide shows that the stroma in question had been sectioned near its edge, where it bulged into the epidermal cell. The subepidermal portion of the stroma is visible, but only in a deeper plane of focus.

Although stroma initiation in *P. phebalii* is also subepidermal, the intracellular elements of the stroma are functionally and visually so pronounced that the mature stroma is most usefully described as intraepidermal.

Subepidermal aggregates of hyphae provide the physical and/or chemical potential necessary to facilitate the breaching of the inner epidermal cell walls by *P. correae* and *P. phebalii*. The hyphal aggregate of *P. phebalii* could be thought of as serving no purpose once the epidermal cell is penetrated, because subsequent stroma development is conspicuously intraepidermal. Accordingly, the subepidermal aggregates in *P. phebalii* would be considered separate from the spore-producing stroma, and stroma initiation would be classed as intraepidermal. In *P. correae*, on the other hand, the hyphal aggregate not only facilitates penetration, but also expands to become an obvious, integral part of the mature stroma. Stroma initiation in this case would be regarded as subepidermal. This interpretation is subjective and arguably erroneous. The stroma of *P. phebalii* is so large, and fills so many epidermal cells, that the subepidermal hyphal mass is not often encountered in transverse sections, and when it is it appears insignificant by comparison with the intraepidermal stromatic mass. However, it increases in size along with the rest of the conidioma, and is contiguous with the intraepidermal stromatic masses. If the *P. phebalii* stroma occupied only one epidermal cell, the subepidermal portion would be seen to be not much different to that of *P. correae*. 
Following this line of thought, a stroma initial could be defined as the *first-formed aggregation of hyphae involved in stroma formation which becomes a substantial part of the mature stroma*. Defined this way, the stroma initials of *P. correae* would be described as subepidermal, but those of *P. phebalii* might be regarded as intraepidermal. The subcuticular stromata formed by *P. loranthi* would be regarded as being initiated beneath the cuticle, while epigenous stroma initials of *P. kennediicola* and *P. hardenbergiae* would be considered subepidermal, even though their later expansion includes the production of stromatic tissue beneath the cuticle.

Alternatively, the stroma initial could be defined as the *first-formed aggregation of hyphae which leads to stroma formation, whether or not it becomes a substantial part of that stroma*. According to this definition, the stroma initial of *P. phebalii* would be described as subepidermal like those of *P. correae* and *P. kennediicola*. The subcuticular stroma of *P. loranthi* would still be described as being initiated under the cuticle because the subcuticular sheet of mycelium from which it develops is formed well before stroma formation, its chief function probably being one of nutrient assimilation.

This latter definition is probably the more biologically sound, in that it recognises the specific function of the hyphal aggregates which herald stroma development and requires no subjective ranking of these aggregates according to their perceived contribution to the mature stroma.

How, then, should stroma position be described? It can not be described simply according to where the stroma was initiated except in cases of obligate stomatal egress. As a stroma develops it often occupies several different tissues, such as the mesophyll layers and the epidermis. To describe epigenous stromata of *P. correae*, as subepidermal, for instance, does not present a useful picture to the reader.

Stromata could be classified according to the overall impression they give to the observer, which means they would be classed according to where the bulk of the stroma is situated. In that case, stromata of *P. phebalii* and even *P. correae* could reasonably be termed epidermal. The stromata of *P. correae* can appear epidermal simply because the intraepidermal portion accounts for the bulk of the stroma, probably because a stroma can expand more easily within the lumen of an epidermal cell than it can within the confines of the palisade mesophyll cell or beneath the cuticle, where cell walls and/or cuticle offer resistance. The subepidermal and subcuticular portions of a *P. correae* stroma are nevertheless true components of the total stroma and their existence tells us much about stroma development in this particular fungus. It is clearly more useful to describe the process of stroma formation of *P. correae* in detail, rather than as simply epidermal. Similarly, although we may describe stroma development in *P. phebalii* as intraepidermal, it is important to note where it was initiated, the way in which the fungus enters and exits from the epidermal cell and the way in which egress occurs, because these are relevant to the morphology of the stroma and the biology of the fungus.

Problems in accurately describing stroma or fruiting body position are not confined to the cercosporoid fungi. The acervuli of *Phloeosporella kitajimae*, for example, are clearly subepidermal in origin and development, yet were described as epidermal, presumably because they occupy the region occupied by the epidermis prior to its displacement (Dianese, Medeiros & Santos, 1993).
These examples suggest that there is no satisfactory short cut to the description of the different types of stromata encountered in this study. The position of stroma initiation, how and where the stroma develops, and how egress is effected, are all important aspects of the biology of the fungus and of the relationship it bears to its host.

I believe that the terms 'epidermal stroma' and 'epidermal conidioma' should be applied only to stromata or conidiomata which develop from intracellular hyphae already present in epidermal cells. This application of the term requires the assimilative hyphae of the fungus to be intracellular, which the hyphae of the cercosporoid fungi are decidedly not.

The different modes of development and egress probably reflect differences in the lytic abilities of the various fungi. For example, hyphae of *P. kennediicola* and *P. hardenbergiae* dissolve a passage between the upper epidermal cells, presumably with the aid of pectinases; *P. correae* and *P. correicola* hyphae dissolve a passage through the periclinal walls of the upper epidermis, which suggests the production of cellulases; and there is evidence of cutinase production by *P. loranthi*. Stomatal egress from substomatal stromata requires no lytic action at all.

The passage of hyphae of *P. phebalii* into the epidermal cell and then into adjacent cells appears to be largely or entirely by mechanical means. One could speculate that *P. phebalii* needs this extensive stroma, occupying a number of contiguous host cells, to provide the mechanical force needed to rupture the overlying epidermis and cuticle. In contrast, each stroma of *P. correae* occupies only one host epidermal cell, and its passage through the outer wall is achieved by lysis rather than physical force.

As pointed out by Kolattukudy (1985), enzymatic digestion and physical force are not mutually exclusive, and it is likely that many of the fungi under study utilise both chemical and physical means in effecting egress. For example, although conidiomata of *P. platylobii* can effect egress by what appears to be mechanical rupture of the epidermis and cuticle, its hyphae also commonly ramify between the epidermal cells, a process which may be aided by the action of pectinases.

The degree to which conidioma development and mode of egress are host-related can best be judged by comparing two or more *Pseudocercospora* species on the same host. The foliar pathogens described on *Correa, P. correae* and *P. correicola*, appeared to provide a suitable example of two similar fungi with quite different habits occurring on the same host. Close examination has shown, however, that not only can they not be separated on the basis of habit (see Chapter 3.12), but that they share special features such as the ability to dissolve a passage through the outer epidermal wall. In view of their similarities, it is proposed that *P. correicola* be reduced to synonomy with *P. correae*.

It can be seen that the essential characteristic of each fungus is not where it sporulates, but how it sporulates; that is, how it overcomes host barriers and emerges from stomatophorous and non-stomatophorous leaf surfaces. For example, a pathogen which is capable of only obligate stomatal egress has the potential to be amphigenous on leaves which bear stomata on both surfaces, but must be
hypogenous on leaves which have only abaxial stomata. An example in this study is provided by the *Pseudocercospora* on *Solanum* - whether or not sporulation is amphigenous or hypogenous depends on host leaf surface morphology, which varies between specimens. *Cercospora nigri* var. *microsporae* on *Solanum nigrum* L. was differentiated from *Cercospora nigri* on the same host partly because it was hypophyllous rather than amphigenous (Bhardwaj & Paul, 1987). No reference was made to host morphology or to possible environmental effects. (The other supposed differentiating feature, shorter conidia, was not supported by the measurements provided).

The essential feature of the *Solanum* pathogen is the stomatal nature of its sporulation, the only characteristic in the present context which has biological significance. Similarly, the amphigenous sporulation displayed by *Pseudocercospora correae* and the *Solanum* pathogen does not indicate similarity. In the first, egress is largely erumpent, while in the second it is obligatorily stomatal.

These examples demonstrate that grouping species of *Pseudocercospora* according to whether sporulation is amphigenous, hypogenous or epigenous would constitute an entirely artificial approach to the taxonomy of the group. Once the mode of egress is understood, observations on where the fungus sporulates may have added significance but 'mode of egress' remains the biologically more significant character. Although the character 'mode of egress' is probably inherent, the usefulness of its application to the taxonomy of this group is uncertain because the number of ways a fungus can breach the leaf epidermis and cuticle is limited, and it is inevitable that any one solution must have evolved in the cercosporoid fungi more than once.

Another problem with using 'mode of egress' as a taxonomic tool is that the many subtly different ways in which egress can occur make it difficult to determine the course of events in each case. Just as the morphology of a fungus within its host may be considered taxonomically useless because it is so hard to observe (Chesters, 1968), so the finer details of development and emergence of fungal structures within the host may be of limited use in taxonomy. This should not, however, inhibit the investigator from making the most detailed observations possible. As pointed out in the introduction to this chapter, many inaccuracies and inconsistencies have crept into the literature simply because critical attention has not always been paid to these details. If stroma position and mode of egress are reported, they should be reported accurately.

There is probably no *Pseudocercospora* which does not utilise stomata for egress one way or another. Obligate stomatal egress in *Pseudocercospora*, however, is nearly always associated with small substomatal stromata, caespitose conidiophores, sparse internal mycelium and a significant initial biotrophic phase. *Pseudocercospora platylobii* is unusual in having large stromata associated with stomata, and is hard to categorise. Casual stomatal egress is generally associated with relatively large stromata which can be subepidermal or substomatal in origin, and both stomatal and erumpent conidiomata are formed. By the time erumpent sporulation occurs there is a well-developed internal mycelium and necrosis of the host tissue.
6.5 CONCLUSIONS

*Pseudocercospora* species can initiate stromata in subepidermal, subcuticular or substomatal regions. While some species rely completely on stomata for sporulation (*obligate stomatal egress*), others utilise them to a lesser extent and commonly produce subepidermal stromata even on stomatophorous surfaces (*casual stomatal egress* in conjunction with *erumpent egress*).

Egress at non-stomatophorous leaf surfaces must be erumpent. It occurs in a number of different ways according to where the stroma is initiated (subepidermally or, less commonly, subcuticularly), how the fungus gains access to the epidermis (by direct entry into epidermal cells, or by passage between adjacent cells), and how the fungus effects egress (by rupture of the epidermis and cuticle, by rupture of the outer epidermal cell wall and cuticle, by passage through the outer epidermal cell wall and rupture of the cuticle or by rupture of the cuticle alone after intercellular passage through the epidermis). Rupture of the host epidermis and cuticle can involve chemical dissolution, physical force or a combination of the two.

Cercosporoid stromata can not be simply intraepidermal. Because the assimilative hyphae are intercellular in these species, some build-up of hyphae must occur beneath the epidermis to enable its penetration, and the subepidermal stroma or hyphal knot formed there becomes part of the mature stroma. True epidermal stromata can develop only in fungi with intracellular assimilative hyphae.

*Pseudocercospora* has become a catch-all genus of enormous diversity, and this diversity is largely an expression of variations in the biology of the species within the genus, as well as of marked morphological diversity. A thorough understanding of the biology of the different cercosporoid fungi is essential to our understanding of individual disease cycles and may eventually be applied to the taxonomy of the group. Application of this knowledge requires the collection of reliable information, and to this end I make three recommendations:

Firstly, we must accurately record where stroma initials are formed (are the initials substomatal, subepidermal or subcuticular?), the manner of stroma development (does it develop only where it was initiated, or does it invade other tissues as well, and if so which and by what means?) and the mode of egress (stomatal or erumpent, with details). This must be done separately for each leaf surface. Such information will be easily obtained in some cases but elusive in others. Where there are doubts, these also should be recorded. While it may be tempting to avoid what may seem to be an unnecessarily time-consuming study of sections, when conidium and conidiophore morphology can be quickly determined for these fungi, the rewards gained from a thorough examination of development in the host are worthwhile and, of course, the task becomes easier with practice.

Secondly, all illustrations of fructifications and other fungal parts should be identified as being hypogenous or epigenous. Because fructifications often differ in form depending on where they are produced, the value of illustrations is seriously diminished by inadequate labelling.
Thirdly, the incidence of stomata on adaxial and abaxial leaf surfaces should be recorded for each host, as a matter of course. Without this information we can not fully understand the behaviour of any cercosporoid fungus, whether it sporulates on one or both leaf surfaces.
Fig. 6.1 Stomatal and erumpent egress in *Pseudocercospora platylobii*

A, immersed, immature substomatal stromata; B, epigenous stroma initial developing beneath the upper epidermis and between the palisade mesophyll cells, which are displaced; C, epigenous stroma which is extending beneath the cuticle on the left hand side and beneath the epidermis on the right. The stroma has encircled an epidermal cell which would normally become incorporated into the large, mature stroma; D, hypogenous conidioma which has presumably disrupted the stoma with which it was originally associated and has become erumpent and sporodochial.
Fig. 6.2 Conidioma development in *Pseudocercospora kennediicola* (figures drawn from 2 μm resin-embedded sections)

A  Substomatal stroma initiation followed by stomatal egress

B  Hypogenous subepidermal stroma initiation followed by erumpent egress.

Fragments of host cells (hatched) are seen within the emerging stroma

C  Hypogenous conidiophores emerging through a stoma and through the nearby epidermis and cuticle. A guard cell is trapped between the two fascicles of conidiophores, which developed from the one stroma.

D  The upper epidermis is breached by intercellular hyphae.
Fig. 6.3 Tangential sections of a lesion on the undersurface of a leaf of *Hardenbergia violacea* infected with *Pseudocercospora hardenbergiae*. The outlines of developing stromata are indicated by dotted lines. Stromata are initiated more or less randomly beneath the epidermis.
Fig. 6.4 The development of subcuticular stromata in *Pseudocercospora loranthe*

(figures drawn from 2 μm resin-embedded sections)

A Inter cellular hyphae breach the epidermis, completely encircling many epidermal cells in the process.

B A subcuticular sheet of hyphae develops and epidermal cells collapse.

C The development of a localised subcuticular stroma results in the rupture of the cuticle.
Fig. 6.5 *Pseudocercospora correae* (figures drawn from 2 μm resin-embedded sections)

A Hypogenous conidiophores or external hyphae develop from a small knot of substomatal hyphae and emerge through the stoma.

B In a developing epigenous (crumpent) conidioma, hyphae have extended from beneath the epidermis through an epidermal cell and into the subcuticular region.
Fig. 6.6 *Pseudocercospora phebalii* The development of the epigenous stroma
(figures drawn from 2 µm resin-embedded sections)

A. A few hyphae transected in 3 adjacent epidermal cells are the first sign of stroma development.

B. Direct passage of hyphae from one cell to the next. (Later stages are seen in Figs 6.14-6.16)
Figs 6.7-6.11 Patterns of stroma development in the *Pseudocercospora* on *Hakea*. BF Bars, 20 μm

6.7 A stroma has been initiated beneath the epidermis, and from there hyphae are penetrating two epidermal cells.

6.8 Several epidermal cells are packed with hyphae. Hyphae have not entered a nearby stoma.

6.9 A stroma which appears to be simply sub-stomatal. Sectioned at right angles to this plane of section it could, however, resemble that in Fig. 6.9.

6.10 A stroma which was probably initiated below the epidermis but which, during expansion, entered a substomatal cavity.

6.11 An erumpent stroma forcibly lifting the outer wall of the epidermis and cuticle. The projecting edge of the plant cell seen to the right of the stroma (arrowed) looks very like the edge of one of the guard cells in Fig. 6.9 (arrowed). The stroma appears to have been initiated below the epidermis, and to have developed there rather than in the substomatal cavity, but the stoma may have been the weak point at which fissure could most readily occur.
Fig. 6.12 Hypogenous conidiomata of *Pseudocercospora pultenaeae*
emerging through stomata (medium arrow) and through fissures in the
leaf surface (broad arrow). SEM Bar, 100 μm

Fig. 6.13 Hypogenous conidioma emergence in *Pseudocercospora correae*.
Conidiophores are emerging between the guard cells of two stomata
(black arrows), but other conidiophores arising from the same stroma
are bursting through the side of one stoma in erumpent fashion (black
on white arrow). SEM Bar, 10 μm
Figs 6.14-6.16 Successive stages of development of epigenous stromata in *Pseudocercospora phebalii*. NIC

6.14 Hyphae have filled two epidermal cells and are invading the cells on either side by direct passage through the periclinal cell walls. Bar, 20 μm

6.15 Pressure from the expanding stroma has ruptured the outer epidermal wall and cuticle, while lateral spread is still proceeding. Bar, 50 μm

6.16 The outline of the emerging stroma reflects its intracellular development. Bar, 50 μm
Fig. 6.17 Hypogenous conidioma initials of *Pseudocercospora platylobii* on *Platyllobium formosum*, seen in tangential section. Each initial is directly beneath a stoma. BF Bar, 100 µm

Fig. 6.18 Stomatal conidioma of *Pseudocercospora clematidis*. The stoma is not disrupted. BF Bar, 20 µm

Fig. 6.19 Hyphae of *Pseudocercospora correae* growing through the outer wall of the upper epidermis (ep) and contacting the cuticle (c). The passage of hyphae appears to be aided by enzymatic action. TEM Bar, 2 µm

Fig. 6.20 Similar to 6.19, but hyphae have begun multiplying beneath the cuticle where a stromatic mass will eventually exert sufficient pressure on the cuticle to rupture it. TEM Bar, 2 µm
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Title: Cercosporoid fungi on Australian native plants

Date: 1994-05


Publication Status: Unpublished

Persistent Link: http://hdl.handle.net/11343/39430

File Description: Chapter 6

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