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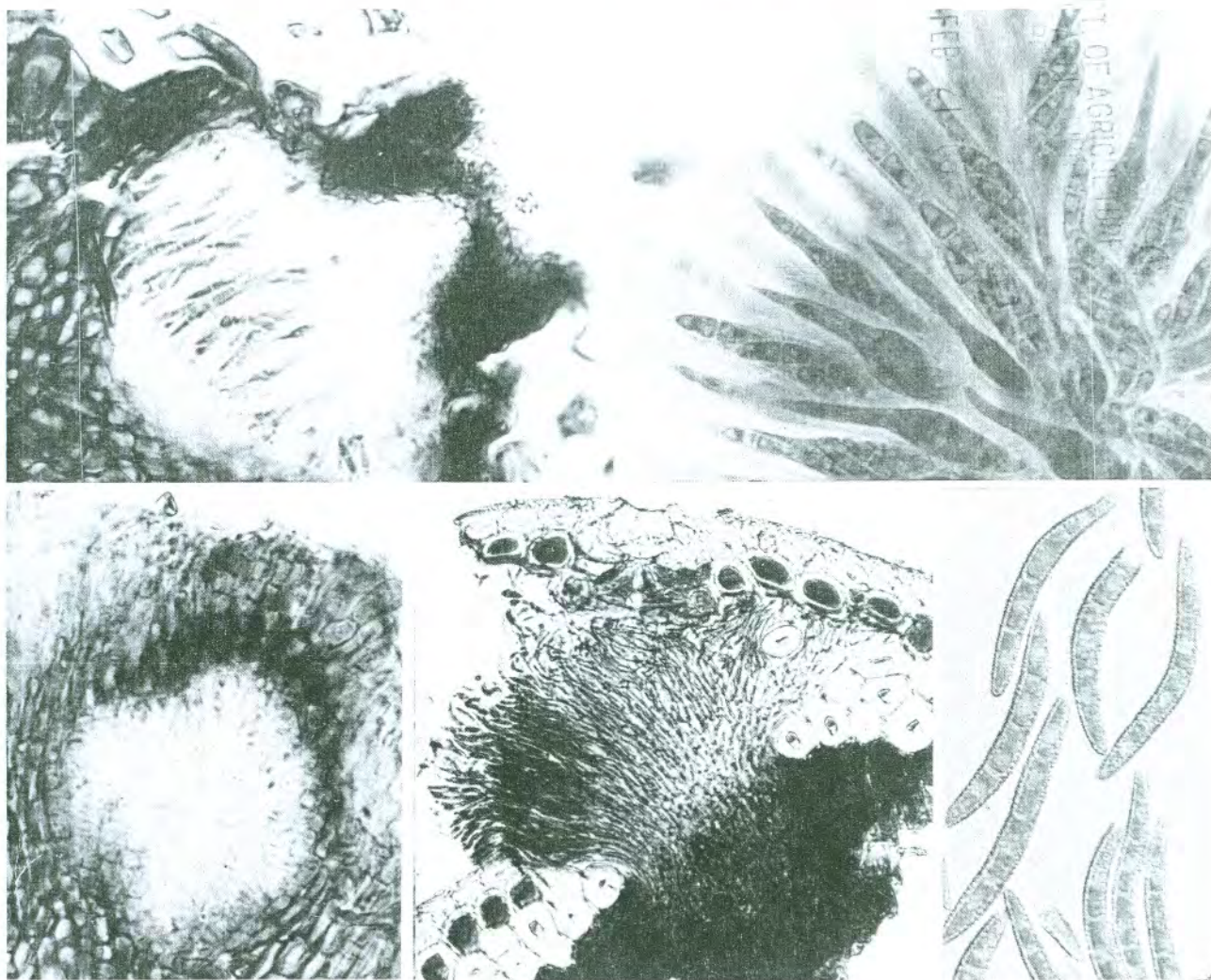
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## The Genus *Mycosphaerella* and its Anamorphs *Cercoseptoria*, *Dothistroma* and *Lecanosticta* on Pines

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## SUMMARY

Three important pine needle pathogens, with teleomorphs assigned to the genus *Mycosphaerella* Johanson, are described: *M. dearnessii* Barr; *M. pini* (E. Rostrup apud Munk) and *M. gibsonii* sp. nov. Historical, morphological, ecological and pathological details are presented and discussed, based on the results of a three-year survey of Central American pine forests and supplemented by an examination of worldwide collections. The fungi, much better known by their anamorphs and the diseases they cause: *Lecanosticta acicola* (Thüm.) H. Sydow (Lecanosticta or brown-spot needle blight); *Dothistroma septospora* (Doroguine) Morelet (Dothistroma or red-band needle blight) and *Cercoseptoria pini-densiflorae* (Hori & Nambu) Deighton (Cercospora or brown needle blight), are considered to be indigenous to Central America, constituting part of the needle mycoflora of native pine species. *M. dearnessii* commonly occurred on pines in all the life zones investigated (tropical to temperate), *M. pini* was locally abundant in cloud forests but confined to this habitat, whilst *M. gibsonii* was rare. Significant, environmentally-related changes were noted in the anamorph of *M. dearnessii* from different collections. Conidia collected from pines growing in habitats exposed to a high light intensity were generally larger, more pigmented and ornamented compared with those from upland or cloud forest regions. These findings are discussed in relation to the parameters governing taxonomic significance.

An appendix is included in which various pine-needle fungi collected in Central America, and thought likely to be confused with the aforementioned *Mycosphaerella* anamorphs are described: *Lecanosticta cinerea* (Dearn.) comb. nov.; *Lecanosticta gloeospora* sp. nov.; *Suttonina guatemaltica* gen. et sp. nov. and *Erythrogloeum pini-acicola* sp. nov.

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Mrs. Eve Rainbow skilfully deciphered and typed the manuscript.

## GENERAL INTRODUCTION

In 1980, a project to survey and catalogue the fungal pathogens associated with Central American pine species in their native habitats was initiated. The pathology of these pines in natural stands is inadequately known even though two of the species, *Pinus caribaea* Morelet and *P. oocarpa* Schiede, have been the subject of considerable silvicultural investigation in view of their increasing importance as exotic plantation trees in both the tropics and subtropics (Greaves, 1978, 1979). Information on endemic pathogens may be of value in helping to predict the long-term performances of Central American pines in exotic situations and thereby possibly prevent some of the pathological disasters which have beset plantations of *P. radiata* D. Don. in various parts of the world.

During the survey, three well-documented and economically important pathogens assignable to the genera *Cercoseptoria* Petrak, *Dothistroma* Hulbary and *Lecanosticta* H. Sydow were encountered. The latter two were particularly widespread and associated with dothideaceous teleomorphs now accommodated in the genus *Mycosphaerella* Johanson (Arx, 1983). A comparative taxonomic study of the Central American collections and diverse collections held in the CMI herbarium confirmed the close relationship of these pathogens which was further endorsed by the discovery of an undescribed *Mycosphaerella* species consistently associated with *Cercoseptoria*.

The results of this taxonomic investigation are presented here together with observations on the ecology and pathology of these highly specialised, needle-colonising fungi with particular reference to Central America. A brief introduction to the survey area and the hosts involved, in addition to an historical review of each fungus are considered essential prerequisites in order to clarify fungal morphology and hopefully, to reach more meaningful taxonomic conclusions.

## THE CENTRAL AMERICAN SURVEY

### The Survey Areas

The major part of the survey work was carried out in Honduras but short collecting trips were made to Belize, Guatemala, Nicaragua and Mexico. Four, two-monthly surveys were made during the period October 1980 to June 1983 and most of the provenance sites (seed sources) of *P. caribaea* and *P. oocarpa*, described in detail by Greaves (1978, 1979) and Robbins & Hughes (1983), were visited.

*P. caribaea* var. *hondurensis* (Sénécl.) Barr. & Golf. occurs along the humid, tropical, Atlantic coastline of Honduras, including the Bay islands (Guanaja), and Nicaragua, in an extensive tract of wet savanna characterised by infertile, acidic, lateritic soils, often referred to as the Mosquitia region. A similar coastal population is found in Belize. In all these countries, smaller inland populations are also present, typically as remnant stands in the dry valleys of the interior, although significant stands do occur on poorer soils up to 700 m a.s.l. in both Belize (Mountain Pine Ridge) and Honduras. This pine species, therefore, has a wide ecological range in terms of rainfall (650-4000 mm annual precipitation) whilst the mean annual temperature is narrow (25-27°C). Above 800 m, *P. caribaea* is replaced by *P. oocarpa*, although there is a transition zone where the distinction between the two species is unclear, perhaps due to interspecific hybridisation (Styles *et al.*, 1982). *P. oocarpa* occupies the niche between 800-1600 m in areas of poor soil and moderate rainfall (1000-1500 mm p.a.), where there is a gradual change from an inland tropical to a subtropical, upland climate (mean annual temperature 13-23°C). Throughout the upper and wetter end of its range, *P. oocarpa* is replaced by high-altitude pine species notably *P. maximinoi* H. E. Moore, formerly *P. tenuifolia* Benth. and often wrongly called *P. pseudostrobus* Lindley (Mittak & Perry, 1979), and *P. tecumumanii* Schwardt., see Barnes & Styles (1983) for taxonomic details of this pine which is now considered to be a subspecies of *P. patula* Schiede & Deppe. *P. tecumumanii* has been consistently confused with *P. oocarpa* but usually it occupies wetter habitats with richer soils. Pine species from relatively inaccessible mountain ranges in Honduras (up to 2800 m) were also investigated, including *P. ayacahuite* Ehrenb. and the legitimate *P. pseudostrobus*. These two species were also observed in the highlands of Guatemala and Mexico, together with *P. michoacana* Martínez and *P. patula*.

The survey covered, therefore, a series of habitats ranging from the humid tropical coast to the dry, inland valleys and up to the subtropical-temperate cloud forest. During the same period visits were made to other countries in Central America (Costa Rica) and the Caribbean (Jamaica) where some of these pine species have been planted as exotics.

### The Collections

Composite needle samples were taken from each locality and three categories were delimited: necrotic needles from regenerating trees; necrotic primary and chlorotic-necrotic secondary needles from mature trees; cast needles from the litter layer. Specimens were air-dried for 3-4 days and sealed in waxed herbarium packets until examination, some 2-6 months later. Needles were examined under a stereoscopic dissecting microscope and hand-cut sections were routinely made of fungal fructifications. Direct plating of the contents of dissected ascostromata and conidiomata proved to be a rapid and successful method of obtaining pure cultures of these needle fungi.

The first survey, carried out towards the latter part of the 1980 wet season, revealed that acervuli characteristic of *Lecanosticta acicola* (Thüm.) H. Sydow (the brown-spot fungus) were common on living needles of pines throughout Honduras. During the 1981 survey, a similar situation was found to exist in Belize and Nicaragua, *L. acicola* being consistently identified in needle samples from all representative pine zones. The closely-related red-band fungus (*Dothistroma septospora* (Dorog.) Morelet) was recorded initially only from a small provenance trial, one of the few planted pine stands in Honduras, leading to the supposition that this pathogen had been introduced with the planting material and that it

was not endemic (Evans, 1982). However, during the 1982 survey more attention was given to the high-altitude pine species and subsequently *D. septospora* was discovered in natural stands from several, isolated mountainous areas suggesting that the fungus was indeed indigenous. This was corroborated during the 1983 survey when comprehensive collections were made from remote, high-altitude sites in Honduras as well as from the highlands of Guatemala. Specimens of *Cercoseptoria pini-densiflorae* (Hori & Nambu) Deighton (the brown blight fungus) were collected from a single locality in Nicaragua and subsequently compared with Asian and African collections either received by the author for identification or conserved in Herb. IMI.

## TAXONOMIC CONSIDERATIONS

### Historical

#### 1. The brown-spot fungus: *Lecanosticta* needle blight

The somewhat confused history of this fungus, and the disease it causes, has been admirably reviewed by Siggers (1944) but it is considered justified in re-evaluating some of the earlier taxonomic background in order to see where and how the confusion has arisen. According to Hedgcock (1929), the brown-spot disease or needle blight of pines has been known in the southern U.S.A. since the 19th Century. The causal agent was described by De Thümen (1878) on *P. variabilis* Lamb. (= *P. echinata* Mill., but wrongly identified fide Hedgcock (1929) and now thought to be *P. caribaea*) from S. Carolina and placed in the genus *Cryptosporium* Kunze (Melanconiaceae). The genus was characterised by innate acervuli and hyaline, aseptate conidia and consequently Saccardo (1884) moved the species with its septe conidia to the genus *Septoria* Fr. (Phomaceae = Sphaerioidaceae). Sydow (Sydow & Petrak, 1922) received blighted material of *P. taeda* L. from Arkansas and erected the new genus *Lecanosticta* to accommodate the causal fungus which he named *L. pini* being unaware of the earlier description and placing it in the patelliform 'Excipulaceae' of the Sphaeropsidales presumably believing it to be pycnidial as had Saccardo. Later, however, he recognised the synonymy but considered that the generic concept of *Lecanosticta* was still valid, typifying it by the erumpent stromata and darkly pigmented conidia (Sydow & Petrak, 1924). Moreover, he also examined material of *Actinothyrium marginatum*, described earlier by Saccardo (1920) on needles of *P. ponderosa* Dougl. from Idaho, and considered that it was conspecific with *L. acicola*, basing this on the linear, dothideaceous stromata and acicular conidia, despite the absence of spore pigmentation in the specimen which he regarded as immature. This erroneous conclusion undoubtedly created the initial confusion, not only concerning the identity of the brown-spot fungus but also that of the red-band fungus, as will be shown later. The descriptive common name has also added to the uncertainty since Dearness (1928) referred to the red-spot symptoms in his emended description of the causal agent, which he felt justified in retaining in the genus *Cryptosporium*, apparently recognising the acervular rather than the pycnidial nature of the conidiomata as interpreted by both Saccardo and Sydow, "While not a typical *Cryptosporium* this fungus fits better here than in *Septoria*". Hedgcock (1929) rejected the opinions of both Dearness and Sydow and chose to accept Saccardo's placement in *Septoria*, mainly because of its cultural characteristics. He also adopted the name brown-spot disease, which he considered to be a more accurate description of the needle symptoms, even though he had initially proposed the red-spot disease terminology (see Dearness, 1928), and proved conclusively that the fungus in question was the causal agent. Siggers (1932), in his initial epidemiological investigations, used the name *Septoria acicola* but later, after a detailed taxonomic analysis, he concluded that *Lecanosticta acicola* was the most appropriate name since stromatal formation and pigmented spores were not within the generic concept of *Septoria* (Siggers 1939; 1944). This binomial has received the general acceptance of both plant pathologists and mycologists (Laut *et al.*, 1966; Sutton, 1980; Arx, 1983) for the anamorph of the brown-spot fungus.

The generic disposition of the teleomorph has been equally puzzling (Barr, 1972). *Oligostroma* ("Phyllachora") *acicola* was first described by Dearness (1926) on needles of *P. palustris* Mill., in association with *L. acicola*. Subsequently, he suggested a teleomorph-anamorph connection (Dearness, 1928) which was endorsed by the collections of Hedgcock (1932) . . . "This species of fungus is constantly associated with *Septoria acicola* and is probably its perfect stage". Earlier, Sydow (Sydow & Petrak, 1922) had noted erumpent stromatal structures and the presence of microconidia (spermatia) in

his description of the anamorph but had not correlated this with ascocarp formation. However, it was left to Siggers (1939) to culturally prove the connection. He considered that the genus *Oligostroma* H. Sydow & Sydow, characterised by immersed, clypeate ascocarps, was not suitable for the brown-spot fungus and referred the erumpent stromata to the genus *Scirrha* Nitschke, making the new combination *Scirrha acicola*. Interest in the brown-spot disease was increasing because of its serious effect on young stands of *P. palustris* in the southern U.S.A., and this prompted Wolf & Barbour (1941) to undertake a detailed study of the causal organism. The latter authors illustrated the true acervular nature of the anamorph, accepting its disposition in *Lecanosticta*, and not *Septoria*, but considering the teleomorph to be best accommodated in the genus *Systemma* Theiss. & H. Sydow. They recognised two families of Dothideales which differed mainly in the final position of the ascostromata: the Dothideaceae, with erumpent forms; the Phyllachoraceae, which included the genera *Oligostroma* and *Scirrha*, in which the fructifications remain covered by the host tissues, and laid much emphasis on ascospore pigmentation, describing the spores as phaeodidymous, and the absence of paraphyses in assigning the fungus to the genus *Systemma*. Siggers (1944), however, vigorously defended his earlier taxonomic decision as to the correct placement of the teleomorph and presented the results of a comparable study of ascostromatal morphology in *Oligostroma*, *Systemma* (which he showed to be synonymous with *Dothidea*) and *Scirrha*, putting much emphasis on the origin of the stroma and its final position in relation to host tissue. He concluded finally, that the teleomorph of the brown-spot fungus agrees most closely in stromatal characters with the type of the genus *Scirrha*, refuting Wolf & Barbour's observations concerning ascospore pigmentation. Accepting their criticism that he had only described in situ ascospores ("immature") in his original diagnosis, he also examined ascospores discharged naturally from the ascus and failed to detect any pigmentation in the spore wall. He questioned the interpretation of Wolf & Barbour as regards to colouration and argued that although some of the spore contents (oil globules) are amber or pale-brown, the ascospores are essentially hyaline. Wolf & Wolf (1947), nevertheless, were still critical of Sigger's evidence believing that his illustrations were of immature stromata and equally immature ascospores, stating that "Mature stromata are exposed and prominently protrude . . ." and that "Discharged ascospores have brown walls as well as brown cell contents". Finally concluding that "It seems unthinkable that this brown-spot fungus could be properly placed in the genus *Scirrha*". Thus, they maintained the fungus in *Systemma* although perversely they noted that teleomorph development is unlike that observed in the type, *Systemma ulmi* (Duval) Theiss. & H. Sydow, and compared it with that of *Cymadothea trifolii* (Pers.) Wolf.

No further reference has been made to ascospore pigmentation by subsequent workers who have generally accepted Sigger's conclusions, although Morelet (1968b) did propose the new combination *Dothidea acicola* probably basing this on a nomenclatural rather than a morphological examination. Barr (1972), later transferred the fungus to the genus *Mycosphaerella* using locule and ascus development as generic concepts rather than the position of the ascocarp, which she regarded as a highly variable character. However, because the new combination was invalid, *M. acicola* (Cooke & Harkn.) Lindau having priority, she established the new name *M. dearnessii* as the type of the section *Caterva*, typified by the stromata developing on dead host tissues and the fusoid ascospores. Despite this proposal, *Scirrha acicola* is still the most widely used name to identify the brown-spot fungus in both mycological and phytopathological literature (Punithalingam & Gibson, 1973; Skilling & Nicholls, 1974; Kais, 1977; Gibson, 1979; Sutton, 1980; Jewell, 1983). Recently, Arx (1983) re-emphasized the correct placement of the fungus in the genus *Mycosphaerella* but questioned the sections proposed by Barr since they were based on inconsistent characters. Arx (1949) and Arx & Müller (1975) recognised only three sections in the genus, as opposed to the two subgenera and ten sections erected by Barr. In the former classification, *M. dearnessii* fits readily into the section *Cymadothea*, accommodating species with stromatic, often aggregated and erumpent ascocarps and fasciculate, cylindrical asci. Previously, Petrak (1941) had placed *Cymadothea* in synonymy with *Mycosphaerella* and it is surprising, therefore, that after examining North American material of the brown-spot fungus he had not realised the similarity, although he did conclude that the fungus could not be placed in *Scirrha*, nor *Oligostroma* and was not typical *Systemma*, stressing that further morphological studies were necessary to clarify the correct generic disposition (Petrak, 1961). It is perhaps even more surprising that Wolf & Wolf (1947), in comparing the analogous ascocarp development of *Cymadothea* and the brown-spot fungus, still maintained the latter in the genus

*Systemma* even though they had concluded that "The type of sexuality in *Systemma ulmi* is quite unlike that recently described for *S. acicola* . . ."

## 2. The red-band fungus: *Dothiostroma needle blight*

The red-band fungus has an equally tortuous history as that of *M. dearnessii* with which it has been repeatedly confused perhaps with some justification since symptomatology and morphology overlap. The fungus was described initially by Doroguine (1911), on needles of *P. montana* Mill. (= ? *P. cembra* L.) from Russia, as *Cytosporina septospora*. He illustrated the erumpent, multiloculate stromata and compared the fungus with *Septoria* but considered that this genus with its single or uniloculate pycnidium was inappropriate and elected to broaden the concept of the genus *Cytosporina* Sacc. to accommodate species with septate conidia. Saccardo (in Trotter, 1931) later transferred it to the genus *Septoriella* Oudem. However, this European record remained in obscurity until Gremmen (1968) and Morelet (1968a) recognised, apparently independently, the similarities of the Russian fungus with the causal agent of the notorious red-band disease of pines in the U.S.A. and Africa. The Russian type specimen may have been overlooked due to the fact that it was not associated with a serious disease, nor with the diagnostic red-banding, or to Doroguine's own misgivings when he later referred the fungus to the genus *Brunchorstia* Eriksson (fide Gremmen, 1968; Morelet, 1968a & b). During his investigations on the red-band disease of pines in France, Morelet requested type material of *Cytosporina septospora* from the Leningrad herbarium but the only available specimen was from a later collection (1914) on *P. sylvestris* L., which he found to be identical to the red-band fungus. In view of the apparent loss of the holotype, Morelet (1969) suggested that the latter material should be designated as neotype. Gremmen (1968) reached his conclusions following an examination of a reported record of *Brunchorstia* disease of *P. ponderosa* in Romania. He compared Doroguine's original description and illustration with the Romanian material and considered them to be identical with the North American red-band fungus, suggesting that the confusion with *Brunchorstia pinea* Karst. which Doroguine had initiated and which other East European workers had perpetuated, was due to resemblances in conidial morphology.

It is now necessary to turn to the North American material in order to complete the nomenclatural jigsaw. Saccardo (1920) described *Actinothyrium marginatum* (Leptostromataceae) on red-banded *P. ponderosa* needles from Idaho but this name was rejected by Sydow & Petrak (1924) as a nomen confusum since it was based on discordant elements. Saccardo had described the fruiting body of *Leptostroma decipiens* Petrak and the spores of another fungus, which they assumed to be immature (i.e. hyaline) conidia of *Lecanosticta acicola*. Thus although they had recognised the close affinities of the brown-spot and red-band fungi, noting similarities in stromatal structure, they had failed to critically analyse conidiogenesis and, by attempting to remedy Saccardo's error, had only added to the confusion which was to be perpetuated by Petrak (1961) in his erroneous identification of *L. acicola* from Austria. Dearness (1928) emended the description of De Thümen, possibly to include the red-band fungus, and Hedgcock (1929) listed *A. marginatum* as a synonym of *L. acicola*.

Finally, however, it was Siggers (1944) who fully appreciated the confusion which existed in the identification of these needle pathogens, "Two or more species of hyaline-spored pine needle fungi are found in herbaria under names applied to the color-spored brown spot fungus", adding that the hyaline spore form is typically associated with pronounced reddish lesions on pines from the northern U.S.A., whilst the brown-spore form has mainly a southern distribution. He did note, nevertheless, some overlap in both distribution and symptomatology but, after examining various U.S.D.A. records of *L. acicola* (and its synonyms), he concluded that none of the northern collections (mainly on *P. nigra* Arn. var. *austriaca* Harrison) were conspecific with *L. acicola*, being distinguished by the highly erumpent stromata, hyaline conidia and red lesions, which he compared to a fungus described by Hulbary (1941) from blighted Austrian pine in Illinois. Thus, he removed *A. marginatum* from synonymy with *L. acicola*. Thyr & Shaw (1964) considered it fortunate that *A. marginatum* had been rejected since this could have been used for the type of the red-band fungus, after emendation of the original description to include only this discordant element. This name was used, in fact, for the initial tentative identifications of the red-band fungus from East Africa (Gibson, 1962). The former authors also re-examined all Sigger's material and agreed essentially with his conclusions, referring the red-band fungus to the genus *Dothiostroma* Hulbary. Slightly earlier, Murray & Batko (1962), during an investigation into a needle

disease in pine nurseries in southern England, had reached a similar conclusion, although *L. acicola* had originally been considered to be the possible causal agent.

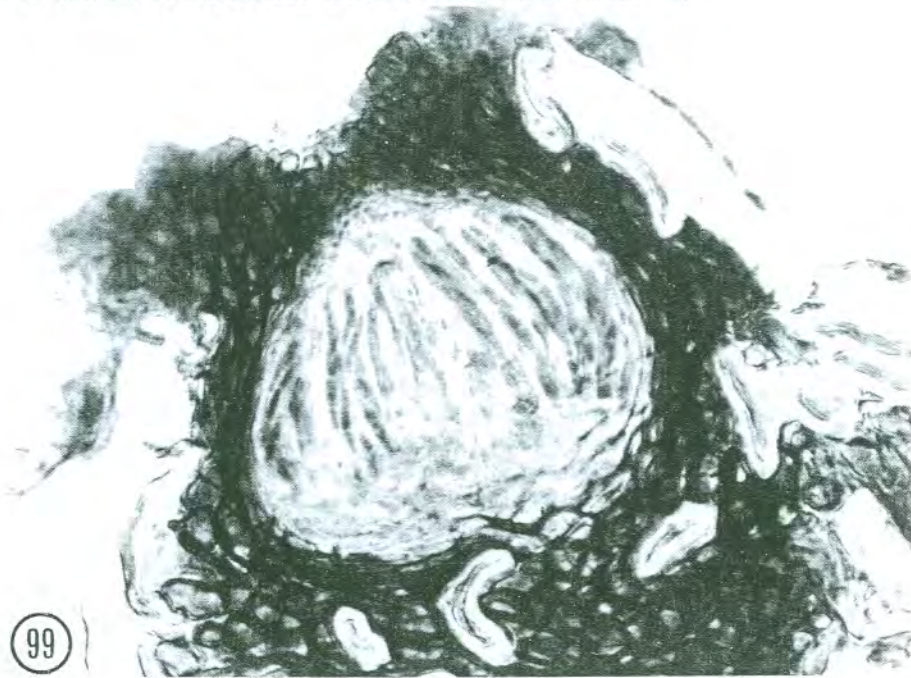
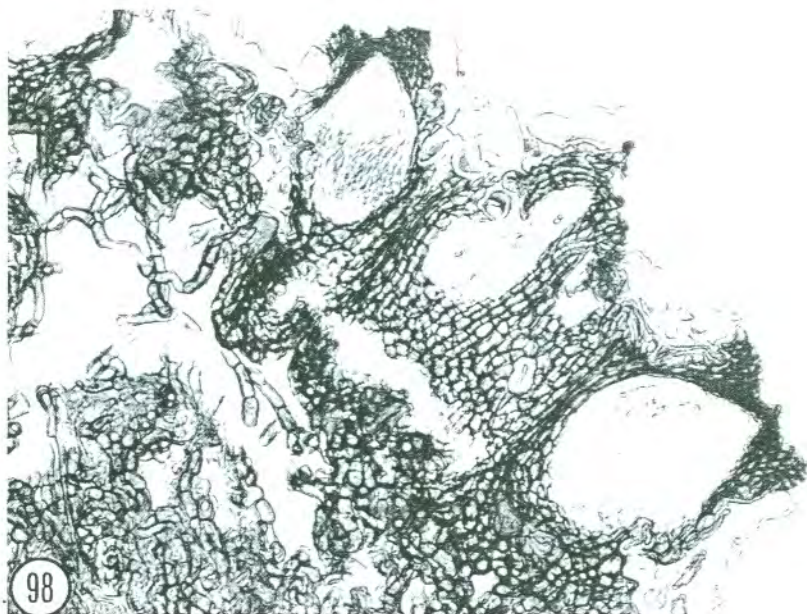
Hulbary (1941) in his study of the needle blight on Austrian pine, described the symptoms as small brown or tan spots and failed to record the red-banding, noted by Thyr & Shaw (1964) in the type material. He placed the new genus in the scolecosporous division of the Phomaceae relating it to the dothideaceous genera *Hemidothis* H. Sydow and *Septocytia* Petrak. This has been universally agreed to be the most suitable placement of the anamorph of the red-band fungus, and the name *Dothistroma pini* was widely used to describe the causal agent of red-band needle blight of *P. radiata* in East Africa (Gibson, 1963), Chile (Dubin & Staley, 1966) and New Zealand (Gilmour, 1967). This binomial is still perhaps in most common usage amongst phytopathologists (Franich *et al.*, 1983) although Morelet's combination has been taken up recently by Sutton (1980) and Arx (1983). The latter author, however, considers *Septoria* to be the correct generic placement of the anamorph. This decision and others relating to varietal division of the species (Thyr & Shaw, 1964; Ivory, 1967), have been rejected by the present author and will be discussed within the context of the morphological investigation.

The teleomorph connection was established by Funk & Parker (1966) in Canada and shortly afterwards by Morelet in France (1967 a & b). In assigning the species to the genus *Scirrhia* (as *S. pini*), Funk & Parker noted some overlapping of teleomorph characters between the red-band and brown-spot fungi but separated them principally on ascostromatal dimensions and the presence or absence of interthecial tissue. In his recent revision of the anamorphs of the genus *Mycosphaerella*, Arx (1983) proposed the new combination *M. pini*.

### 3. The brown needle fungus: *Cercospora* needle blight

A serious needle disease was first reported in 1913 from southern Japan on various exotic and native pine species, including *P. pinaster* Ait., *P. thunbergii* Parl., and the causal agent was later described from seedlings of *P. densiflora* Sieb. & Zucc. as a new species of *Cercospora*, *C. pini-densiflorae* Hori & Nambu (in Nambu, 1917). Although reports of the fungus are relatively scarce until recently, it apparently proved to be a major obstacle to pine establishment in parts of Japan and Taiwan (Ito, 1972). During the 1960's, the pathogen was recorded from East Africa, causing serious defoliation of *P. radiata* (Mulder & Gibson, 1972), and later from Malaysia (Ivory, 1975) and the Philippines (Kobayashi *et al.*, 1979). Analogies were drawn with *Dothistroma* needle blight and distinctive bands, usually grey-brown in colour, are common symptoms on needles affected by *Cercospora* needle blight (Ito, 1972; Gibson, 1979). In 1976, Deighton revised the concept of the genus *Cercospora* Fres. and transferred the brown needle fungus to the genus *Cercoseptoria* Petrak on the basis of the unthickened conidial scars and the acicular conidia.

There have been no previous reports of a teleomorph connection for *C. pini-densiflorae* but, during an examination of material deposited in Herb. IMI, *Mycosphaerella* ascostromata were consistently observed. This led to a more detailed study of all pine specimens conserved under *Mycosphaerella* or *Cercoseptoria*. Specimens deposited as *M. pinicola* (Fautr.) Naoum., reported to be the causal agent of a debilitating needle blight of *P. massoniana* Lamb. in Hong Kong (Leather, 1968), were found to have conidiomata of *C. pini-densiflorae* in association with the ascostromata, subsequent examination of the *Cercoseptoria* folder revealed that the fungus had been sent several years later from the same pine nursery in Hong Kong. Further analysis of East African material deposited under *M. pinicola* and unidentified *Mycosphaerella* spp. demonstrated the presence of erumpent ascostromata, similar to the red-band and brown-spot fungi, in association with conidiomata of *C. pini-densiflorae*. The type of *M. pinicola* (ex Herb. K) on *P. austriaca* from France proved to be incompatible with the modern concept of the Dothideaceae, consisting of groups of scutate, superficial ascocarps probably belonging to the Microthyriaceae. The hyaline, 1-septate, oblong ascospores are reminiscent of *Mycosphaerella*, hence the original disposition of this species in the '*Sphaerella*' complex. However, in the brief diagnosis of Roumeguère (1891) and Saccardo (1895), the ascocarps are said to be flattened (appliqués) and the asci ventricose (ventrues, difformes) and, therefore, readily distinguishable from the subepidermal, erumpent, dothideaceous ascostromata and essentially cylindrical asci of the *Mycosphaerella* sp. associated with *C. pini-densiflorae*, and purported to be its teleomorph. This species is considered to be undescribed.



Figs 98-99. *Mycosphaerella gibsonii*, holotype, IMI 92286, showing deep-seated mycelium and stroma, locules and ostiolar region ( $\times 250$ ,  $\times 650$ ).

### 3. *Mycosphaerella gibsonii* H. Evans sp. nov. (Figs 98-120)

*Etym*: Named in honour of Dr. I. A. S. Gibson who has been intimately involved with this group of pine pathogens.

*Ascostromata* foliicola, variabilia, brunnea vel atra, sub epidermide immersa; innata, discreta, uniloculata, globosa, (50–) 70-90 (–120)  $\mu\text{m}$  diametro vel erumpentia, linearia, multiloculata, 150-800 (–1400)  $\times$  70-125 (–160)  $\mu\text{m}$ , et 90-150  $\mu\text{m}$  altitudine, interdum in fasciae formata; parietibus pseudoparenchymatosis, e cellulis crassitunicatis 3-8 (–12)  $\mu\text{m}$  diametro compositis. *Loculi* globosi vel lageniformes, (45–) 50-75 (–95)  $\times$  55-75  $\mu\text{m}$ , ostiolati, periphysati, in ordines longitudinales. *Asci* bitunicati, clavati vel cylindrici, (33–) 35-38  $\times$  5.5-7  $\mu\text{m}$ , apice obtusi, vel rotundati, raro saccati, 32-36  $\times$  6-8  $\mu\text{m}$ , octospori, oblique biseriati; textura intertheciali absens vel praesens. *Ascospori* hyaline, 1 septati, ellipsoidei vel cuneati, (7.5–) 8.5-11 (–12.5)  $\times$  (1.8–) 2.2-2.8  $\mu\text{m}$ , guttulati. Anamorphosis *Cercoseptoria pini-densiflorae*.

*Spermogonia* praesens, affinis *Asteromella*; *spermatia* hyalina, bacilliformia, 2.3  $\times$  1  $\mu\text{m}$ .

Holotypus in foliis *Pini radiatae*, Rondo Forest Reserve, Lindi, Tanzania, Mar. 1962, I. A. S. Gibson, IMI 92286.

*Ascostromata* variable, dark brown to black, innate, discrete, subepidermal, uniloculate, globose, (50–) 70-90 (–120)  $\mu\text{m}$  diam, to erumpent, linear, multiloculate, 150-800 (–1400)  $\mu\text{m}$  in length, 70-125 (–160)  $\mu\text{m}$  wide and 90-150  $\mu\text{m}$  deep, occasionally uniting laterally in bands; stroma of pseudoparenchymatous, thick-walled cells, 3-8 (–12)  $\mu\text{m}$  diam. *Locules* globose to flask-shaped, (45–) 50-75 (–95)  $\times$  55-75  $\mu\text{m}$ , ostiolate, periphysate, often with an apical stromatic shield, 70-90  $\mu\text{m}$  diam, in longitudinal series. *Asci* bitunicate, clavate to cylindrical, (33–) 35-38  $\times$  5.5-7  $\mu\text{m}$ , with a thickened, bluntly rounded apex, rarely saccate, 32-36  $\times$  6-8  $\mu\text{m}$ , 8 spored, obliquely biseriate. Interthecial tissue present or absent. *Ascospores* hyaline, 1 septate, ellipsoidal to cuneate, (7.5–) 8.5-11 (–12.5)  $\times$  (1.8–) 2.2-2.8  $\mu\text{m}$ , guttulate.

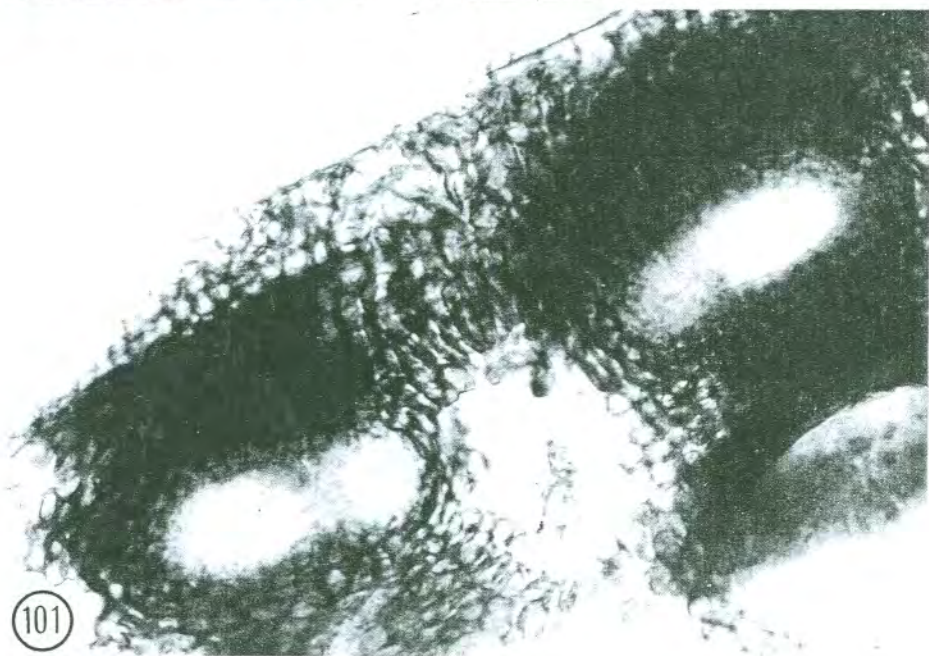
*Asteromella* spermogonial state present; *spermatia* hyaline, rod-shaped, 2.3  $\times$  1  $\mu\text{m}$ .

*Holotype*: ex needle of *Pinus radiata*, Rondo Forest Reserve, Lindi, Tanzania, Mar. 1962, I. A. S. Gibson, IMI 92286.

*Paratypes*: ex needles of: *P. radiata*, Cherangani, Kitale, Kenya, I. A. S. Gibson, Sept. 1962, IMI 95092, as *Mycosphaerella pinicola*; *P. caribaea*, Kimakia, Kenya, I. A. S. Gibson, Aug. 1962, IMI 95451, as *M. pinicola*; *P. radiata*, Lushoto, Tanzania, D. E. Etheridge, Dec. 1963, IMI 104005, as *M. pinicola*; *P. radiata*, Stapleford, Salisbury, Zimbabwe, J. O. Whiteside, Aug. 1965, IMI 112710, as *Cercoseptoria pini-densiflorae*; *P. massoniana*, Tai Lung nursery, Hong Kong, R. I. Leather, 19 July 1966, IMI 122031, as *M. pinicola*; *P. caribaea*, Abra, Luzon, Philippines, H. I. Speechley, July 1980, IMI 250111; *P. merkusii* Jungh & de Vriese, Than Long, Vietnam, I. A. S. Gibson, 7 Aug. 1980, IMI 281637.

Ascostromatal morphology is highly variable and probably governed by both host and climate. Needles of East African *P. radiata* specimens are colonised by an extensive stroma, frequently investing the entire cortex (Fig. 98) distorting and crushing the medulla. Non-pigmented, immature stromatic tissues may aggregate appearing as white blisters on the needle surface, uniting laterally and longitudinally in a continuous, erumpent band. The epidermis usually ruptures by a medium longitudinal slit or along stomatal lines. Occasionally, two regular, longitudinal splits occur so that an epidermal dome or lid is lifted above the needle surface as described for *M. dearnessii*. Conspicuous shield-like stromatic tissue ('pseudoclypeus') may accumulate around the ostioles imparting a roughened appearance to the apical region of the ascostromata (Fig. 99, also Figs 104, 105) evidence suggests that this is an amalgamation of pseudoparenchymatic sporodochial cells, the remnants of the suspected *Cercoseptoria* anamorph (Fig. 100). Ostioles are circular to ovoid, 15-20  $\mu\text{m}$  diam, and lined with periphyses (Fig. 101). Non-staining, hyaline, thin-walled, lipid-filled, oblong cells may remain as packing between the asci (Figs 102A, 103), but this interthecial tissue is disorganised, probably constituting unused food reserves and, since it is not consistently present (Fig. 104), it is felt unwise to place any taxonomic significance on it. Ascospores are typically 4-guttulate and of variable size and shape (Fig. 102).

In IMI 95092, the erumpent, black ascostromatal bands are particularly conspicuous and the cortex is entirely replaced by a compact tissue of pseudoparenchyma and dark, swollen, mucilage-covered hyphae. More regular, brick-like cells occur laterally uniting adjacent ascostromata forming a composite



Figs 100-101. *M. gibsonii*, holotype, IMI 92286 ( $\times 650$ ):

Fig. 100. Ascostromatal locules with remnants of *Cercoseptoria* conidiogenous cells (arrowed) around apex;  
Fig. 101. Longitudinal section through ascostromatal apex, viewing down into periphysis-lined ostioles, note amalgamation of ascostromata.

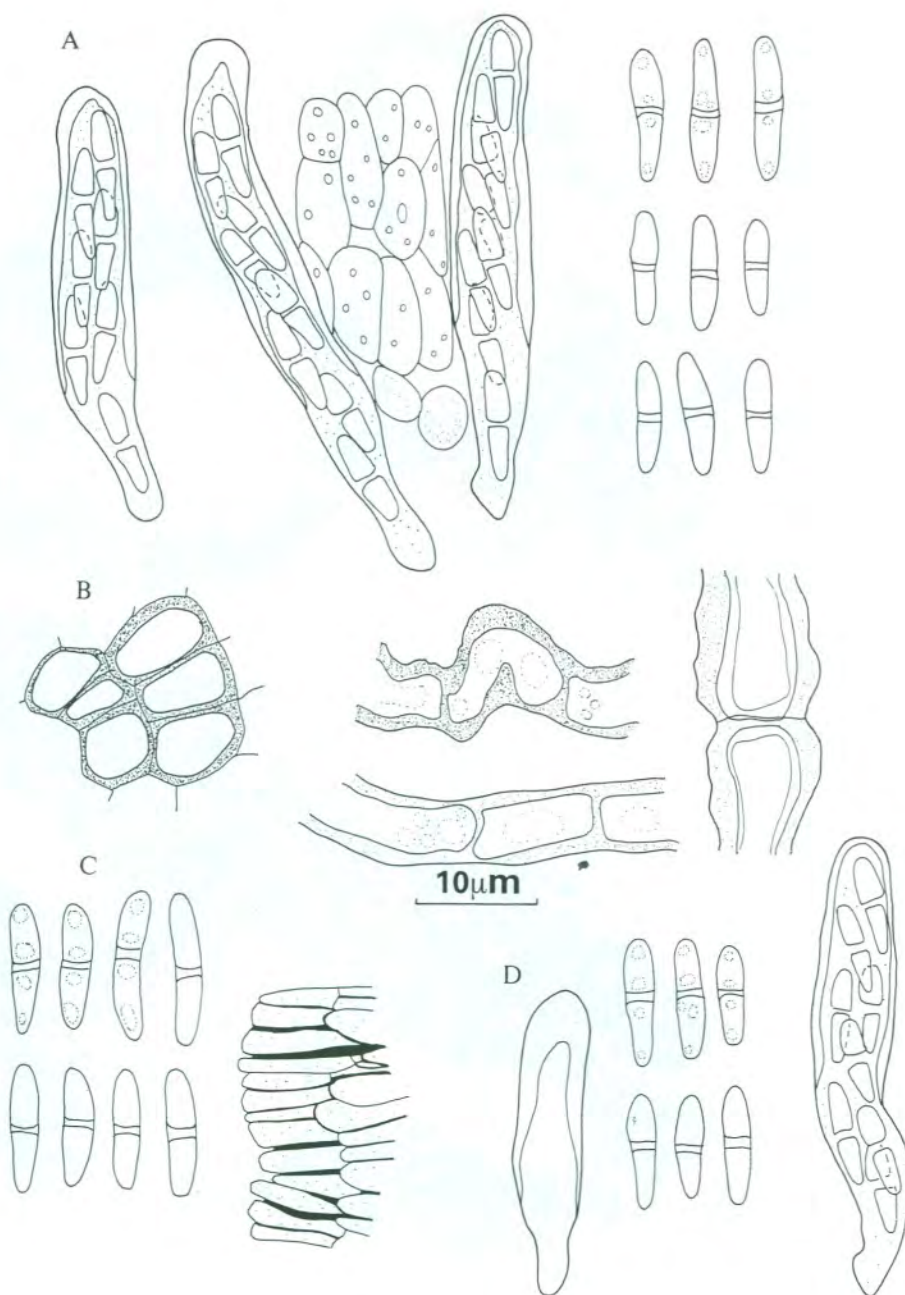
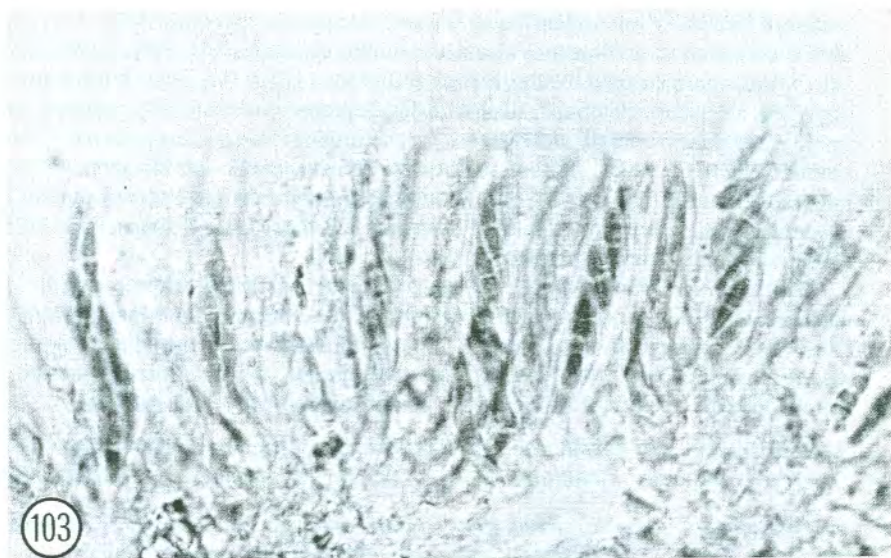


Fig. 102. *M. gibsonii*, ascus and ascospore morphology:  
 A, Holotype, *P. radiata*, Tanzania, IMI 92286;  
 B, Stromatal cells and hyphae from holotype;  
 C, Paratype, *P. merkusii*, Vietnam, IMI 281637, also showing tooth-like cells  
 around apex of ascostroma;  
 D, Paratype, *P. caribaea*, Philippines, IMI 250111.



Figs 103-104. *M. gibsonii*, holotype, IMI 92286 ( $\times 1000$ ):

Fig. 103. Asci with interthecial packing tissue;

Fig. 104. Locule and apical shield, showing asci, apparently without interthecial tissue, and ascospores.

structure internally but appearing as separate structures externally (Fig. 105). Small, solitary, globose, almost astromatic fructifications also occur on this specimen (Fig. 106). A well-developed internal stroma with white, spermogonial locules is present in IMI 112710 (Fig. 107) but mature ascostromata were not observed. In Asian collections, stomatal development is considerably reduced, and the ascostromata are predominantly uniloculate and discrete. In the original Hong Kong material of *M. pinicola* (IMI 122031; Leather, 1968), the black, globose, uniloculate ascostromata occur in aggregations or bands but remain as integral structures, and there is no longitudinal or lateral stomatal development. Few ascostromata were encountered on specimens from the Philippines but those from Vietnam (IMI 281637) show aggregations of black, mainly immature ascostromata.

Apart from IMI 95092, all the *M. gibsonii* specimens listed are associated directly or indirectly with conidiomata of *Cercoseptoria pini-densiflorae*. In the holotype and IMI 112710, conidiogenous cells are found on a contiguous ascostromatal sheath, forming where the epidermis is ruptured by median or longitudinal slits (Fig. 108) and, as described earlier, often remaining around or contributing to the ostiolar region. This is considered to be the anamorph of *M. gibsonii*.

***Cercoseptoria pini-densiflorae*** (Hori & Nambu) Deighton, *Mycol. Pap.* 140: 167, 1976.

= *Cercospora pini-densiflorae* Hori & Nambu (apud Nambu, 1917; translation Tanaka, 1918).

*Conidiomata* stromatic, silvery-grey to dark green or black, substomatal and emerging through the stomata, or initiating from an extensive, deep-seated stroma, composed of dark, thick-walled pseudoparenchyma which ruptures the epidermis with a median or two longitudinal slits. *Conidiogenous cells* developing directly on this stroma in a dense fascicle or from a well-defined sporodochium up to 150 µm diam and 60 µm in height; subhyaline to green or pinkish brown, clavate to cylindrical, 20-30 × 2.5-3.5 µm, producing grey-green conidial tufts, polyblastically, sympodially. *Conidia* hyaline at first, then grey-green-pale brown, smooth, thin-walled, cylindrical, (12-) 20-60 (-80) × 2.4 µm, 1-6 (-10) septate, rounded to pointed at apex with a truncate base.

#### *Material examined:*

##### Nicaragua:

*P. oocarpa*, Santa Cruz, Estelí, 1200 m, 2 Nov. 1981, IMI 281636.

On secondary needles of regenerating trees, only limited material collected. A more intensive survey in 1982 in this locality failed to reveal the species.

*Conidiomata* variable, some discrete occupying only the substomatal chamber, others forming a well-defined sporodochium (Fig. 109) or developing from a deep-seated stroma, up to 75-110 µm deep and 140-180 µm wide (Fig. 110). Spermogonia develop as an integral part of the latter structure (Fig. 111) but ascostromata were not seen.

*Conidia* hyaline to green, (2-) 3 (-5) septate, usually curved, (30-) 35-50 (-58) × 2.5-3.5 µm, MCL = 42.6 µm (Figs 112-113, 114A).

Traces of *C. pini-densiflorae* were also found in an exotic stand of *P. caribaea*, San Ramon, Alajuela, Costa Rica, Apr. 1980, but conidia were too few to allow for significant measurements.

##### Tanzania:

IMI 92286, as *Mycosphaerella gibsonii*, see above.

*Conidiogenous cells* arising directly from ascostromata or from discrete sporodochia, 30 µm diam, 50-70 µm high, emerging through stomata or from a deep-seated stroma (Figs 115-116) grey-dark green, linear sporodochia, 90-150 × 40-60 µm. *Conidia* mainly cylindrical, 3 septate, (12-) 22-40 (-65) × 2.3 µm, MCL = 40.2 µm, but clavate, 1 septate forms also present (Fig. 11B).

*P. radiata*, Lupembe, Njambe, D. E. Etheridge, IMI 103170.

*Conidiomata* grey, in groups or scattered, associated with reddening on distal part of primary needles.

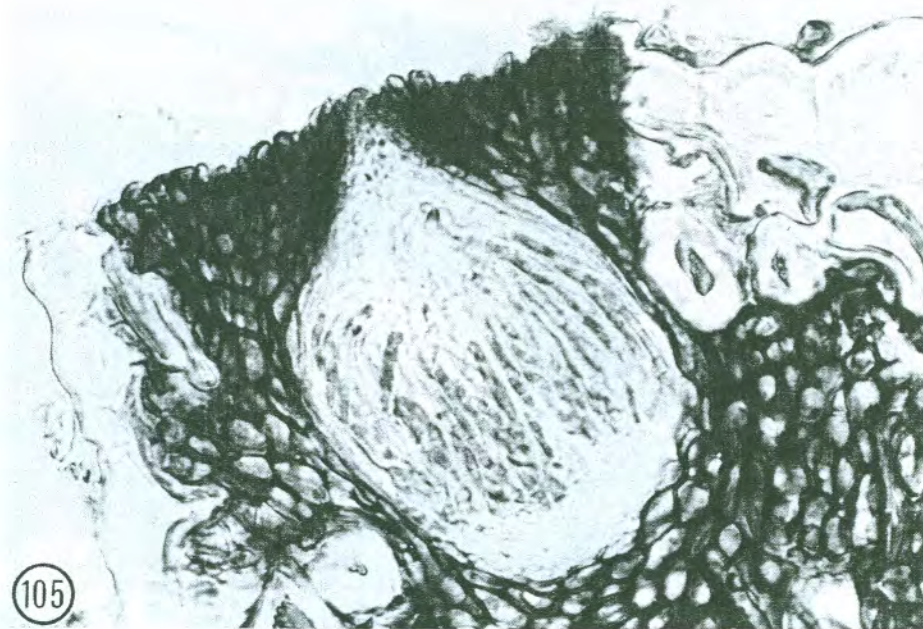
*Conidia* pale green, cylindrical, stout with a broadly rounded apex, 3-6 septate, (25-) 30-60 (-72) × 3.4 µm, MCL = 50.8 µm (Fig. 114C).

##### Zambia:

*P. radiata*, Dola Hill, M. H. Ivory, Oct. 1975, IMI 197660.

On needles with reddish-brown necrosis.

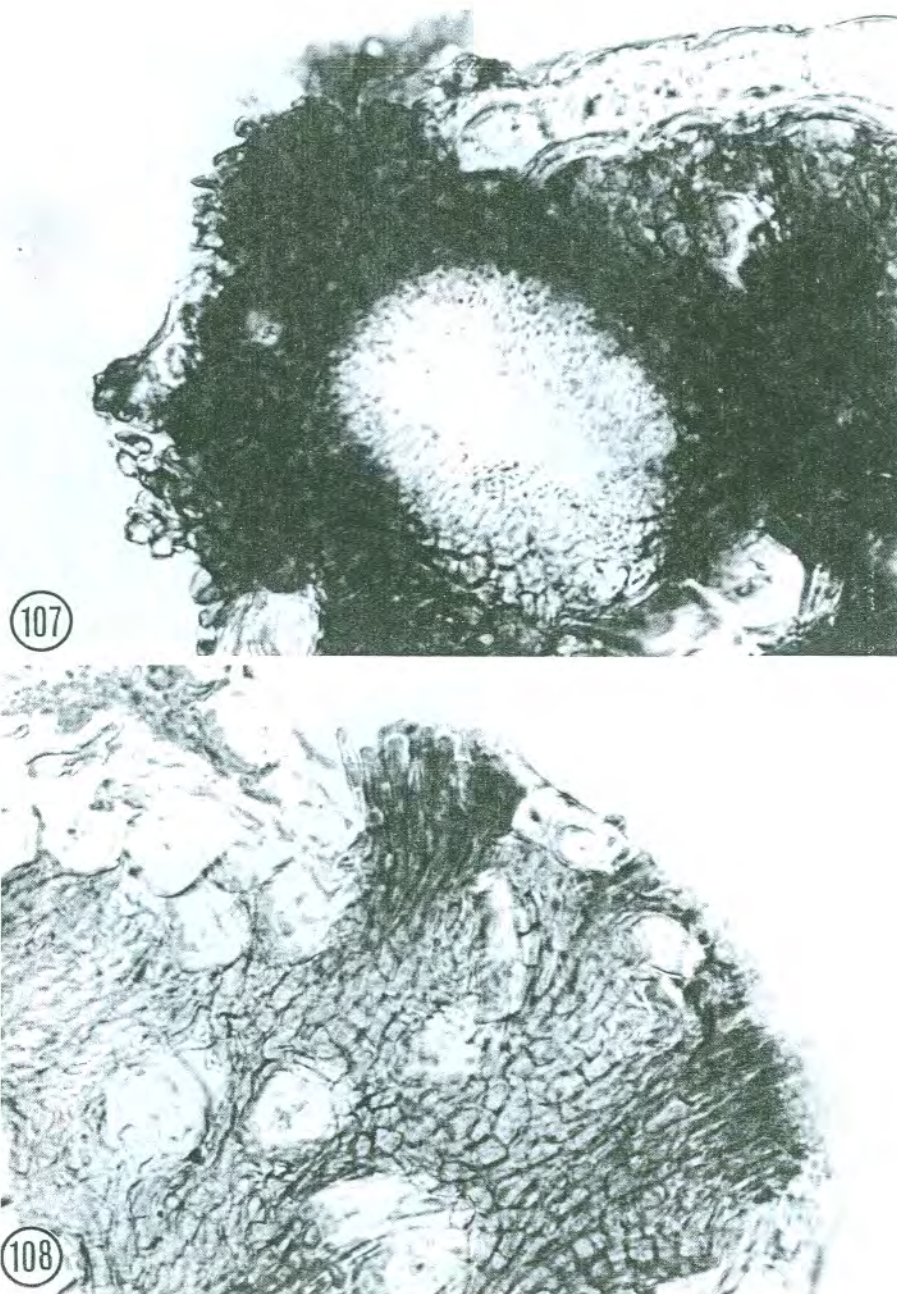
*Conidia* cylindrical, stout, 3-8 (-10) septate, (38-) 45-60 (-68) × 2.8-3.6 µm, MCL = 48.9 µm.



Figs 105-106. *M. gibsonii*, paratype, IMI 95092 ( $\times 650$ ):

Fig. 105. Part of extensive multiloculate ascostromatal band, note periphysate ostiole;

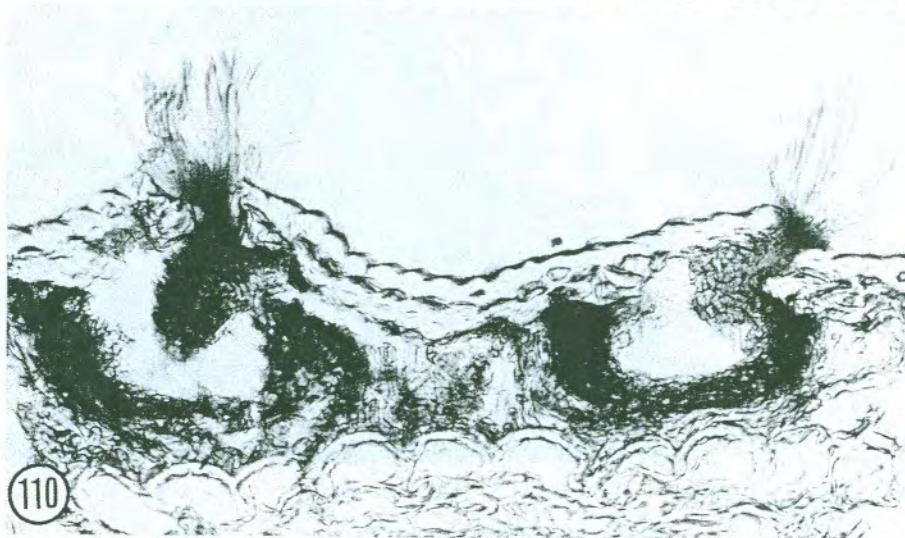
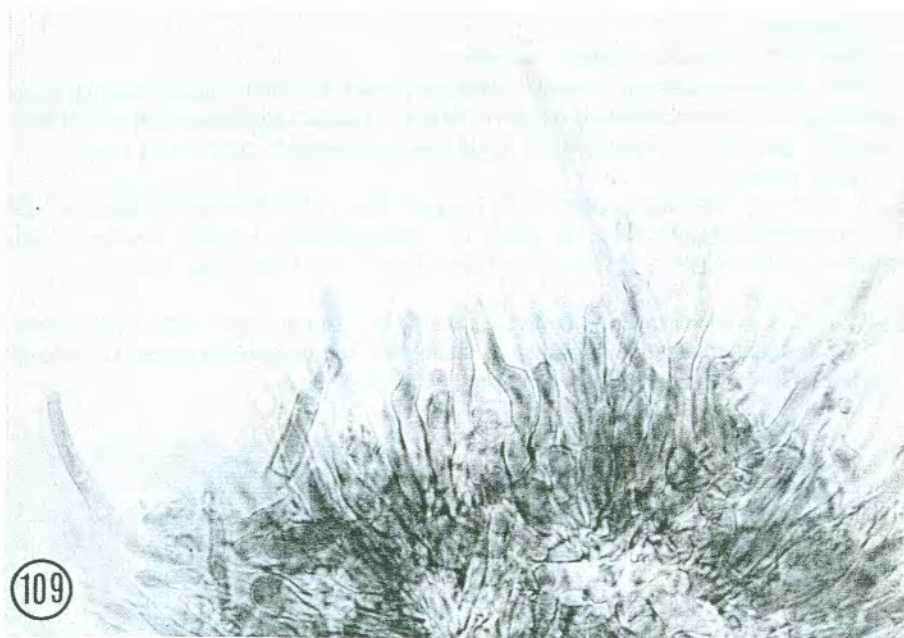
Fig. 106. Discrete, uniloculate ascostroma.



Figs 107-108. *M. gibsonii*, paratype, IMI 112710 ( $\times 650$ ):

Fig. 107. Spermatogonial locule;

Fig. 108. Conidiogenous cells forming on exposed stroma after rupture of host epidermis.



Figs 109-110. *Cercoseptoria pini-densiflorae*, on *P. oocarpa* from Nicaragua:  
Fig. 109. Sporodochium on needle surface, showing sympodial, polyblastic conidiogenous cells ( $\times 1000$ );  
Fig. 110. Deep-seated stroma, with spermogonial locules, producing conidiogenous cells in caespituli, emerging through median or lateral longitudinal splits in epidermis ( $\times 250$ ).

## Zimbabwe:

IMI 112710, as *M. gibsonii*, see above

Red, necrotic secondary needles with grey-black stromatic bands bearing immature ascostromata, spermogonia and conidiomata (Figs 117-118). Conidia cylindrical with a rounded apex and swollen, truncate base, 3-8 septate,  $(40- ) 45-68 (-75) \times 3-4 \mu\text{m}$ , MCL =  $53.6 \mu\text{m}$ .

## Hong Kong:

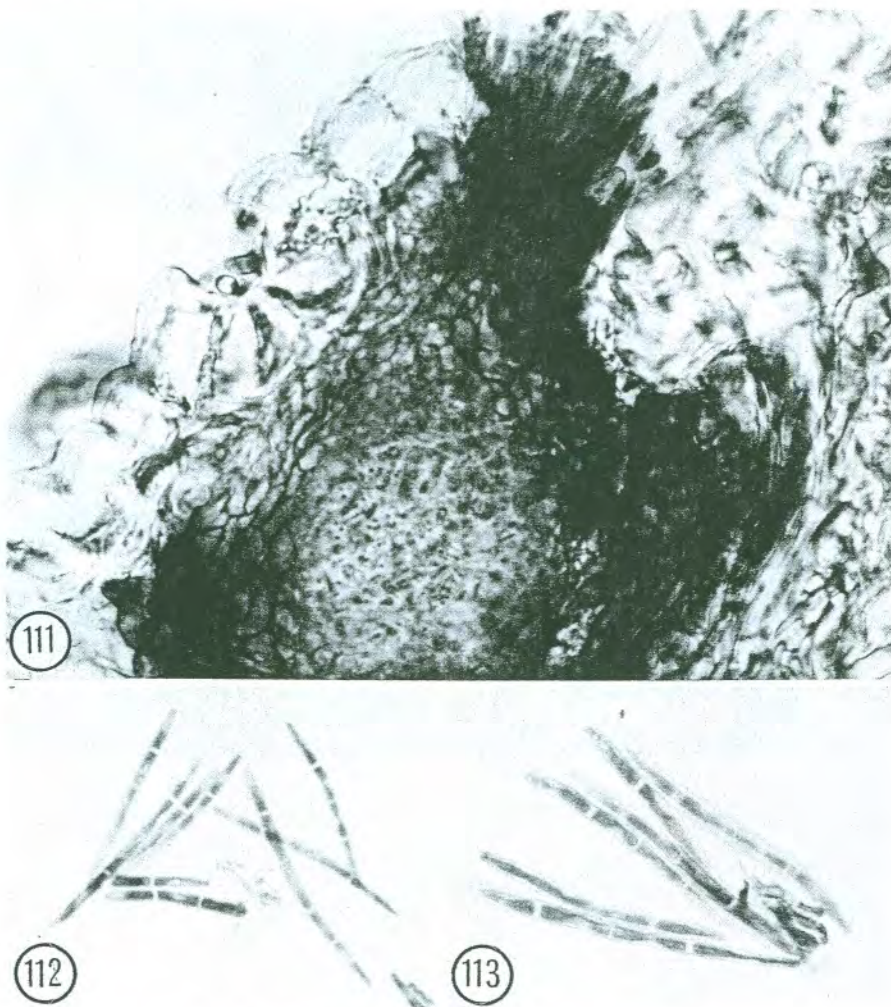
*P. caribaea*, Tai Lung nursery, R. I. Leather, Nov. 1967, IMI 133795 (also IMI 122031, *M. gibsonii*).

Conidiomata scattered or in bands on yellowish-grey, necrotic needles. Conidia cylindrical, 1-4 septate,  $(12- ) 15-45 (-58) \times 2.5-3.5 \mu\text{m}$ , MCL =  $30.1 \mu\text{m}$  (Fig. 114D).

## India:

*P. radiata*, Amarkantah, Madhya Pradesh, R. Reddy, Apr. 1972, IMI 167108.

Discrete and stromatic conidiomata, some with spermogonial locules. Conidia cylindrical, mainly 4 septate,  $(30- ) 34-48 (-52) \times 2.5-3 \mu\text{m}$ , MCL =  $40.3 \mu\text{m}$ .



Figs 111-113. *C. pini-densiflorae* on *P. oocarpa* from Nicaragua:

Fig. 111. Caespitulum emerging directly through ruptured epidermis and not through adjacent stoma, note spermogonial locule forming within stroma ( $\times 650$ );

Figs 112-113. Conidia ( $\times 650$ ,  $\times 1000$ ).

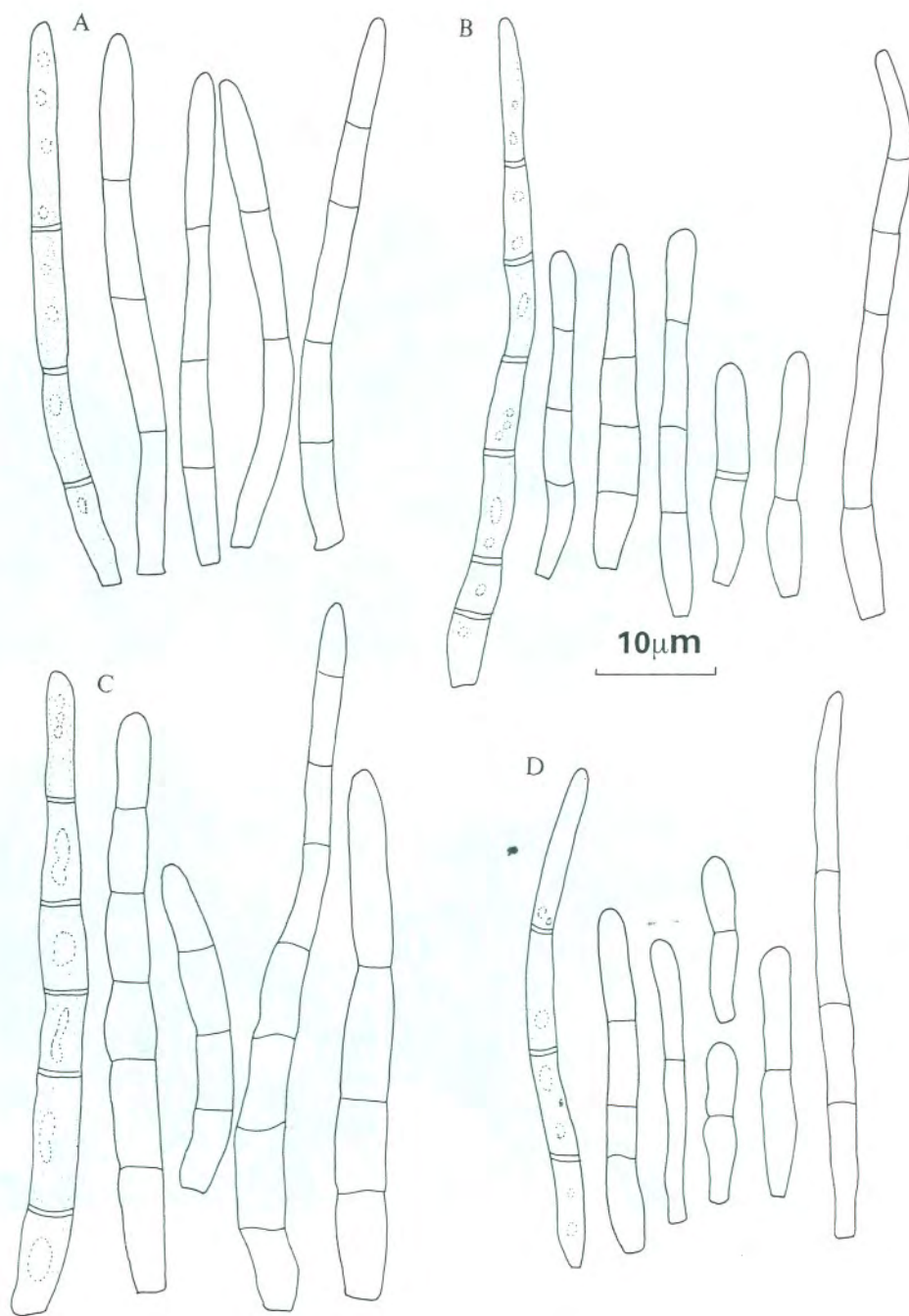
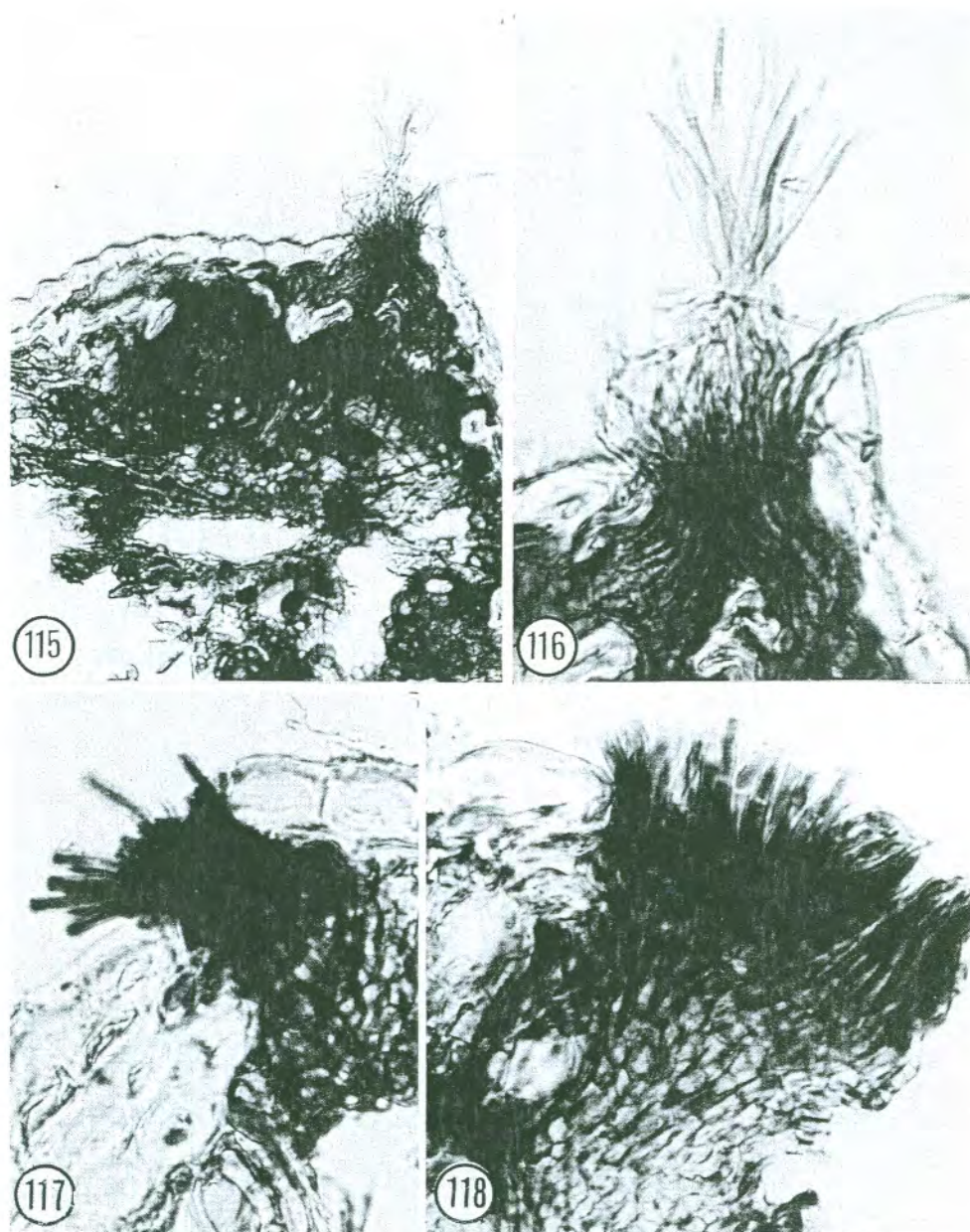


Fig. 114. *C. pini-densiflorae*, conidial morphology on:  
 A, *P. oocarpa*, Nicaragua, IMI 281636;  
 B, *P. radiata*, Tanzania, IMI 92286;  
 C, *P. radiata*, Tanzania, IMI 103170;  
 D, *P. caribaea*, Hong Kong, IMI 133795.



Figs 115-118. *M. gibsonii*, African collections with associated *C. pini-densiflorae* conidiomata:  
Figs 115-116. Deep-seated ascostroma bearing fascicles of conidiogenous cells, holotype, IMI 92286 ( $\times 250$ ,  $\times 650$ );  
Figs 117-118. Conidiogenous cells forming where stroma ruptures host epidermis, paratype, IMI 112710 ( $\times 650$ ).

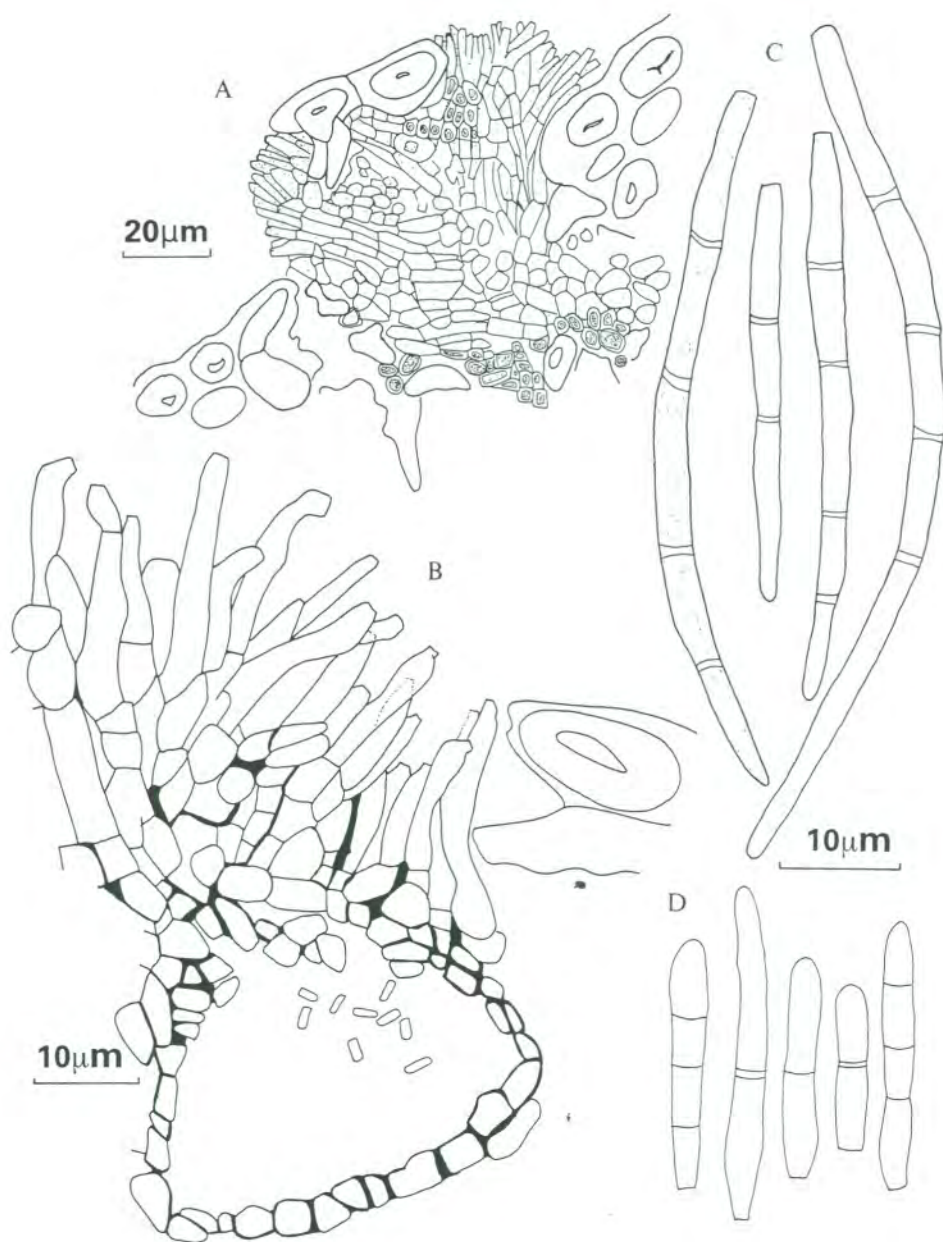


Fig. 119. *C. pini-densiflorae*, morphology of Philippine isolate, IMI 250111:  
 A, Conidioma, showing deep-seated, erumpent stroma;  
 B, Mature conidioma (sporodochium) with spermogonium;  
 C-D, Variation in conidial size, the former type (C) predominates.

## Sri Lanka:

*P. radiata*, Talawakele, Central Province, B. C. Sutton, Jan. 1973, IMI 173410.

Reddening of distal region on primary needles, diffuse banding; grey-yellow necrosis on older needles. Conidia few, cylindrical, stout, 3-6 septate,  $35-55 \times 3-4.5 \mu\text{m}$ .

## Philippines:

IMI 250111, as *M. gibsonii*.

Conidiomata numerous, scattered, on brown necrotic needles, typically discrete arising from stomata, or occasionally from a deep-seated stroma containing spermogonia, forming dense cushions (sporodochia) (Fig. 119A-B).

Conidia predominantly narrow cylindrical, 2-6 septate,  $(35-40-70 (-75) \times 2.5-3 \mu\text{m}$ , MCL =  $51.2 \mu\text{m}$  (Fig. 119C); significantly smaller, clavate-cylindrical, 1 (-3) septate conidia also present,  $14-22 (-28) \times 1.8-2.5 \mu\text{m}$ , MCL =  $19.5 \mu\text{m}$  (Fig. 119D).

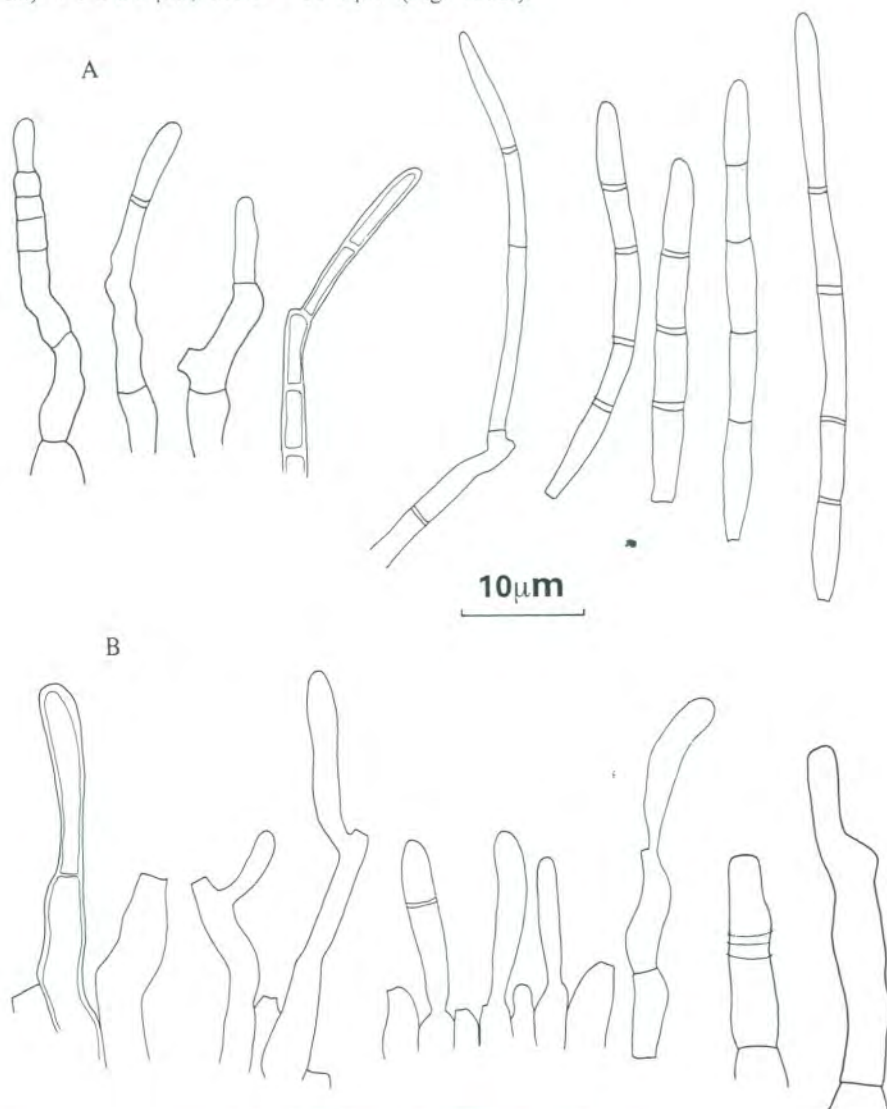


Fig. 120. *C. pini-densiflorae*, conidiogenesis:

A, Conidiogenesis and conidia in culture, IMI 281637;

B, Conidiogenesis on host, note faint annellations on older conidiogenous cells, IMI 92286.

# Vietnam:

*P. merkusii*, Than Long, S. Uhlig, 1974, IMI 202929.

Conidiomata stromatic in grey to red, necrotic areas.

Conidia narrow cylindrical, 2-6 septate, (35-) 45-60  $\times$  2-3  $\mu$ m, MCL = 50.1  $\mu$ m.

# Japan:

*P. pinaster*, Kyoto, A. Ueyama, Apr. 1962, IMI 92836.

Greyish-yellow needle blight; conidiomata discrete, substomatal. Conidia cylindrical, 1-7 septate, (25-) 30-55 (-60)  $\times$  2.5-3.5  $\mu$ m, MCL = 41.2  $\mu$ m.

IMI 92835 on *P. densiflora* similar to above but stromatic tissue abundant, containing spermogonia, and forming black bands alternating with yellow-orange necrotic bands.

# Cultures

Colonies grey to greyish-green or black, attaining 1.8-2.2 cm after 15 days on PCA-UV at 25°C; low, compact, grey mycelium becoming pulvinate in centre, black reverse. Most isolates non-sporulating but IMI 281637 formed either lilac-grey mycelial segregants with ill-defined conidiophores or compact, green, sporodochial-like aggregations bearing olivaceous, narrow cylindrical conidia, 3-10 septate, (32-) 40-65 (-90)  $\times$  1.8-2.5  $\mu$ m (Fig. 120A). On PCA seeded with sterile pine needles, this isolate and IMI 250111 become strongly stromatic producing rows of spermogonial stromata on the needle surface, 140-180  $\times$  70-100  $\mu$ m.

# Conidiogenesis

Conidiogenesis holoblastic, initially solitary, becoming polyblastic, sympodial (Fig. 120B). Faint annellations infrequently present on old conidiogenous cells from the host and in culture indicating that percurrent development also occurs. Conidia appear to be produced in dry fascicles but a faint mucilaginous covering is sometimes evident.

# Discussion

*M. gibsonii* has been variously received and identified at the CMI as *Mycosphaerella* sp. ("probably new") and *M. pinicola*. The East African collections on *P. radiata* are macroscopically and microscopically similar to both *M. dearnessii* and *M. pini*. The Asian collections, however, usually on pines with less robust needles, are markedly less stromatic and ascostromata when present, are typically uniloculate and weakly erumpent. The ostiolar region of *M. gibsonii* differs slightly in that it is more pronounced, often made up of long, tooth-like cells which are probably thickened and pigmented conidiogenous cells. Ascus and ascospore sizes overlap with those of the other two species and hence these characters cannot be used satisfactorily in order to distinguish between them.

The purported anamorph is *Cercoseptoria pini-densiflorae* but single ascospore cultures are necessary to confirm this association, assuming that a sporulating colony is obtained.

However, the genus *Cercoseptoria* is an accepted anamorph of *Mycosphaerella* (Arx, 1983) and the occurrence of conidiogenous cells on the ascostromata of the holotype of *M. gibsonii* together with the presence of ascostromata and spermogonia on worldwide collections of *C. pini-densiflorae* and the induction of the spermogonial (*Asteromella*) state in culture, is strong circumstantial evidence to support this hypothesis.

Conidial morphology is variable but, although there are indications that distinct spore types do exist, there are no readily definable categories as in *M. dearnessii* and *M. pini*. Various Japanese workers have compared geographic and host collections of the fungus and give conidial ranges of 12-90  $\times$  1.5-3  $\mu$ m (Ito, 1972; Kobayashi *et al.*, 1979; Suto, 1979); mean length and septation were similar to those recorded during the present study (40-50  $\mu$ m, 3-4 septate).

Chupp (1954) and Ellis (1976) accepted the species in the genus *Cercospora* and it is still most frequently referred to this genus, even though Deighton (1976) transferred many *Cercospora*-like fungi with unthickened conidial scars to other genera, placing the brown needle fungus in *Cercoseptoria*. The latter is typified by acicular conidia, tapering gradually towards the apex from the truncate base, and densely fasciculate conidiogenous cells arising from a prominent substomatal stroma. Stromatal structure is a useful diagnostic character, and obviously related to the dothideaceous nature of the teleomorph, but

conidial morphology, which Deighton places much emphasis on, "... showing no sign of a slightly obclavate shape", is a much more tenuous character. Chupp, in fact, describes the conidia of Formosan specimens of *C. pini-densiflorae* as obclavate to obclavato-cylindric, in sharp contrast to Deighton's primary distinguishing character for separating *Cercoseptoria* from *Pseudocercospora*. Similarly, the short, clavate, 1-septate conidia and the stout conidial forms from some East African collections do not conform to the concept of *Cercoseptoria* spores "... narrow (not more than 3 µm wide), acicular, tapering gradually from close to the base towards the apex but not obclavate ..." (Deighton, 1976).

Both Kobayashi *et al.* (1979) and Suto (1979) found that *C. pini-densiflorae* in inoculation tests could infect and sporulate on non-*Pinus* hosts, including species of *Abies*, *Cedrus*, *Larix* and *Picea*, although pathogenicity was restricted. Interestingly, a very similar stromatic needle fungus, *Mycosphaerella laricina* (Hart.) Neg., is an increasingly important pathogen of European larch (*Larix decidua* Mill.) in the U.S.A. (Patton & Spear, 1983) and conidial measurements of the, as yet unnamed, *Cercoseptoria* anamorph overlap with those of the pine fungus. Erumpent, stromatic conidiomata, spermogonia and ascomata are common to both species and reveal an obvious close relationship. The authors described the conidiomata of *M. laricina* as acervuli, however, it is highly unlikely that conidiogenesis is initiated before the stroma ruptures the epidermis and the fructifications, therefore, are probably best regarded as sporodochia or caespituli (Deighton, 1976).

### ECOLOGICAL CONSIDERATIONS

All three species are considered to be indigenous to Central America but, whereas *Mycosphaerella dearnessii* and *M. pini* are common representatives of the pine mycoflora, *M. gibsonii* appears to be rare. The discovery of the purported *Cercoseptoria* anamorph of the latter species in the natural pine stands of Nicaragua is an unusual record since historically the fungus was thought to have had a strictly Asian distribution before its relatively recent spread to East Africa (Ito, 1972; Gibson, 1979). The Central American collection, from a remnant stand of *P. oocarpa*, indicates that the fungus is adapted to an upland subtropical climate and the African and Asian records in Herb. IMI would seem to confirm this supposition. However, conclusions as to its natural distribution in Central America are speculative since the species was not regularly collected. The single Nicaraguan collection was from hanging needles on young regenerating trees and contained the anamorph together with an associated *Asteromella* state. It is probable that the teleomorph occurs on older, cast needles but in this particular locality, where most needles are colonised by *M. dearnessii*, it may be difficult to distinguish between ascostromata of the two species in the litter layer. Ascstromata were not encountered in any of the Japanese specimens examined and there have been no previous reports of a teleomorph connected with *Cercoseptoria pini-densiflorae* in Japan. It would seem unlikely, therefore, that the teleomorph occurs in that country, where the pathogen has been intensively studied because of its economic importance. The teleomorph also appears to play an insignificant part in the disease epidemiology in Vietnam and the Philippines. Conversely, a serious pine needle blight reported from Hong Kong apparently involved only the *Mycosphaerella* stage (Leather, 1968), although the anamorph was subsequently recorded from the same locality. The East African records, almost exclusively on *P. radiata*, consisted of collections of both teleomorph and anamorph, either separately or in direct association. The development of complex ascostromata in well-defined bands may be a direct response to climate and/or host.

*M. dearnessii* has a particularly wide host and habitat range in Central American pine forests occurring on: *P. caribaea* in the humid, coastal, tropical savanna and in the dry, inland valleys; *P. oocarpa* in both wet and dry, subtropical, upland habitats; *P. tecumumanii* and *P. maximinoi* in the increasingly wet cloud forest areas; high-altitude pines in the cooler, almost temperate, regions of Honduras and the highlands of Guatemala and Mexico. Each change of habitat appears to involve a significant variation in fungal morphology, particularly in conidial pigmentation or ornamentation and size. It is still uncertain, however, if these forms could be best regarded as morphotypes, resulting from phenotypic plasticity, or ecotypes, involving genetically adapted forms. In Fig. 56, conidial size is plotted against altitude. As can be seen, only large spores, which are typically heavily pigmented and ornamented, occur on *P. caribaea* at low elevations (below 600 m) and it is hypothesized that this is a direct consequence of the high solar radiation in these tropical habitats. In the wet savannas of the Caribbean coast, the conidia are exuded in

dark green or black mucilaginous cirri, whilst in the semi-arid, inland pine stands, there is evidence that the conidia are more pigmented and thicker-walled and surrounded by less mucilage, often occurring loosely on the needle surface rather than in compact, mucilaginous aggregations. This may be an adaptation to both wind as well as to the normal rain-splash dispersal. Ascstromata were also collected with greater frequency in these drier zones. At the upper altitudinal limit of *P. caribaea* (700-800 m), where there is mixing and putative hybridisation with *P. oocarpa*, a second conidial type can be delimited, showing a marked reduction in size, pigmentation and ornamentation. Since both spore forms may be present on the same needle, although apparently in separate acervuli, it has not been proven if they represent variants of a heterogeneous race or distinct races. The large-spored form, however, becomes progressively less common in the higher, wetter sites where *P. oocarpa* merges with *P. maximinoi* and has not been found in any of the *P. tecumumanii* needle collections. Nevertheless, the interpretation of a single small-spored type becomes untenable in these *P. oocarpa* stands because of the great range in conidial morphology. In a 25-30 km journey through almost continuous *P. oocarpa*, from the high Sierra above La Paz (1600 m) to Marcala (1100 m), three or four types of conidia were distinguishable; varying in size, septation, wall ornamentation and pigmentation. Conversely, in Honduran collections of *P. tecumumanii*, conidia are extremely uniform being consistently small, 1-septate and weakly pigmented and ornamented, whilst on the high-altitude *P. ayacahuite*, conidial morphology does not correspond to any of the previously delimited types. The aforementioned small-spored isolate appears to be dominant in the *P. oocarpa* stands in Nicaragua and Guatemala and this is particularly evident in the upland, Nicaraguan *P. tecumumanii* collections. The latter are also characterised by the presence of ascstromata on green needles.

Dramatic variation in conidial morphology was demonstrated in a short 15-20 km transect in the Omoa range of Honduras but was also observed, over much smaller distances, in the semi-arid inland valleys, where the topography rises steeply to cloud or wet, montane forest. Clearly, sunlight and rainfall would seem to be the most important environmental variables governing spore form, particularly the composition of the wall. Thus in the high UV light habitats, the conidia are heavily pigmented, particularly in the drier areas, where wall thickness may also increase in response to low humidity. Build-up of melanin granules and other products in the spore wall may contribute to the apparent reduction in mucilaginous covering observed in such dry areas. In the mist-covered montane regions, colonised by *P. maximinoi* and *P. tecumumanii*, periods of intense sunlight are probably of short duration and confined to the drier months of the year. Thus, there would be no continual pressure to develop heavily-pigmented, thick-walled conidia, although there may be seasonal factors operating perhaps accounting for the variability of conidial form in *P. oocarpa* stands which occupy contrasting habitats, from dry, transition (700-800 m) to lower, cloud forest areas. On the highest peaks, however, exposure to UV light probably increases significantly and melanin incorporation in the spore wall may be essential for prolonging or maintaining conidial viability.

Spore size may also be determined by a complex relationship between the amount and distribution of annual precipitation, sunlight and host density. Large, heavily pigmented conidia may survive for longer periods than similarly pigmented small conidia and this would be of selection value in dry *P. caribaea* stands or where host populations are scattered as in the wet but widely-spaced stands of the Mosquitia coast. Fire is responsible for much of the irregular host distribution and thinness of stands in this region but is less common in upper montane forest where stands tend to be correspondingly denser. Thus, small, easily-splashed conidia may be the most effective dispersal units; wall thickness and pigmentation varying according to each particular situation from almost non-pigmented, thin-walled in permanent cloud forest to heavily pigmented, thick-walled in forests subjected to more seasonal change.

Whether or not these spore forms can be considered as ecotypes or morphotypes has not been conclusively proven. All records of the fungus outside of Central America correspond essentially to the large-spored form and there have been no reports that the small-spored form occurs in the U.S.A., either in the field or in culture isolates. This circumstantial evidence does suggest, therefore, that variation is stable and genetically-controlled and hence ecotypic. This stability is reflected in the large-spored collections from varying habitats both in the U.S.A. and other countries into which it has obviously been introduced, viz. Colombia, and possibly Canada and Yugoslavia. Earliest reports of the pathogen were from pines, including *P. caribaea*, in the subtropical, lowland, southern states of the U.S.A. (Hedgecock,

1929) and this typically southern distribution was emphasized later by Siggers (1944). Not until relatively recently has the fungus been recorded in the northern states (Skilling & Nicholls, 1974) and Canada (Laut *et al.*, 1966), indicating a non-indigenous occurrence and a rapid, possibly ecotypic, adaptation to the colder, temperate regions of North America. Kais (1972) noted physiological differences between these geographic isolates but not in spore morphology. Significantly, however, the teleomorph has not been found in any of these northerly collections and hence it would not appear to play a part in the fungal life cycle in cold climates. It is suggested here that *M. dearnessii* has a strictly Central American origin and that the large-spored form was introduced into the southern U.S.A. during the last century on *P. caribaea* imported from the Caribbean pine islands or from the Central American mainland.

*M. pini* has a much more restricted distribution in Central America being confined to the cloud forest region, and was not encountered below 1500 m. On the previous reasoning, therefore, it would appear that this species with hyaline, thin-walled conidia is perfectly adapted to such high humidity, low sunlight habitats. The initial collections, from hanging needles on trees in relatively exposed situations, were mainly teleomorphic and ascospores would seem to be functioning as primary dispersal propagules. In later collections, however, the anamorph was dominant, the conidiomata developing either as open acervuli or closed pseudopycnidia. The latter would obviously be less efficient in terms of spore production but stomatal formation may be of added survival value during unfavourable conditions protecting and enclosing the hyaline, thin-walled conidia which may be dispersed over a much longer period of time. In contrast to *M. dearnessii*, therefore, it is the conidiomata of *M. pini* which adapt to the environmental pressures, being open and acervular in optimum (overcast or cloudy) conditions and stomatal and locular (pseudopycnidial) during periods of high solar insolation or low humidity.

Conidial size and septation are generally variable within any one collection of *M. pini*, nevertheless, consistently small, clavate, 1-septate conidia were found in certain collections from both Honduras and Guatemala. Varying proportions of similar spores are represented in other collections, being particularly evident in material from the U.S.S.R. As in the case of *M. dearnessii*, spore size may be directly linked to dispersal efficiency within a particular habitat, being controlled by climate, topography and host distribution. Once again, there is no evidence as to the nature of this morphological variation, and whether or not it is an example of phenotypic or genotypic adaptation.

Interestingly, in the countries where *M. pini* has become a major needle pathogen (Chile, East Africa, New Zealand), the teleomorph has not been reported. It would seem that ambient conditions continually favour the anamorph, and selection pressures are low.

The fungus can survive and grow at relatively low temperatures. In Ecuador, plantations of *P. radiata* in the shadow of Cotopaxi are subjected to frosts and perhaps significantly, stomatal development, including the formation of pseudopycnidia and spermogonia, appears to be much more advanced in these collections compared with those at lower elevations (Evans & Oleas, 1983). Murray & Batko (1962), investigating an isolated outbreak of red-band disease in pine nurseries in Dorset, reported that needle necrosis took place during the winter and spring months, although severe infection was correlated more with the warm, wet summers. The combination of mild winters and occasional humid summers, which occurs in this part of the U.K., probably promotes sporadic activity of the fungus which is almost certainly near the limit of its geographic range.

Red-band disease is endemic on native North American pine species in the north-western states of the U.S.A. and Canada suggesting that *M. pini* is indigenous to that region. Nevertheless, the possibility of an earlier introduction of the pathogen from Central America, where on the evidence of the present study it is considered to be an indigenous species, should not be over-ruled. The earliest known record of the fungus from the U.S.S.R. may indicate the initial route of its introduction into Europe. Undoubtedly the rapid and worldwide dissemination of *M. pini* is directly related to the importation of exotic pines and suggests an efficient dispersal mechanism, most probably stomatal tissue, containing teleomorph or anamorph locules, amongst seed debris.

#### PATHOLOGICAL CONSIDERATIONS

*M. dearnessii* is omnipresent on pines in Central America but rarely can it be identified with a serious blight condition. Yellow, orange, red or brown necrotic spots and bars occur on older, secondary needles causing some premature needle cast. Acervuli and occasionally spermogonia may develop on green

needles with limited accompanying necrosis and clearly this obligate parasite has developed an almost symbiotic relationship with its host in natural pine stands. Severe needle yellowing and necrosis were observed but typically on debilitated trees or on hosts at the extremes of their altitudinal range. Pine nurseries in Honduras have invariably been established adjacent to natural pine stands but, although surrounded by mature trees carrying a heavy fungal inoculum, brown-spot symptoms were not observed on nursery seedlings during the survey, even though the disease is notably more severe on young plants (Siggers, 1932; Wolf & Barbour, 1941). Earliest reports of *M. dearnessii* as a serious needle blight pathogen are on longleaf pine (*P. palustris*) in the Gulf States of the U.S.A. (Hedgcock, 1929), and this is additional evidence to support the hypothesis that the fungus is an exotic in this region, attacking susceptible (non-adapted) indigenous pine species. Brown-spot blight continues to be the most important disease of *P. palustris* in southern U.S.A. and is the main limiting factor to the establishment of this pine species throughout most of its natural range (Henry, 1954; Jewell, 1983). Luttrell (1949) also associated it with a serious decline of *P. ponderosa* in Missouri, confirming Siggers' earlier observations that acervuli and conidia could be found throughout the year whilst ascostromata developed predominantly during the winter on fallen needles. Siggers (1944) recorded the most northerly distribution of *M. dearnessii* as Ohio and, although it was not reported from Wisconsin until the 1960's, earlier accounts of the fungus from this area later being ascribed to *M. pini* (Prey & Morse, 1971), the pathogen is now a major constraint to the growing of *P. sylvestris* (for Christmas trees) in several north-central States (Nicholls & Hudler, 1972; Nicholls *et al.*, 1973). In contrast to southern populations of the pathogen, conidia are produced only in the spring and summer (May-Sept.) and ascospores apparently play no part in disease epidemiology since ascostromata have not been reported (Skilling & Nicholls, 1974; Nicholls *et al.*, 1973).

*M. dearnessii* appears to be a highly adaptable pathogen with a wide ecological tolerance and host range, it has even been recorded on white spruce, *Picea glauca* (Moench.) Voss, (Skilling & Nicholls, 1974). This adaptability has been demonstrated during the Central American survey. Recent reports from the Altiplano of Colombia have shown it to be the cause of a severely debilitating needle cast of *P. radiata* (Gibson, 1980; Waller, J. M., pers. comm.). Examination of this material has revealed the presence of abundant ascostromata on hanging needles and a diffuse reddening or banding, normally diagnostic of *M. pini* infections. It seems, therefore, that *M. dearnessii* poses a threat to exotic pine plantations not only in the tropics and subtropics but also in temperate regions. However, the Central American pine species because of prior exposure and adaptation to the pathogen are likely to remain resistant, particularly if they are grown within their normal altitudinal range.

The results of the Central American survey also show that *M. pini* is an inhabitant of cloud forests, being adapted to subtropical-warm temperate, misty climates. On *P. maximinoi* in these habitats, the fungus was causing a red necrosis of both primary and secondary needles on regenerating trees but typically in exploited or exposed positions. Thus, this host appears to be susceptible within its natural range, possibly when predisposed by habitat disturbance. Nevertheless, needle cast was not severe and there was no evidence of *M. pini* on older forest trees. *P. tecumumanii* and *P. michoacana* were also identified as natural hosts but necrosis was limited.

Records of *M. pini* in other geographic areas demonstrate that the pathogen assumes importance only in subtropical high altitude or warm temperate climates and never in tropical habitats. In Central America, *P. caribaea* has not been exposed to *M. pini*, since it rarely occurs above 600-700 m. When this pine species is planted as an exotic in upland, humid situations, as in Jamaica (1100-1300 m) and Costa Rica (1100-1200 m), it has proved to be highly susceptible. Conversely, *P. oocarpa* (almost certainly including provenances of *P. tecumumanii*), *P. michoacana*, *P. ayacahuite* and *P. "pseudostrobus"* (= *P. maximinoi*) have been listed as being resistant or immune to *M. pini* in exotic habitats (Gibson, 1979), and all these species would have been exposed to the fungus in high-altitude Central American pine forests and hence possess some resistance. Obviously this would not apply to *P. caribaea* which should not be growing in habitats conducive to the pathogen.

The results of the Central American survey clearly show why *M. pini* has been so successful in adapting to high-altitude, subtropical-tropical, exotic pine plantations. The impact of the pathogen in such plantations in East Africa, Chile and New Zealand has been thoroughly reviewed by Gibson (1972, 1974, 1979) and various reports of its devastating effect, particularly on *P. radiata*, have appeared since (Pas, 1981; Eldridge *et al.*, 1981; Ford, 1982; Evans & Oleas, 1983). The natural range of *P. radiata* is a narrow

coastal strip of southern California from sea level to 300 m where it is relatively disease free, but at higher altitudes in northern California, the species is said to be prone to disease (Hepting, 1971). *M. pini* is probably the main cause of disease in these plantations (Cobb *et al.*, 1968; 1969). Once again, this provides evidence of a tree species being grown outside of its natural range, albeit only slightly, but in a region conducive to the pathogen which the host had never previously been exposed to. Thus, although *P. radiata* has proved to be a valuable plantation tree in exotic situations, adapting to a variety of habitats, its susceptibility to *M. pini* has proved to be a major limiting factor. *P. nigra* var. *austriaca* is another susceptible host on which the fungus was first recorded as the causal agent of a serious needle blight in central U.S.A. by Hulbary (1941) and later by Peterson (1965). Severe defoliation attributable to *M. pini* was also reported on *P. contorta*, *P. monticola* and *P. ponderosa* in Idaho (Thyr & Shaw, 1964). In British Columbia, *M. pini* was identified initially in 1963, causing a severe needle cast of exotic pine species, and was shown later to be widespread on both exotic and native pines (Parker & Collis, 1966). *P. radiata* proved to be particularly susceptible and suffered heavy mortality.

The apparent susceptibility of North American pine species within their natural range and the relatively recent discovery of the pathogen in this region seem to favour the theory that *M. pini* was introduced into a new host population and subsequently rose to prominence. It could also be argued that changing forestry practices, notably the introduction of highly susceptible hosts in plantation monocultures, had altered fungal behaviour and increased pathogenicity. The exact natural range of *M. pini*, therefore, is open to speculation, although on the evidence of the present study, Central America must be considered to be a centre of origin.

The occurrence of red-band needle blight in temperate Europe (U.K., France, Austria) has been more of academic rather than pathological interest and it has not been reported in epiphytotic proportions in this region. A combination of overall low temperatures and low humidity may restrict development of this essentially non-temperate fungus. Reports from other European and Asian countries is sparse and often confused, *M. dearnessii* usually being mistakenly implicated, but from the herbarium material received at CMI from Spain, Greece, Yugoslavia and the U.S.S.R., showing classic red-banding and severe needle necrosis, there is evidence to suggest that it may be locally important on certain hosts in humid Mediterranean climates.

*M. gibsonii* is a major obstacle to the natural regeneration and nursery cultivation of pines in southern Japan, including native species such as *P. densiflora*, *P. luchuensis* Mayr and *P. thunbergii* Parl. (Ito, 1972; Suto, 1979). The same species has been reported as causing severe defoliation of *P. radiata* in East Africa (Mulder & Gibson, 1972) and various pines in Malaysia (Ivory, 1975), the Philippines (Kobayashi *et al.*, 1979), Vietnam (Uhlig, 1973) and Hong Kong (Leather, 1968). Reports of this pathogen from the New World have been unsubstantiated (Gibson, 1979) until the present study. However it does not appear to have established itself as an important needle pathogen of pines in the American continent. Its occurrence on *P. oocarpa* in Nicaragua suggests that it is best adapted to a subtropical, upland habitat, possibly in less humid and warmer situations than *M. pini*, and as such is a potential threat to exotic plantations in the Southern Hemisphere.

## CONCLUDING DISCUSSION

The teleomorphs of all three species are morphologically very similar and difficult to distinguish without the aid of additional characters. There is such considerable overlap in both ascus and ascospore form that separation cannot be based on these structures. To a certain extent, the same is true of ascostromatal structure which varies within any one species according to a complex of factors, including host and climate. In general, however, stromatal development is much more controlled or organised in *M. dearnessii* so that the initially innate stroma becomes erumpent in stages, splitting the epidermis uniformly, invariably with two longitudinal slits either side of the stomatal lines. Thus, a flap or lid is lifted above the needle surface exposing the rows of ostiolate locules. Formation of stromatic tissue in *M. pini* is more vigorous and disorganised so that the fructifications burst through the epidermis irregularly, sometimes becoming almost completely erumpent and, although they typically aggregate in distinct bands on the needle, they less readily form into the integral, unified, multiloculate structure which is