# DISEASES OF COWPEA AND OTHER LEGUMES CAUSED BY *Pseudocercospora cruenta* and *Cercospora canescens*

by

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## ABSTRACT

The cultural and pathogenic variability and the host-pathogen relationships of isolates of two leaf spot pathogens of cowpea, Cercospora canescens and Pseudocercospora cruenta were investigated.

To study the physiological specialization of C. canescens, which has been recorded on several legumes, fourteen isolates from leaves of cowpea (Vigna unguiculata), lima bean (Phaseolus lunatus), bambarra groundnut (Voandzeia subterranea), mung bean (Vigna radiata) and groundnut (Arachis hypogaea) were examined.

Colony morphology, growth, and sporulation of the isolates on agar media varied considerably both within an isolate and between isolates; and each characteristic was influenced by the type of medium. The isolates produced either a pink-purple or glaucous green pigment on potato dextrose agar (PDA). Compatibility tests of the two groups of isolates based on an intermingling of mycelia, did not show a clear pattern of interrelationships.

Pathogenicity and cross-infection among the isolates of C. canescens was tested by inoculating both detached and intact leaves of selected leguminous and non-leguminous plant species in the glasshouse. Inocula obtained from agar culture was found to be less virulent, inducing chlorotic to

'green island' lesions on most of the cowpea cultivars tested. When inocula from infected leaves were used, lesion development was obtained readily, and isolates were observed to cross-infect other legume species as well as the non-leguminous plant species okra (Hibiscus esculentus) and sugar beet (Beta vulgaris). Similar pathogenicity tests with an isolate of Cercospora beticola from sugar beet and selected leguminous plants, showed that C. beticola infected cowpea, producing lesions similar to those observed on sugar beet in the field. The significance of the results are discussed in relation to the suggestion by some authors that a single Cercospora species occurs on several hosts.

The virulence of an isolate of Pseudocercospora cruenta and the reaction of 20 selected cultivars of cowpea of diverse origin was evaluated by inoculating detached and intact leaves. A great deal of variation in the disease reaction (lesion type, size and sporulation index) was observed for the cultivars. The influence of plant genotype and leaf age on symptom expression was studied on leaves of three age categories and three cultivars varying in susceptibility to infection with P. cruenta. Symptoms were observed to develop readily on more mature foliage and the size of the lesions formed were also large and of a spreading type on leaves that were 8-12 weeks old for the highly susceptible cultivar. To determine the nature of the host-pathogen interactions in relation to susceptibility/resistance, the behaviour of conidia of P.

cruenta was examined in leaf diffusates, in leaf extracts, and on leaf surfaces of the three genotypes x leaf age combinations. The results obtained indicated that leaf age and plant genotype had little effect on the pre-penetration growth of P. cruenta. It was concluded that no consistent differences or trends were obtained in the present study that could account for the differences in the symptom expression observed. These results are discussed in relation to reports in the literature of an inhibition of conidial germination and germ tube growth attributable to biochemical changes on young susceptible cowpea leaves and on both young and mature resistant leaves.

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## CONTENTS

## LIST OF TEXT TABLES

PAGE

## LIST OF PLATES

PAGE

## LIST OF FIGURES

## 1. GENERAL INTRODUCTION

Grain legume crops, characterised by their high protein content, are an important food source of protein of high biological value and of energy, minerals, vitamins and roughage. In addition, they provide nitrogen-rich residues in the soils thus enhancing the productivity of other crops grown in association with them.

In many developing countries in the tropics, legumes supply most of the plant proteins which are not only the main, but also the cheapest source of dietary proteins in areas where meat is scarce and too expensive for a large proportion of the population (Okigbo, 1978).

In the traditional farming systems of the tropics such as the rotational bush fallows of tropical Africa, the wet rice farming systems of South-east Asia, and the compound or homestead gardens of both areas, legumes are found as minor crops intercropped with the starchy major cereal or root and tuber staples. Legumes are also relay cropped through cereal crops such as sorghum in the semi-arid tropics and they are able to mature with the late season residual moisture after the sorghum has been harvested. Farmers in South and South-east Asia, frequently plant legumes after rice in rainfed lowland rice fields. In semi-arid West Africa, cowpea is traditionally intercropped with millet or sorghum. As soon as the rains become well established in early to late June, the cereal is planted; then in mid to

late July cowpeas are planted. If the planting of the legumes coincides with heavy rains, then 'Cercospora leaf spots' develop. These are caused by Cercospora canescens and Pseudocercospora cruenta, and have been recorded on a wide range of leguminous crops including cowpea, mung bean, bambarra groundnut, winged bean, hyacinth bean and many others (Mulder and Holliday, 1975). Foliar symptoms of Cercospora leaf spots, typically develop late in the growth of the crop and often after flowering as reported for cowpea (Verma and Patel, 1969; Schneider and Sinclair, 1975; Fery et al., 1977; Vakili, 1977) and mung bean (Mew et al., 1975).

Because Cercospora leaf spot diseases develop late, it has often been assumed that they are relatively unimportant, but recent studies on the epidemiology and potential crop losses caused by these diseases have shown that as much as 40-60% loss may occur, adversely affecting various components of yield (Schneider et al., 1976; Fery et al., 1977). Cercospora leaf spots frequently cause premature defoliation and this is important because yield losses in various legumes are greatest when defoliation occurs early in the pod-filling period (Enyi, 1975). According to Emechebe and Shoyinka (1985), crop losses caused by Cercospora leaf spots of cowpea have received far more attention than those caused by any other foliar disease of this crop. Until recently the pathogens causing the leaf spot diseases were regarded as species of Cercospora: C. canescens and C. cruenta. Cercospora cruenta is now known

as Pseudocercospora cruenta, having been transferred to the genus Pseudocercospora by Deighton (1976). The two species are quite distinct, C. canescens being very similar to C. apii, with hyaline conidia whereas P. cruenta has olivaceous conidia and a quite different type of conidiophore (Plate 4). On cowpea, studies by several workers have shown that leaf spot caused by P. cruenta is more important than C. canescens. However, C. canescens has been recorded as causing leaf spots on many other food legumes. It has been recorded as causing a severe leaf spot of bambarra groundnut (Voandzeae subterranea) in Ghana (Teyegaga and Clerk, 1972), and Zambia (Kannaiyan, 1988, personal communication). C. canescens has also been recorded as a pathogen of bambarra (ground bean) in Malawi (Subrahmanyan, 1983). According to Mew et al. (1975) C. canescens leaf spot is a major disease of mung bean in most of South-east Asia, and Poehlman et al. (1973) (cited by Thakur et al. (1980)) described the leaf spot caused by C. canescens as a threat to mung bean production in Indonesia, Thailand and Colombia. The most serious disease of mung bean identified at the Asian Vegetable Research and Development Centre AVRDC, is C. canescens leaf spot. During the rainy season from June to September infection of susceptible varieties generally causes complete defoliation which greatly reduces yields (Mew et al., 1975).

Although C. canescens and P. cruenta have been recorded as causing leaf spots on several tropical food legumes, no

studies have been made on the pathogenic variation within either of the species so that the specificity of leaf spot resistance remains unknown. Similarly, little is known of the underlying mechanisms of Cercospora leaf spot resistance (Allen, 1983). Past studies indicate that the relatively late development of symptoms may relate to biochemical changes in the host with age. On Cowpea, Schneider and Sinclair (1975) found for C. canescens that conidial germination and germ tube growth were each inhibited on the surface of young, but not of old leaves of a susceptible cultivar Prima. Similar inhibition was caused by diffusates from apical but not from basal leaves of a susceptible cultivar and by diffusates from both apical and basal leaves of a resistant cultivar. Ekpo and Esuruoso, (1977), also found that extracts from leaves of old plants of cowpea, (a susceptible cultivar, "New Era") enhanced germination of P. cruenta conidia whereas extracts from young plants did not. The above observations, indicate that susceptibility of cowpea to Cercospora leaf spot diseases may in part depend on a pre-penetration phenomenon, influenced by host plant maturity (Allen, 1983).

The aims of the research described in this thesis were threefold;

(1) to examine the cultural variability among isolates of C. canescens obtained from cowpea originating in three different countries, and among isolates from mungbean, bambarra groundnut, lima bean, and groundnut

originating from the Philippines, Zambia, Ghana and Malawi respectively; and to study isolates of C. canescens from the 4 hosts, in an attempt to determine if they could be regarded as strains of the same species or if they are different species;

(ii) to determine if the isolates from the different hosts were pathogenically similar and to explore their host range;

(iii) to determine the nature of host-parasite relationships, by examining and characterizing histologically external growth and penetration, internal colonization and subsequent sporulation of the fungi in host plants.

# 2. REVIEW OF LITERATURE

## 2.1 Tropical food legumes

The Leguminosae, comprises three sub-families, the Caesalpiniodeae, the Mimosoideae and the Papilionoideae; the first two consist mainly of tropical trees and shrubs with few economically important species. It is the members of the Papilionoideae that are of much greater agricultural importance. They are of value, firstly for their nitrogen-rich plant material for consumption by man and his animals, and secondly for the nitrogen-rich residue they leave in the soil after fixation of atmospheric nitrogen, thus enhancing the productivity of other crops grown in association with them. The human food they supply, is of three kinds: edible tubers; leaves, green pods and unripe seed (vegetable legumes); and ripe dry seeds (grain legumes, pulses). The Papilionoideae are classified in ten botanical tribes, three of which together contain the major vegetable and grain legumes. They are Fabae which includes the genera Vicia, Pisum, Lens and Cicer; the Phaseolea, which includes Phaseolus, Vigna, Glycine and Cajanus; and the Hedysarea which contains Arachis (Allen, 1983). Evans, (1979) lists twenty principal food legume species grown in tropical agriculture, at different elevations and rainfall zones, but the legumes vary in their adaptation to the extent that with the exception of India, no one tropical country is a major producer of more than three of the species of legumes. The most important food legumes grown in tropical Afro-Asia, include:- Cowpea (Vigna unguiculata), bambarra groundnut

(Vigna subterranea = Voandzeia subterranea); Chickpea (Cicer arietinum), mungbean or green gram (Vigna radiata = Phaseolus aureus) pigeon pea (Cajanus cajan), Soybean (Glycine max) winged bean or Goabean (Psophocarpus tetragonolobus) African yambean (Sphenostylis stenocarpa), groundnut (Arachis hypogae) and lima bean (Phaseolus lunatus). According to Sellschop, (1962) the most important leguminous crops in terms of production and consumption in Africa are groundnut, cowpea, and bambarra groundnut in that order. Cercospora leaf spot diseases caused by C. canescens and P. cruenta are of economic importance on cowpea and bambarra groundnut.

Cowpeas are predominantly a nutritionally important but minor component of subsistence agriculture in the semi-arid and sub-humid tropics of Africa and to a lesser extent, of India and Asia. The crop is grown for its mature seeds and/or its immature fruits and leaves (which are used as vegetables). The haulms are also fed to livestock (Steele et al., 1985). Cowpea in Africa is cultivated under diverse soil and climatic conditions and is traditionally grown with cereals. The bulk of production comes from small holdings in the semi-arid zones of West Africa, particularly in Nigeria, Burkina Faso and Senegal (Muleba and Ezumah, 1985). Cowpea is grown in many parts of Asia as a monoculture in rotation with cereals intercropped with maize, sorghum, pearl millet, cotton, cassava, sugarcane and relayed in standing rice (Pandey and Ngarm, 1985).

Bambarra groundnut is an indigenous African leguminous crop and one of the most important pulses grown on the continent. It is the only other important food legume in the West African region apart from cowpea. Bambarra groundnuts are grown for their edible seeds which are used as a nutritious pulse and not as an oil-seed. The fresh seeds are eaten in an unripe state and the dried seed is eaten after soaking and boiling. Several cultivars are recognized in Africa, differing in the shape of the leaves and the size, hardness and the colour of the seeds; the greatest variation is found in Togo and Zambia. Bambarra groundnut is adapted to a wide range of soils, especially light or sandy loams, and does well on very poor soils where most other crops fail. The crop features prominently in traditional farming systems in Africa as an intercrop of cereals and root crops, although it is planted as a sole crop in some areas. In a typical bambarra groundnut type of legume culture, the pure crop is usually planted at a very close spacing to form a dense mat, compared to the more common intercrop culture as practiced with cowpea and Lima bean (Stanton, 1968).

The other important food legume on which Cercospora leaf spots caused by C. Canescens and P. Cruenta are economically important, is mung bean or green gram (Vigna radiata = Phaseolus aureus). Mung bean is an important pulse crop in Asia, where the dried beans are boiled and eaten whole or after splitting into half. The green pods are eaten as a vegetable, the haulms are used as fodder and the

husk and split beans are useful in livestock feed. The crop is also grown for hay, green manure and as a cover crop. Farmers in South and South-east Asia, frequently plant mung bean in the rotation of rice with legumes in the lowland rice fields.

## 2.2 The genus Cercospora and related genera

### 2.2.1 Origin and Taxonomy

The genus Cercospora was created by Fresenius in 1864 (cited by Chupp, 1953) based on the species found on celery, (Apium graveolens), to include hyphomycetes with tail-like multiseptate spores borne on fasciculate conidiophores. Since then a number of related genera have been added, and Cercospora has therefore grown to become one of the largest of the genera of fungi (Fajola, 1978a). Ellis, (1971) points out that it had been customary for plant pathologists to describe as a new species any Cercospora found on a host plant for the first time, most species being named after the hosts on which they were found. Deighton and Leakey (1964), gave a description of the true Cercospora spp. morphologically similar to Cercospora apii, and pointed out that relatively few of these are distinct. Although a few species of Cercospora are host limited, there is considerable evidence to contradict the validity of using a host as a criterion for their distinction (Johnson and Valleau, 1949; Sobers, 1968). According to Deighton (1976) the majority of the species which have been included in the genus fall into two distinct taxonomic categories:- those in

which the old conidial scars on the conidiophores are thickened to a greater or lesser degree though always showing a distinct thickening even if this is only present as a rim; and those in which the scars are unthickened being no thicker anywhere than the wall of the conidiophore. There are variations in the thickening of the scars in the first category which may allow some further taxonomic divisions. The hilum on the conidium is thickened or unthickened in correspondence with the scar on the conidiophore. Thickened scars occur in the type species Cercospora apii and in the many similar species: C. beticola, C. nicotianae, C. canescens, C. kikuchii and C. ricinella. Sobers (1968), Deighton (1967, 1973) and Fajola (1978a, 1978b) consider those species of Cercospora having acicular, hyaline conidia with a dark hilum scar as belonging to the 'true Cercospora'. Further evidence suggesting interrelationships between the species with hyaline acicular conidia, have been based on growth rate and other cultural characteristics, in particular, the production in culture on PDA of a pink, maroon to purple pigment. Lynch and Geoghegan (1977) examined a total of 15 species of Cercospora for their ability to produce the pigment in culture on PDA exposed to a light intensity of 110 lux at 25$^{o}$C. Ten of the fifteen species, all with hyaline acicular conidia, including, C. beticola, C. apii, C. coffeicola, C. kikuchii and C. zebrina produced varying amounts of the pigment which diffused into the medium whereas the five other species with pale olivaceous to straw coloured conidia, C. vaginae from

sugarcane, C. elaedis from oil palm and C. pini-densiflorae from pinus spp., C. concors from potato (Solanum tuberosum) and C. ferruginea from Artemisia vulgaris, did not produce the pigment.

Similar observations were made by Fajola (1978c) when he examined twenty isolates of 17 species of Cercospora from 16 different hosts from Nigeria. Twelve of the isolates, including, C. apii, C. canescens, C. kikuchii, C. columnaris, C. nicotianae, C. citrullina and C. ricinella produced the pigment which coloured the surrounding medium red. The degree of production varied in the different isolates, with C. ricinella producing the greatest amount and C. nicotianae very little. Eight of the isolates from seven different hosts including C. cruenta from cowpea, C. abelmoschi from okra, C. caribae and C. henningsii from cassava which all have distinctly coloured conidia, did not produce any pigment. These species have been transferred to the related genera of Pseudocercospora, Cercosporidium and Phaeoramularia by Deighton, (1976). Roy (1982) also observed that ten Cercospora species from hosts other than soybean produced an intense maroon to purple pigment in culture and on inoculated soybean seeds, indistinguishable from that produced by Cercospora kikuchii which causes purple seed stain of soybean. He suggested that the ability of the species to produce a purple pigment in culture is also expressed in their ability to stain soybean seeds as suggested by Jones (1959) and Kilpatrick and Johnson (1956).

The species which produced hyaline acicular conidia with truncate bases on necrotic leaf spots of surface sterilized leaf discs on PDA, included, C. canescens, C. citrullina, C. zebrina, C. malayensis and C. sorghi. These observations, as suggested by Fajola (1978c) appear to confirm the trend in Cercospora taxonomy, in which those species having hyaline acicular conidia with dark hilum scar are considered to belong to the true Cercospora (Deighton, 1967; Sobers, 1968; Deighton, 1973; Fajola, 1978a, 1978b). In culture on PDA, most of the species with coloured conidia have raised dark to medium grey colonies (Pseudocercospora cruenta = C. cruenta Cercospora arachidicola, Phaeisariopsis personata) that grow to about half the size of C. apii colonies after about two weeks (Sobers, 1968). Steinkamp et al. (1981) have however, pointed out that the ability to produce Cercosporin, the pink to purple pigment is scattered through the genus Cercospora as well as through a complex of related genera, thus qualifying earlier statements that cercosporin is limited to the genus Cercospora (Lynch and Geoghehan, 1977), or is invariably produced by all species properly placed in the genus (Fajola, 1978a, 1978c). Venkataramani (1967) isolated cercosporin from Cercosporidium personatum, (=Phaeisariopsis personatum), and Melouk and Schuh (1987) also described, two of four isolates of Cercospora arachidicola, obtained from peanut grown in Oklahoma, USA, as "pink" because cercosporin was detected in cultures of the isolates maintained on peanut oatmeal agar. Petrie and Vanterpool, (1978) have also reported that Pseudocercosporella capsellae, the cause of

white and grey stem in members of the Cruciferae in Western Canada, produces cercosporin *in vitro*. The literature is however, not clear as to whether the production of cercosporin relates in all instances to a pink colouration of the medium. Chandrasekaran and Rangaswami (1960) reported that their isolate of *C. cruenta* produced a characteristic pink soluble pigment in both complex and organic synthetic media, and Rangaswami and Chandrasekaran (1962) also observed that among four species of *Cercospora* recorded on cucurbitaceous hosts in South India, *C. madrasensis* and *C. chidambarensis* both with hyaline acicular conidia produced varying quantities of a pink pigment in both synthetic and complex agar media. However, *C. citrullina* isolated from *Mormodia charanti* and *C. citrullina* var. *trichosanthei - anguinae* from *Trichosanthes anguina* also producing acicular hyaline conidia, did not produce any soluble pigment in any of the media tested. Balis and Payhe (1971) pointed out that various isolates of *C. beticola* differed greatly in their ability to produce cercosporin, and Assante *et al.* (1977) observed an isolate of *C. beticola* that did not produce cercosporin. Latterel and Rossi, (1983) have noted that *Cercospora zea-maydis* the cause of grey leaf spot of corn (*Zea maydis*), produces relatively broad and large conidia that are hyaline. The growth habit of the species ranges from black densely sporulating cushion-like colonies to white, cottony mycelial growth, one type often mutating from the other. Intermediate types include grey moderately sporulating colonies often with pink, red or purple pigment

depending on the substrate due to the formation of cercosporin crystals. It is therefore possible that non-pigmented sectors could overgrow the pigmented, and often sporulating colonies of most of the species. Lynch (1975) and Lynch and Geoghegan (1977) obtained stable cercosporin strains from C. beticola, C. apii, C. malvicola, C. zebrina, C. kikuchii and C. coffeicola but not from C. sesami, by continuously sub-culturing the various strains under illumination and selecting for Cercosporin production by sub-culturing red pigmented sectors arising on PDA.

### 2.2.2 Cercospora species recorded on tropical food Legumes

Chupp, (1953) listed ten species of Cercospora as pathogens of food legumes in the genera Dolichos, Glycine, Phaseolus and Vigna. These included: C. columnaris (= Isariopsis griseola), C. cruenta, C. dolichi, C. flagellifera, C. canescens, C. kikuchii, C. glycines, C. vanderysti, C. caracella and C. sojina.

Ellis (1971) listed 16 species of Cercospora as occurring in the family Leguminosae in general. Of these C. zonata, C. canescens, C. radiata, C. kikuchii, C. loti, and C. zebrina were species that formed hyaline conidia. The species with straw-coloured, pale brown or olivaceous brown conidia included:- C. arachidicola on Arachis spp., and C. cruenta mainly on Vigna spp. Allen (1983), includes about 20 species of Cercospora-like species which he considers are reliably described. C. arachidicola, Phaeisariopsis

personata, C. canescens, C. kikuchii, Phaeisariopsis griseola, Pseudocercospora cruenta, Cercospora sojina and other related species in the newly created genera of Cercoseptoria, Mycovellosiella and Stenella. C. arachidicola and Phaeisariopsis personata, which cause the serious diseases of groundnut, early and late leaf spot respectively are the most intensively studied. Others of major economic importance are P. griseola which causes angular leaf spot of bean; and C. kikuchii the cause of purple seed stain of Soyabean. These fungi are all widespread, wherever their respective hosts are grown. Pseudocercospora cruenta causes a leaf spot of cowpea and other legumes. C. canescens is considered as a comparatively weak pathogen with a wide host range, occurring often in mixed infections with Corynespora cassiicola, a much more weaker pathogen (Wei, 1950; Vakili, 1977).

### 2.2.3 Symptoms and disease cycles of leaf spot diseases caused by Pseudocercospora cruenta and Cercospora canescens.

Pseudocercospora cruenta induces angular shaped leaf spots which are delimited by the venation of the leaf. Although the disease is most frequently seen on the leaves, it may also occur on the stems. On the leaves, the lesions begin as chlorosis on the adaxial leaf surface which becomes dotted with necrotic spots, that in turn enlarge until the whole area is necrotic and brown (Plate 1A,B). In size, the diseased areas may vary from a few millimetres to a

centimetre in diameter, and the outline may be rather regular to very irregular. Chandrasekasan and Rangaswami (1960) reported diffuse chlorotic haloes around leaf spots on cowpea caused by P. cruenta in India. However, Amin et al. (1976) did not observe haloes around the irregular leaf spots, and suggested that the haloes around lesions may depend on the cultivar as well as on nutritional and environmental factors. Vakili (1977) concluded that the shape of the lesions depended on the leaf morphology of a cultivar and climatic conditions. When conidia are being formed, the lesions are usually irregular in outline and reddish brown in colour when viewed from the adaxial leaf surface of the leaflet (Plate 1C). On the abaxial leaf surface, the lesions are irregular in outline and reddish brown in colour at first, but when the pathogen is sporulating abundantly they are dark to black due to the presence of numerous conidiophores and conidia (Plate 1D). The conidiophores arise in a fascicle from stromata and are usually simple but sometimes branched. They are mid-pale olivaceous brown with small scars. The conidia are also olivaceous to brown, normally conclorous with the conidiophores (Mulder and Holliday, 1975). Latham (1934) described the disease cycle of P. cruenta on cowpea, and indicated that in the field the fungus grows intercellularly for some time before the mycelium becomes intracellular when the leaf spots become necrotic. After the pathogen has become established in the leaf, a single hypha branches and re-branches to form a loosely interwoven intercellular stroma. The stromata, usually develop in the sub-stomatal

PLATE 1

Symptoms of Pseudocercospora cruenta leaf spot of cowpea.

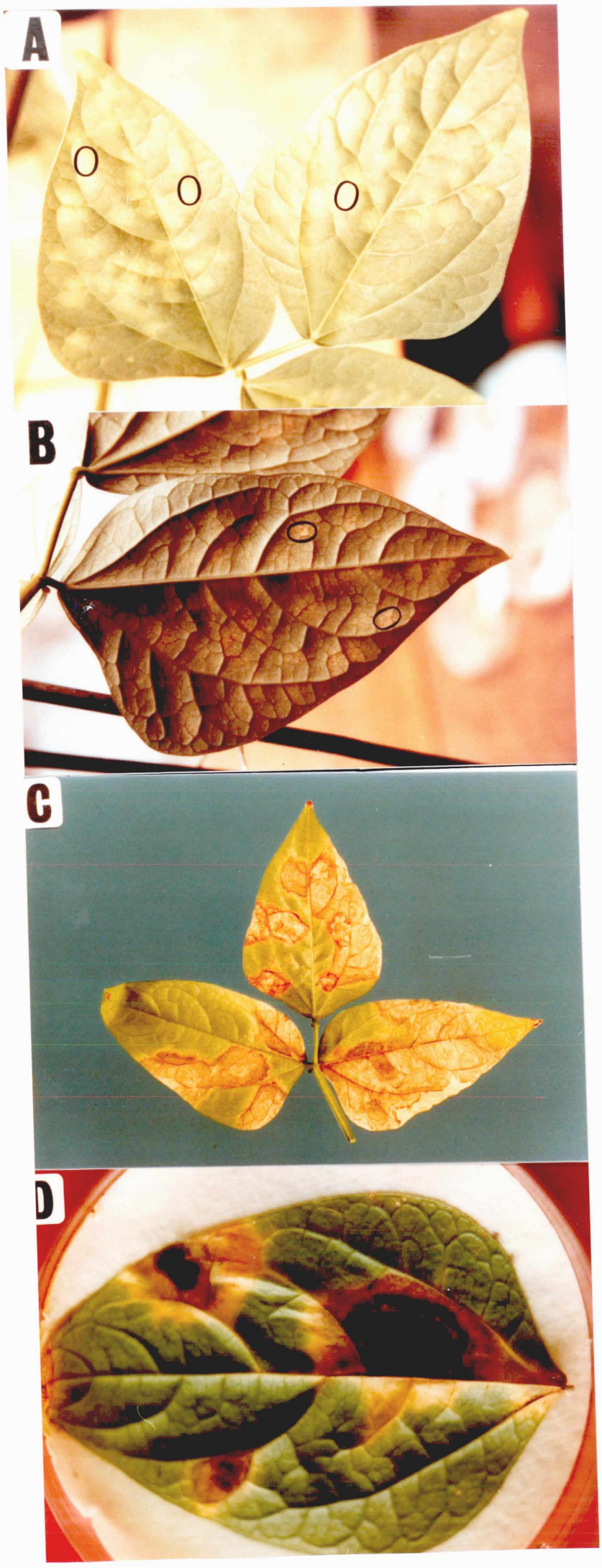
A. Chlorosis on adaxial leaf surface.

B. Necrotic spots developing in the chlorotic lesions.

C. Coalescence of necrotic lesions.

D. Dark sporulating areas of the lesions on the abaxial leaf surface, in Petri dish moist chamber.

Plate I



cavities but sometimes at other places. From the stromata arise the loosely fasciculate conidiophores. On old leaves small, black fruit bodies which resemble pynidia are formed. These spermogonia discharge numerous pycnospore-like bodies having the appearance of spermatia, which when mature are hyaline and rod-shaped (Plate 5). Perithecia develop from old sub-epidermal stromata, the mature perithecia being slightly beaked. Allen, (1983) notes that although P. cruenta and the groundnut leaf spot fungi; Cercospora arachidicola and Phaeisariopsis personata, produce perithecia their perfect stages Mycosphaerella arachidis, and Mycosphaerella berkeleyi have apparently never been found under African field conditions.

Cercospora canescens, unlike P. cruenta induces circular to irregular, cherry red to reddish brown lesions up to 10mm diameter on both leaf surfaces (Plate 2). On cowpea, the shape and size of the lesions also depend on the cultivar (Plate 3). When there are a large number of infections per leaflet, the lesions remain small and round before coalescing and covering the whole leaf. When numerous lesions are formed they cause the leaves to become chlorotic and absciss. The fungus sporulates on both surfaces of the leaf but more abundantly on the abaxial leaf surface. There is no reference in the literature to a detailed study of the disease cycle of infections by C. canescens, and unlike P. cruenta, no other spore types have been reported other than conidia. Conidiophores of C.

PLATE 2

Symptoms of Cercospora canescencs leaf spots on:-

A. Cowpea (Ex. Zambia).

B. Bambara groundnut (Ex. Zambia).

C. Mungbean (Ex. Philippines).

PLATE 3

Variation of C. canescens leaf spot lesions on differing cowpea cultivars originating from:-

KP. Ghana (Agricultural Research Station, Kpong).

$ZB_1$. and $ZB_2$. (Msekera Regional Research Station, Chipata).

TZ. Tanzania (Naliendele Oil Seed Research Project Mtwara).

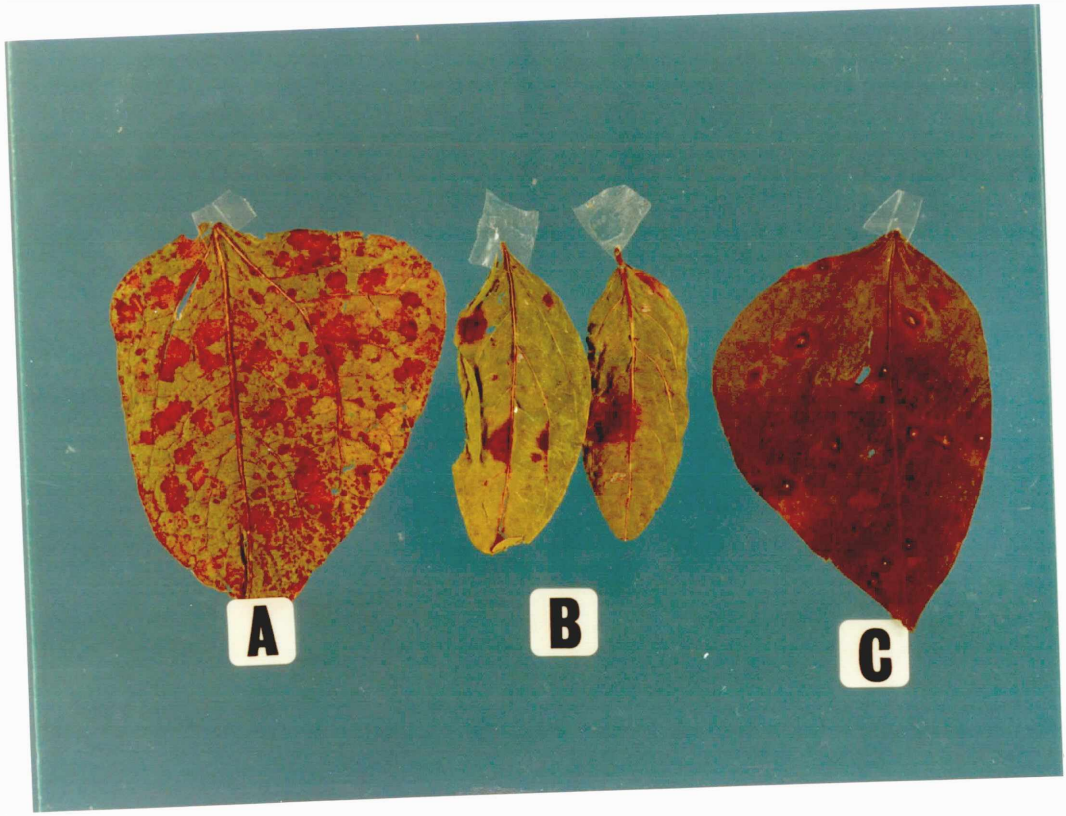



Plate 2

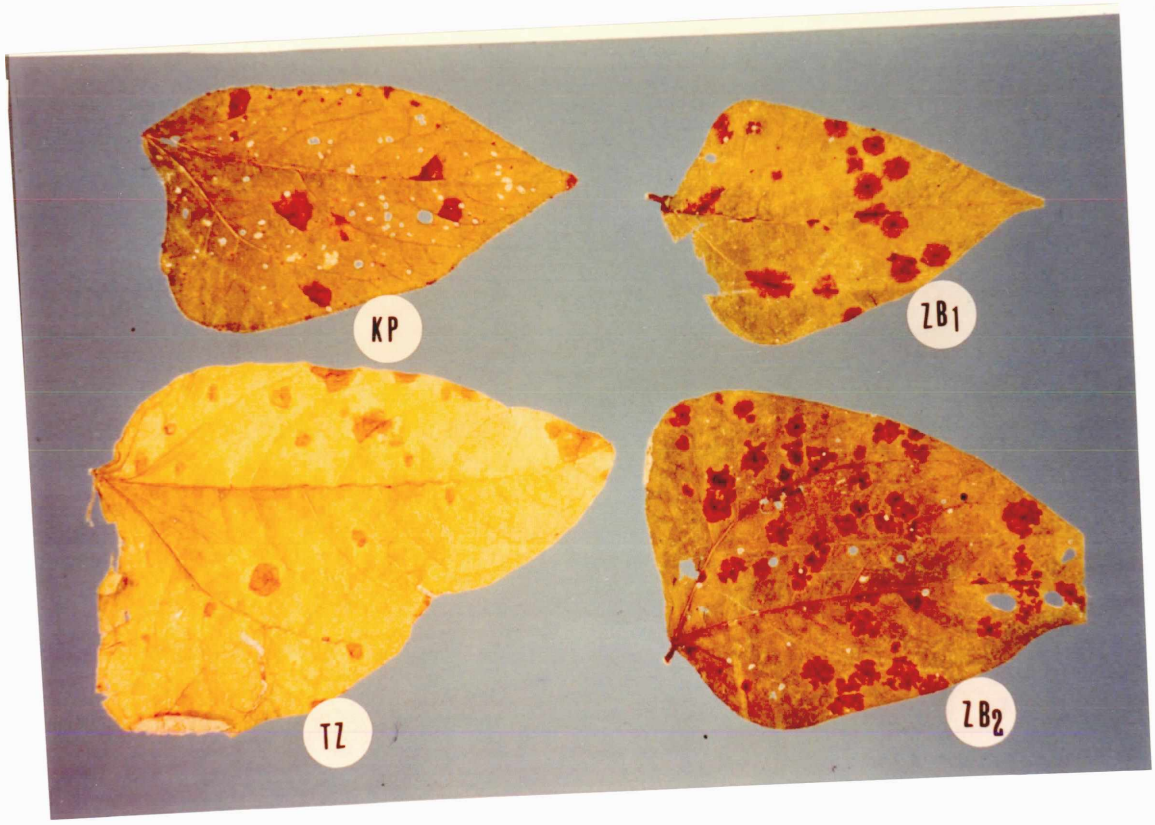



Plate 3

PLATE 4

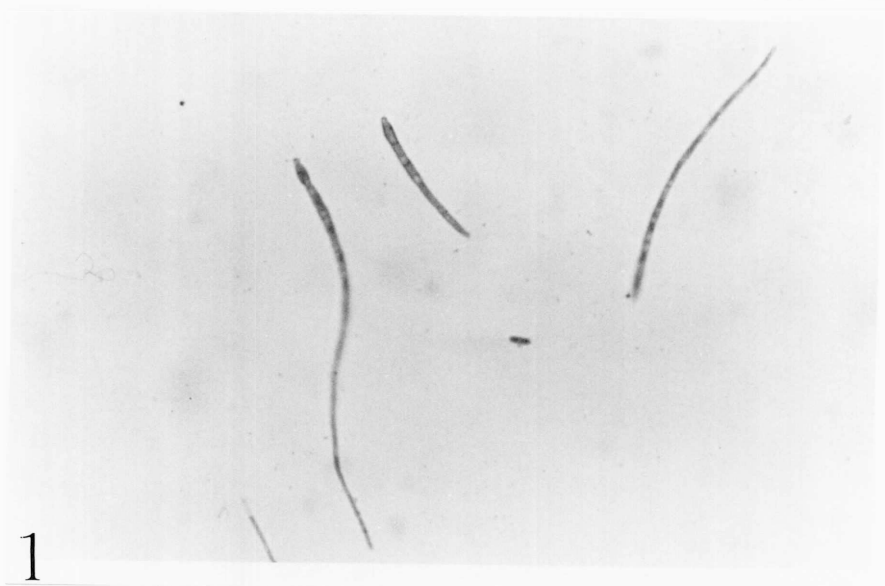
Morphology of conidiophores and conidia of Cercospora species causing leaf spots on cowpea.

Pseudocercospora cruenta (Figs. 1 and 2)

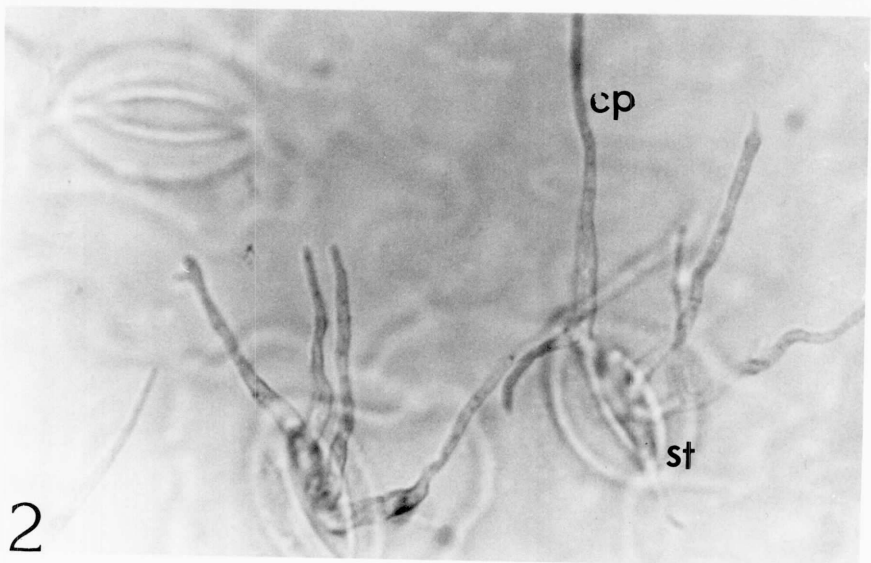
1. Conidia.
2. Conidiophores.

Cercospora canescens (Figs 3 and 4).

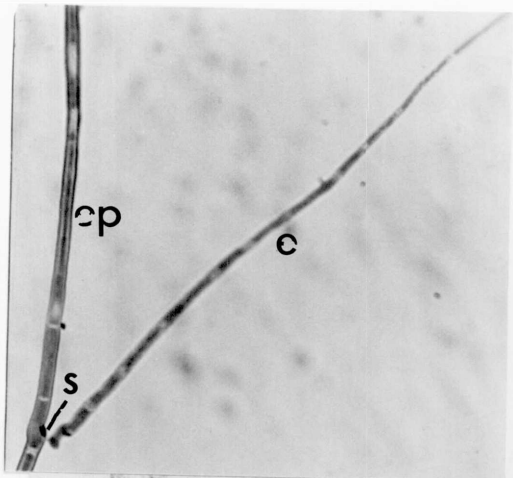
3. A conidium (C) showing basal scar (S) and the corresponding scar on conidiophore (Cp) showing point of attachment of conidium.
4. A fascicle of conidiophores with several geniculations (g), conidial scars (s) and detached conidia (c).



1




2

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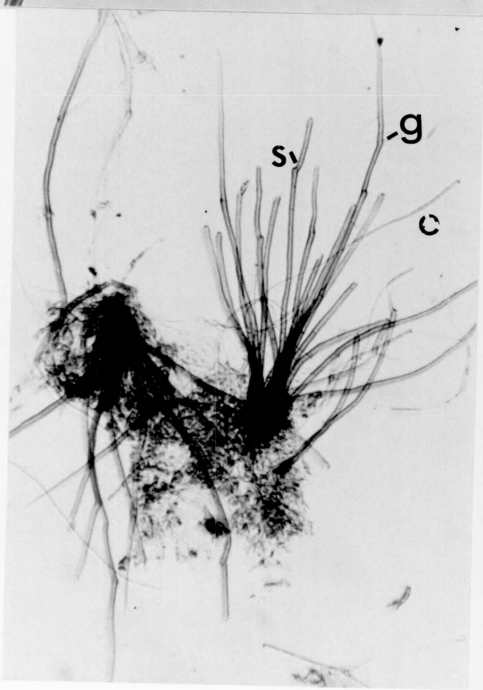



4

Plate 4

PLATE 5

Photomicrographs of cleared whole leaf pieces of field infected cowpea showing the spore types in the life cycle of Pseudocercospora cruenta

1. Hyphal branching and the formation of stromata (Str).
2. Developed stromata in sub-epidermal tissue.
3. Fascicles of conidiophores emerging through stomata.
4. View showing conidiophore fascicle with detached conidia (c).
5. Conidiophores arising from the walls of stromata =(sporodochium).
6. Spermogoina on leaf surface.
7. A mature spermogonium showing the ostiole.
8. A mature spermogonium discharging spermatia.

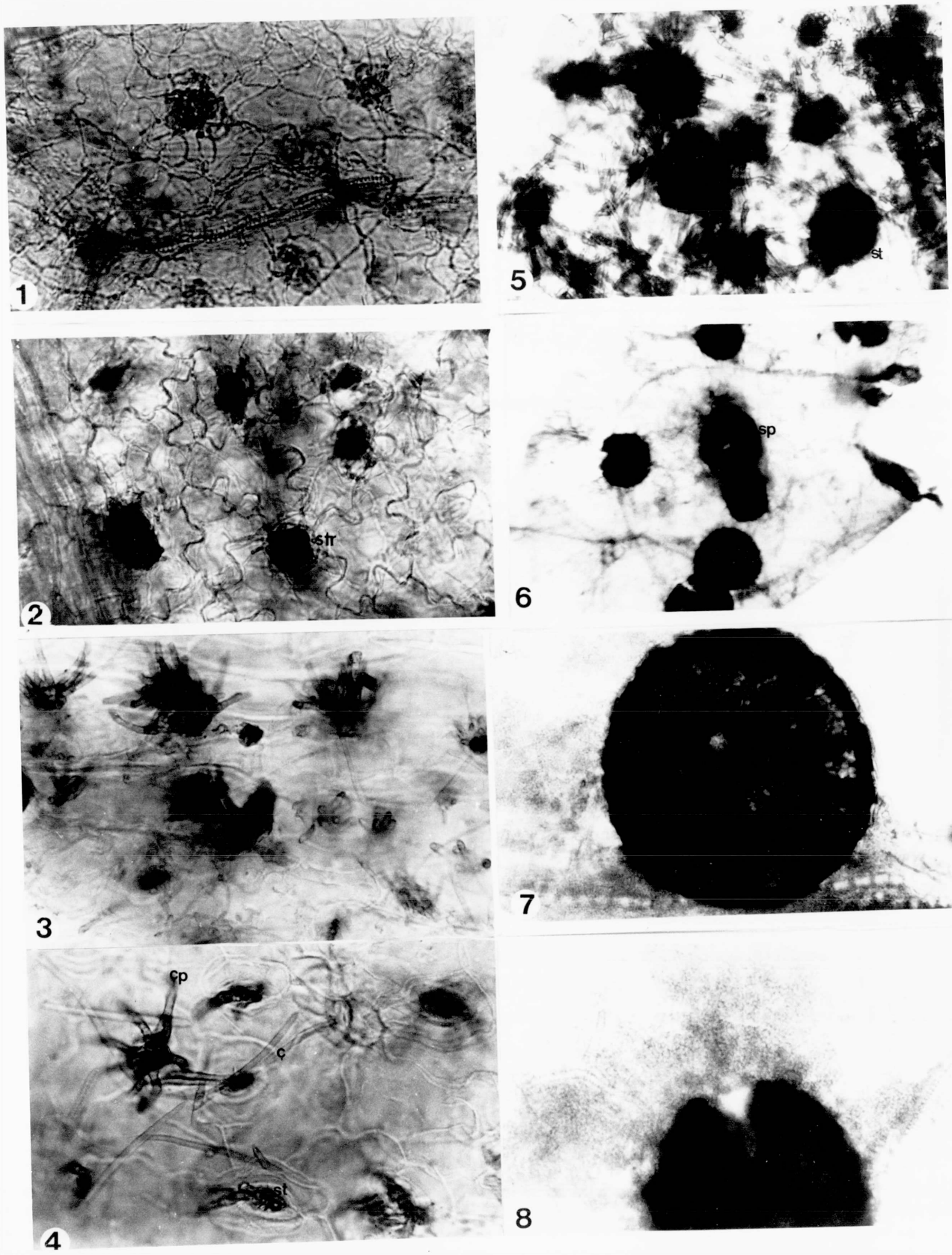



Plate 5

canescens on various leguminous hosts, arise in fascicles sometimes dense, uniform in colour, pale to medium brown, mostly straight, geniculate but rarely branched. They are multi-septate and have a medium to large conidial scar present on a rounded apex. The conidia are acicular or cylindric obclavate, hyaline with an acute apex and a truncate base. They are distinctly multiseptate with a thickened hilum scar (Plate 4, Fig. 3).

### 2.2.4 Host range and pathogenicity of P. cruenta and C. canescens

According to Chandrasekaran and Rangaswami (1960) Saccardo first recorded C. cruenta on Vigna catjan (syn. Vigna sinensis. Since then various leguminous plants including; French bean (Phaseolus vulgaris) cowpea (Vigna unguiculata) hyacinth bean, (Dolichos purpureus = Lablab purpureus), moth bean (Phaseolus aconitifolia = Vigna aconitifolia), mung bean (Vigna radiata), lima bean (Phaseolus lunatus) and black gram (Phaseolus mungo) have been included as hosts for P. cruenta. (Host-pathogen Index Review of Applied Mycology 1921-1960). Chandrasekaran and Rangaswami (1960) reported the occurrence of P. cruenta on Vigna catjan for the first time in India, and in a study of the species of Cercospora occurring in the locality of Chidabram Madras State, observed that P. cruenta occurred only on Vigna catjan, whereas C. canescens was commonly found on Dolichos lablab, Phaseolus mungo and Phaseolus aureus. In artificial inoculations they found that P. cruenta readily

infected the leaves of V. catjan, and caused only light brown spots with characteristic haloes on D. lablab. It failed to infect any of the other leguminous and cucurbitaceous plants tested. They concluded that P. cruenta was highly specific to cowpea. Verma and Patel (1969) investigated the ability of a cowpea isolate of P. cruenta, from the dry arid regions of Rajasthan in India, where the crop is grown as a rainfed crop, to infect plants other than cowpea - fifty-six (56) different host plants belonging to thirty genera, and including several leguminous plants in the genera; Phaseolus, Dolichos, Cyamopsis, Arachis, Vicia and Pisum. They observed typical angular shaped lesions only on Trifolium alexandrium, Chenopodium murale, Lactuca sativa, Dolichos lablab, Datura festuosa and Vigna sinensis var. sesquipedalis. Amin et al. (1976) obtained infection of the common bean, Phaseolus vulgaris cv. Bountiful on detached leaves incubated in moist chambers as well as on intact plants. On cowpea typical angular leaf spots developed in six to eight days on detached leaves. In a field experiment, the disease caused severe defoliation of an entire sprinkler-irrigated field, of cowpea. However, crops of pigeon pea, (Cajanus cajan) and mung bean (Vigna radiata) growing in adjacent plots were not affected. Ellis (1976), lists P. cruenta as causing reddish-brown spots on leaves and stems of Canavalia, Lablab, Phaseolus and Vigna species. Allen (1983) considers bean (P. vulgaris) cowpea, hyacinth bean, and many others as natural hosts of P. cruenta.

Cercospora canescens, has been recorded as causing leaf spots on several leguminous hosts. The symptoms caused by C. canescens are however not particularly characteristic and are often similar to those caused by other Cercospora species (C. vanderysti and C. dolichi) on leguminous hosts in the genera Phaseolus and Vigna (Mulder and Holliday, 1975). According to Chupp (1953), C. canescens infects a number of species in the genera Phaseolus and Vigna, such as the common bean (P. vulgaris) lima bean (Phaseolus lunatus), cowpea (Vigna unguiculata) and soyabean (Glycine max). Ellis (1976) lists the following genera of cultivated legumes, as hosts of C. canescens: Arachis, Cajanus Cyamopsis, Dolichos, Glycine, Phaseolus, Pisum and Voandzeae. Allen (1983) includes, soyabean, common bean, bambarra bean, groundnut, mung bean, lima bean and hyacinth bean as natural hosts of C. canescens. Of the above listed legume species, C. canescens leaf spot has been recorded to be of economic importance on cowpea (Schneider, 1973; Schneider et al., 1976; Vakili, 1977). Schneider (1973) recorded a yield reduction of 18% and Williams (1975) reported a cowpea grain yield reduction of 20% attributable to C. canescens in Ibadan (IITA, 1973). Mew et al. (1975) stated that leaf spot caused by C. canescens is a major disease of mung bean in most of South east Asia, and Poelhman et al. (1973) cited by Thakur et al. (1980), have described the disease as a threat to mung bean production in Indonesia, the Philippines, Thailand and Columbia. Teyegaga and Clerk (1972) recorded C. canescens as causing a severe leaf spot of

bambarra groundnut in Ghana in all regions where the crop is grown. Other records of C. canescens on leguminous hosts include a report by Nowsher et al. (1978) in which C. canescens was described as causing white to sub-circular whitish-grey leaf spots, up to 0.5mm in diameter surrounded by a reddish-brown border on winged bean, (Psophocarpus tetragonolobus) in Bangladesh.

McDonald (1966, 1978) cited by Mercer (1977) and Allen (1983) respectively, stated that C. canescens occasionally infects leaves of groundnut (Arachis hypogae), but is of no economic importance. In Nigeria, McDonald (1966) found that the fungus was associated with lesions of early and late leaf spot diseases ('Tikka') on groundnuts caused by Cercospora arachidicola and Phaeisariopsis personata respectively. Fowler (1970) confirmed that C. canescens had been found to cause typical leaf spots in the riverain areas, Mokwa and Samaru, but it seemed to be of little importance and has not been found in the more northern areas. According to Subrahmanyan (1983) C. canescens has also been observed in a very few cases to be associated with lesions of Phaeisariopsis personata at ICRISAT, Hyderabad, India. Mercer (1977) recorded a higher number of C. canescens spores compared with those of C. arachidicola from spore-trapping experiments carried out beside a plot of groundnut at Chitedze in Malawi, but noted that C. canescens was only a weak pathogen of groundnuts. Subrahmanyan (1983) also observed C. canescens associated with lesions of C.

arachidicola on groundnut leaves in Malawi, and tested the infectivity of the fungus isolate compared with other isolates of C. canescens from ground beans (Voandzeia subterranea), cowpea and common bean. No disease developed after 25 days when healthy detached, rooted groundnut leaves were inoculated. He concluded that C. canescens was not pathogenic to groundnut but may be associated with early leaf spot lesions on groundnut probably as a saprophyte.

## 2.3 Growth and Sporulation of Cercospora species

In culture, several of the species of Cercospora with hyaline conidia, produce greyish colonies which have been variously described as, regular, irregular, serated, tufty effuse and compact (Berger and Hanson, 1963; La, 1963). Many of the species have however, also been found frequently to produce sectors varying from the parent culture, when subcultured on potato dextrose agar; and is particularly apparent when cultures are incubated in the light (Berger and Hanson, 1963; Lynch and Geoghegan, 1978). The sectors have varied in colour from white and pale olive through various shades of grey.

Sporulation on artificial media by species of Cercospora has been well studied (Diachun and Valleau, 1941; Berger and Hanson, 1963; Ekpo and Esuruoso, 1978; Chen et al, 1979; Vathakos and Walters, 1979; Yeh and Sinclair, 1980; Jackson, 1981). However, attempts to induce in vitro sporulation, have not always been successful. Nevertheless, the problem

of *in vitro* production is probably more apparent than real (Holliday, 1980). Several workers have found that rigorous selection from colonies derived from single conidia demonstrates the existence of genotypes that sporulate adequately *in vitro* (Nagel, 1934; Calpouzos, 1955; Jones, 1958; Goode and Brown, 1970). Goode and Brown (1970) postulated that genes necessary for sporulation were either lost through mutation or ceased to be expressed when *Cercospora* spp. were subcultured for several generations on agar media. Other research work has shown that several of the species grown on agar produce sectors of variants, usually fluffy non-sporulating mycelia which overgrow the original sporulating mycelia. Unless special precautions are taken in transferring from the sporulating cultures, the non-sporulating variants predominate and as a result the sporulating forms are lost (Berger and Hanson, 1963a; Latterel and Rossi, 1983).

Various decoction media have been used to induce sporulation in *Cercospora* spp. and a decoction medium for one species may not be suitable for another. Roy (1982) observed that the type of *Cercospora* reproductive unit sub-cultured, the medium from which it was derived and the medium upon which it was sub-cultured affected sporulation. In general, the transfer of mycelium yielded only non-sporulating hyphae. Environmental conditions also influence the growth and sporulation of *Cercospora* species. (Berger and Hanson, 1963a; Calpouzous and Stallknecht, 1965, 1967;

Staverly and Nimmo, 1968). Striking interactions between light and temperature, have been found to influence the sporulation of certain species of Cercospora. Calpouzous and Stallknecht (1965) reported that on sugar beet molasses, sporulation of C. beticola, is stimulated by light at 15°C and depressed by light at 30°C, and that either response may occur at 22.5°C. Chen et al. (1979) noted that sporulation of C. kikuchii was stimulated by light at 15°C and depressed by light at 30°C. Mew et al. (1975), however, observed that between 15°C and 28°C the degree of sporulation of a mung bean isolate of C. canescens was positively correlated with temperature, and the production of conidia was promoted by light. Various media have been quoted as supporting the sporulation of C. canescens in culture. Kilpatrick and Johnson (1956) reported that C. canescens and ten other species of Cercospora with hyaline acicular conidia sporulate well on carrot leaf decoction agar if mycelial fragments are streaked over the surface of the agar just before solidification, and that a deep medium favoured profuse sporulation. Schneider et al. (1973) also reported that non-sporulating cultures of C. canescens and seven other species maintained under oil for several months produced conidia when a mycelial suspension was spread over antibiotic potato dextrose carrot agar (PDCA) and incubated under alternating periods of 12 hours under near UV light followed by 12 hours darkness at 20-25°C. Ekpo and Esuruoso (1978) tested ten media, (cowpea seed carrot agar, cowpea seed potato agar, V-8

juice agar, potato dextrose agar, cowpea leaf decoction plus potato dextrose agar, nutrient agar Czapex Dox agar, malt extract agar, cowpea leaf decoction agar and cowpea stem decoction agar) for their effect on growth and sporulation of cowpea isolates of C. canescens and Pseudocercospora cruenta. They observed that there was little or no mycelial growth and little or no conidia production on most media. They concluded that Oxoid agar, supplemented with V-8 juice supported best growth and production of conidia. Flooding V-8 juice agar with a conidial suspension resulted in a multiplicity of tiny colonies and increased sporulation. More active growth of mycelium, but less production of conidia was observed at an agar concentration of 3% than at lower concentrations. Mew et al (1975) compared different media for the sporulation of a mung bean isolate of C. canescens, and observed that the fungus isolate produced conidia readily but not abundantly on potato-dextrose agar (PDA). Carrot leaf juice-oatmeal agar was the best medium for sporulation followed next by mung bean leaf juice oatmeal agar. Teyegaga and Clerk (1972) however, did not obtain conidia of a bambarra groundnut isolate in culture, but maintained the fungus on seedlings in the glasshouse.

## 2.4 Infection host-parasite relationships and mechanisms of resistance to infection

### 2.4.1 Host penetration and disease development

Latham (1934) described the infection of cowpea leaves by P. cruenta and stated that it was not clear whether the

fungus infects the host by direct penetration of the epidermal cell walls or through the stomata. But once the germ tube had entered, the fungus developed slowly. The mycelium is at first intercellular and becomes intracellular when the attacked cells are dead. At the holonecrotic stage, both inter- and intracellular mycelium are found in the infected host tissue. There is no further reference in the literature to studies on the infection of cowpea and/or other leguminous plants directly with P. cruenta nor C. canescens. Baxter (1956), Latch and Hanson (1962) and Berger and Hanson (1963b) however investigated the method of penetration of leaves of forage legumes, (alfalfa and clover) by Cercospora davisii and Cercospora medicaginis, related taxonomically to C. canescens, and noted that germ tubes penetrated the host through stomata. They reported that conidia germinate within three hours of incubation on leaf surfaces and that germ tubes branched repeatedly as they grew and that some penetrated the leaves without forming appressoria. Often however, germ tubes passed directly over stomata without entering. Price and Munro (1978) studied the infection of winged bean Psophocarpus tetragonolobus by Pseudocercospora psophocarpi (related taxonomically to P. cruenta) and observed that conidia germinate and that germ tubes and hyphae grow over the leaf surface to considerable lengths without penetrating through the stomata on both surfaces of leaf disks and of leaves of potted winged bean plants in the glasshouse.

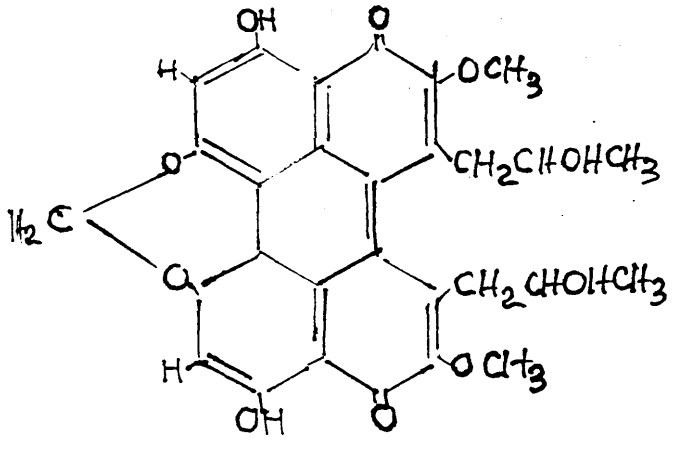
Observations on the infection of other hosts by Cercospora species have shown that the fungus penetrates host leaves through the stomata (Pool and McKay, 1916; Goos and Tschirch, 1963; Solel and Minz, 1971; Rathaiah, 1976, 1977; Beckman and Payne, 1982, 1983). The results of investigations that have examined the mechanisms and factors affecting the germination of conidia and penetration of the host, indicate that Cercospora species require long leaf wetness periods and/or a high relative humidity for infection and disease development. The conidia of some Cercospora species however, grow extensively and randomly over the leaf surfaces when exposed to a continuously humid environment. Interruption of leaf wetness, provided by periodic misting in dew chambers with daily dry intervals of up to six hours duration is more conducive to stomatal penetration. A continuously saturated atmosphere favours extramatrical growth of germ tubes and hyphae but markedly depress the number of penetrations (Goos and Tschirch, 1963; Rathaiah, 1977; Beckman and Payne, 1982). Periods of high relative humidity are however important for post-penetration development of disease in some Cercospora leaf spot diseases.

Goos and Tschirch (1963) observed that the rate of disease development in Cercospora leaf spot of banana appeared to be related to the moisture relations of the plant and was affected by watering treatments. Berger and Hanson (1963) hypothesized that post-infection exposure to

high humidity increased disease severity because the increased water content of tissues or the high relative humidity around them favoured the spread of the pathogen in the host.

2.4.2 Mechanism of pathogenesis by Cercospora spp. and the role of Cercosporin

Studies of the infection and host-pathogen interaction in several Cercospora leaf spot diseases have shown that several species do not penetrate the cell walls of their host plants, but grow throughout disease development in the intercellular spaces (Latham, 1934; Rathaiah, 1976, 1977; Solel and Minz, 1977; Beckman and Payne, 1982). Jenkins (1938) however observed that cells in developing lesions on groundnut leaves are killed in advance of mycelium of C. arachidicola allowing inter- and intracellular growth of the pathogen. Cercosporin, 1,12-bis (2 hydroxypropyl)-2,11-dimethoxy-6-7 methylene dioxy- 4,9-dihydroxy perylene- 3,10-




Structure of Cercosporin

quinone is a non-specific toxin produced by many species of

Cercospora, (see Section 2.2.1) and it has been reported to be important in the interactions of Cercospora spp. with its hosts. When purified cercosporin is applied to a wide range of host species, it reproduces typical necrotic symptoms of the disease. All plants that have been tested are sensitive to cercosporin, including those resistant to the disease (Balis and Payne, 1971; Fajola, 1978c).

Fajola (1978c), Steinkamp et al. (1981), Daub (1984) and Gwin et al, (1987) have independently observed that by applying cercosporin isolated from cultures of C. canescens, C. beticola, C. nicotianae and C. zea-maydis to cowpea, sugar beet, tobacco and maize cells respectively, ion leakage and necrotic lesions are produced that parallel lesions induced by the fungi on their respective hosts in vivo. Daub (1982), noted that cercosporin isolated from C. beticola and C. nicotianae was toxic to tobacco cells only when they were incubated in the light. Daub, (1987) concluded that two lines of evidence suggest that cercosporin plays an important role in diseases caused by Cercospora spp.

First, cercosporin is light activated, and light has been shown to be important for the development of disease symptoms in several Cercospora diseases (Calpouzos and Corke, 1963; Calpouzos, 1966; Calpouzos and Stalknecht, 1967). Second, treatment of sugar beet leaf tissue with cercosporin results in ultrastructural changes similar to

those in plants infected by Cercospora beticola (Steinkamp et al., 1979, 1981) and these changes (particularly membrane damage) are consistent with the known mode of action of cercosporin. Thus, an understanding of resistance mechanism to cercosporin may allow for the future development of novel control measures for these damaging diseases.

### 2.4.3 Sources and nature of resistance to Cercospora leaf spot diseases

Cowpea germ plasm has been evaluated for resistance to Cercospora leaf spots, and numerous cultivars with resistance to P. cruenta have been identified (Fery et al., 1976). Some of these are resistant to both P. cruenta and C. canescens and some but not all sources of resistance are effective at several widely different locations (Allen, 1983). Several accessions have been screened in India (Verma and Patel, 1969), Puerto Rico (Vakili, 1977), and Nigeria (Williams, 1977) for resistance to both leaf spot pathogens, and Fery et al. (1976, 1977) found that resistance to P. cruenta is governed by at least two separate genes. However, little is known of the underlying mechanisms of Cercospora leaf spot resistance (Allen, 1983).

There is evidence to suggest that the expression of resistance to Cercospora leaf spots of cowpea varies with the growth stage of host plants. Schneider and Sinclair (1975) observed that C. canescens leaf spot occurred only after the onset of flowering although sufficient inoculum was

present, and temperature and moisture were favourable for disease development. They found that the germination of conidia and germ tube growth were each inhibited on the surface of young, but not of old leaves of a leaf spot-susceptible cultivar. Similar inhibition was caused by diffusates from apical but not basal leaves of the susceptible cultivar, Prima, and by diffusates from both apical and basal leaves of the resistant cultivar Lalita. Ekpo and Esuruoso (1977) obtained somewhat similar results with P. cruenta. They found that leaf extracts from old plants of a susceptible cultivar, New Era, tended to enhance the germination of conidia whereas extracts from young plants did not. Each investigation indicated that susceptibility in part depends on a pre-penetration phenomenon such as a pre-formed fungitoxic compound, the concentrations of which are influenced by host plant maturity.

# 3. MATERIALS AND METHODS

## 3.1 Plant Material

### 3.1.1 Growth of Plants of food legumes

Seeds of cowpea and other food legumes obtained from the sources listed in Table 3.1 were grown in 10cm and 20cm plastic pots, filled with John Innes Compost No. 1, to the third trifoliate leaf stage or to beyond flowering stage. The third trifoliate leaf stage for most of the legumes was reached after 3 to 4 weeks growth in the glasshouse with an ambient temperature of 25 ± 5°C. During the winter months (December to March), the conditions of high temperature and good light intensity were provided by the use of 2 x 3 kilowatt fan heaters and 3 x 400 w high pressure metal halide lamps in poot luminaries and the glasshouse was double glazed. The heaters were thermostatically controlled to give a temperature range of 25 ± 5°C. The lights were controlled by an automatic time switch which maintained a photoperiod of 12 hours (0.600-18.00hrs). The plants were watered once every three days by flooding capillary mats placed on the glasshouse benches. Capillary mats placed on the floor the glasshouse were watered daily to maintain a high relative humidity. During the summer months the windows of the glasshouse were usually opened during sunny days to maintain an ambient temperature of 25 ± 5°C, and the floor watered twice daily to maintain a high relative humidity.

Table 3.1 Description of Cowpea Cultivars used in Pathogenicity tests

| | COWPEA VARIETY/ LINE | SEED COLOUR/ TEXTURE* | SOURCE/ORIGIN | REACTION TO FIELD INFECTION by ** | |
|---|---|---|---|---|---|
| | | | | C. canescens | P. cruenta |
| 1. | Adua Ayera | R/s | Ghana | S | S |
| 2. | Amantin | Br,Bl Mottled/S | Ghana | S | S |
| 3. | Caroni | R/s | Ghana | S | S |
| 4. | Caloona | R/s | Ghana/ Australia | S | S |
| 5. | Ife Brown | Br/Rr. | Ghana/IITA*** | S | S |
| 6. | TVX 3236 | C,T/Rr. | Ghana/IITA | S | S |
| 7. | IT82D 875 | R/s | Ghana/IITA | MR | MR |
| 8. | IT82D 885 | R/s | Zambia/IITA | MR | MR |
| 9. | IT82D 889 | R/s | Zambia/IITA | MR | MR |
| 10. | Muliana | T,Br Mottled/S | Zambia | S | ? |
| 11. | New Era grey | Br/Bl/15 | Zambia | | |
| 12. | Colossus | T/Wr | USA | ? | S |
| 13. | Colossus 80 | T/Wr | USA | ? | R |
| 14. | Ala. 963.8 | C/s | USA | ? | R |
| 15. | TARS 36 | R/S | Puerto Rico | R | R |
| 16. | TARS 48 | C/S | Puerto Rico | R | R |
| 17. | TARS 62 | W/S | Puerto Rico | R | R |
| 18. | Sudan | T/S | Malawi | S | ? |
| 19. | Sel. 8 | T/S | Malawi | ? | ? |
| 20. | Sel. 1603 | T/S | Malawi | ? | ? |

* Seed colour R = red,
T = tan,
W = white,
Br = brown,
C = cream,
M = mottled

Seed coat texture

R = rough
S = smooth
W = wrinkled

**Disease reaction score country of origin

? = not specified
S = susceptible
MR = moderately resistant
R = Resistant

*** IITA International Institute of Tropical Agriculture

Table 3.2 Description of legume species other than cowpea, used in host range and pathogenicity experiments of *P. cruenta* and *C. canescens*.

| LEGUME SPECIES | VARIETY NAME/CODE | SEED COLOUR | ORIGIN/SOURCE |
|---|---|---|---|
| 1. Lima bean (*Phaseolus lunatus*) | Henderson bush | brown white | USA/Ghana |
| 2. Bambarra groundnut (*Voandzeia*) *subterranea*) | CV18 | white/ pink | Crop Science Dept. University of Ghana |
| | Mbawa Sel 8 | speckle black) brown) | Malawi |
| 3. Mung bean (*Vigna radiata*) | MG50-10A | Green ) | IRRI |
| | CES 2F-1 | Yellow) | Philippines |
| | Ex Legon | Green | Ghana |
| 4. Black gram (*Phaseolus mungo*) | Unknown | black | Southall Market England |
| 5. Winged bean (*Psophocarpus tetragonolobus*) | Ex Kade | brown | Ghana |
| 6. Moth bean (*Phaseolus aconitifolia*) | Unknown | tan | Southall Market England |
| 7. Pigeon pea (*Cajanus cajan*) | Unknown | | Southall Market England |
| 8. Soy bean (*Glycine max*) | S.J.5 | | Ex Thailand/ Imperial College, Weed Science Dept, Silwood Park. |
| | Bragg | | Mississippi, U.S.A. |
| 9. Groundnut (*Arachis hypogae*) | TMV 2 | tan | ICRISAT |
| | Chitembana | dark tan | Malawi, ICI |
| | Tamnut '86 | purple | Jealotts Hill |
| 10. Common bean (*Phaseolus vulgaris*) | | brown | Malawi |

Table 3.3 Description of non-leguminous plant species used in host range and pathogenicity of C. canescens.

| Plant species | Variety/cultivar | Source |
|---|---|---|
| 1. Sugar beet (Beta vulgaris) | Julia<br>Rono beet | ICI Jealotts Hill<br>Bracknell |
| 2. Celery (Apium graveolens) | "Cutting" | Dr. D.C.A. Pink<br>NVRS. Wellesbourne |
| 3. Tomato (Lycopersicon esculentus) | Wosowoso | Crop Science Dept.<br>University of Ghana |
| 4. Aubergine (Solanum melongena) | Black beauty | Crop Science Dept.<br>Univ. of Ghana |
| 5. Okra (Hibiscus esculentus) | 'Local' | Crop Science Dept.<br>Univ. of Ghana |
| 6. Cotton (Gossypium hirsutum) | Not known | Silwood ex Malawi |

Table 3.4 Source of *Cercospora* isolates

| Cercospora species/ Isolate | Host from which isolate was obtained | Date of first isolation | Source of Samples of |
|---|---|---|---|
| Reference Code | | | Infected leaves |
| 1. *Cercospora canescens* | | | |
| $KP_1$ | Cowpea cv. Amantin | Feb 1986 | A.R.S. Kpong, |
| $KP_2$ | Cowpea cv. Caroni | " | Ghana |
| $KP_3$ | Cowpea cv. Adua ayera | " | " |
| TZ | Cowpea cv.? | " | Dr. J.H. Simons Mtawara, Tanzania |
| $ZB_1$ | Cowpea cv? | July 1986 | Dr. J. Kannaiyan |
| $ZB_2$ | Cowpea cv? | " | Chipata, Zambia |
| $ZB_3$ | Cowpea cv. Muliana | " | |
| LB | Lima bean (*P. lunatus*) | Feb 1986 | Crop Science Dept. University of Ghana. |
| IMI 185 292 | Common bean (*P. vulgaris*) | 1972 | CMI ex Nigeria |
| IMI 185 306 | Lima bean (*P. lunatus*) | 1972 | CMI ex Nigeria |
| VS | Bambarra groundnut (*Voandzeia subterranea*) | July 1987 | Dr. J. Kannaiyan Zambia |
| VR | Mung bean (*Vigna radiata*) | July 1987 | Dr. T.W. Mew IRRI, Philippines |
| AH | Groundnut (*Arachis hypogaea*) | July 1987 | Chitedze Agricultural Research Station Malawi. |
| 2. *Pseudocercospora cruenta* | | | |
| Pc KP | Cowpea cv. Caloona | July 1986 | A.R.S. Kpong, Ghana. |
| AC 5114 | Cowpea,cv. unknown | Not recorded | ICI, Jealotts Hill |
| 3 *Cercospora beticola* | | | |
| Cb K897 | Sugar beet | 1975 | ICI, Ex USA |
| 4. *Cercospora arachidicola* | | | |
| Ca K923 | | 1976 | ICI, Ex USA |

3.1.2 Growth of non-leguminous plants (Table 3.3).

Seeds of tomato, pepper, aubergines, and celery were sown in seed trays and seedlings were transplanted into 10cm plastic pots when they were about 10cm tall. Seeds of cotton, okra, and sugar beet were sown directly in 10cm pots filled with John Innes Compost with one plant per pot.

## 3.2 Cercospora Isolates

### 3.2.1 Origin

Leaf samples infected by Cercospora species were obtained from Ghana, Tanzania, Zambia, Malawi and the Philippines. The details of the source including the host legume from which each isolate was obtained and the date of first isolation is outlined below and summarised in Table 3.4.

(a) Isolates $KP_1$, $KP_2$ and $KP_3$, were obtained by inoculating 4-week-old cowpea plants in the glasshouse with a macerate suspension of infected cowpea leaves (apparently infected with Cerospora and Corynespora species) collected from the field at the University of Ghana's Agricultural Research Station, Kpong, Ghana,; the Cercospora isolates were then isolated from the glasshouse infected plants.

(b) Isolate TZ was isolated from infected cowpea leaves obtained from Dr. J.H. Simons, Naliendele Oil seed Research Project, Mtawara, Tanzania. (Plate 3).

(c) Isolate LB was isolated from infected lima bean (Phaseolus lunatus) collected from the garden of the Crop Science Department, University of Ghana.

(d) Isolates $ZB_1$, $ZB_2$ and $ZB_3$ were isolated from infected cowpea leaves in the same field but showing different types of leaf spot lesions (Plate 3, $ZB_1$ and $ZB_2$); obtained from Dr. J. Kannaiyan of the Msekera Research Station, Zambia.

(e) Isolate VS was isolated from infected bambarra groundnut leaves, again provided by Dr. J. Kannaiyan (Plate 2B).

(f) Isolate VR was isolated from infected mung bean leaves (Plate 2C) provided by Dr. T.W. Mew, International Rice Research Institute, Langua, Philippines.

(g) Isolate AH was isolated from groundnut leaves apparently associated with 'tikka' leaf spot lesions. The infected groundnut leaf samples were obtained from the Chitedze Agricultural Research Station, Malawi.

(h) Isolates IMI 185292 and IMI 185306 were obtained as agar slant cultures on corn meal agar; they were originally isolated from common bean (P. vulgaris) and lima bean (P. lunatus) in 1972 from Nigeria respectively.

(i) Pseudocercospora cruenta

Pc (kp) - was isolated by inoculating cowpea plants in the glasshouse with a macerate of infected leaves collected at A.R.S. Kpong, Ghana as in (a).

(j) Cercospora arachidicola and Cercospora beticola cultures were obtained from ICI Ltd., Jealotts Hill, Bracknell, Berkshire.

3.2.2 Single spore Isolation

Leaf samples infected with Cercospora species, were

washed in running tap water and rinsed twice in sterile distilled water. The leaves were then blotted dry and incubated in sterile Petri dish-moist chambers at 25 ± 1°C for 24 to 72h to induce sporulation. Fascicles of conidiophores usually grew out from the leaves after 48h and formed conidia which appeared as 'white hairs' at the tips of the pale-brown to brown conidiophores under a binocular dissecting microscope. Conidia were picked off with the tip of a sterile transfer needle to marked areas on plates of water agar in Petri dishes. Bacteria-free hyphal-tip transfers were made from the periphery of three-day old single spore colonies to PDA slants and incubated at 25°C for 1 week, after which time the cultures were stored by one of the methods described below.

3.2.3 Preservation of Cultures

(i) Storage on agar slants; the cultures grown on agar slants for a week were stored in an incubator at 4°C.

(ii) Storage on agar strips in sterile distilled water; Strips of agar containing mycelium of the isolates were cut from 1-week-old cultures on PDA with a sterile transfer needle and transferred to McCartney bottles containing 20cm$^3$ of sterile distilled water.

(iii) Storage on agar strips under mineral oil

One-week-old cultures of the isolates on PDA slants were covered to a depth of 1cm above the top of the slant with sterile mineral oil (BP) and stored at room temperature 20-22°C.

(iv) Storage as frozen cultures

Cultures of the isolates grown on PDA, V8-A, CLDA and MOA for growth rate studies and cultural characteristics, were stored at -20°C.

(v) Storage of infected leaf material

Samples of infected leaf material with distinct lesions, were dried in between layers of sterile absorbent tissue (Kimwipe) and stored either at 4°C and -20°C.

## 3.3 Culture media

### 3.3.1 Agar media

A Potato dextrose agar (PDA) contained 12g of Oxoid PDA per litre of distilled water.

B Potato carrot agar (PCA) contained 20g each of grated potato and carrot, and 20g of agar dissolved in 1 litre of tap water. The grated potato and carrot were boiled in tap water for one hour and strained through two layers of cheese cloth. The agar was added to the filtrate and boiled to dissolve the agar.

C Potato dextrose carrot agar (Schneider et al., 1973) (PDCA)

Potato-dextrose-carrot agar was prepared by boiling 20g of grated carrots in 1 litre of water for 1 hour and using the broth in place of water to prepare oxoid PDA by dissolving 20g of the agar in 1 litre of the broth.

D Cowpea leaf decoction agar (CLDA);

Cowpea leaf decoction was prepared by boiling 150-200g

fresh weight of cowpea leaves per litre of distilled water and straining through two layers of cheese cloth; 20g of agar was then dissolved in 1 litre of the decoction on a water bath.

E Cowpea leaf decoction-potato dextrose agar (CLPDA)

Cowpea leaf decoction was prepared by boiling 150g of 5-week cowpea leaf in 1 litre of distilled water, and the filtrate used to prepare 1.2% PDA (by dissolving 12g of Oxoid PDA in 1 litre of cowpea decoction).

F Mung bean leaf extract - Oatmeal agar (MOA)

Mung bean leaf extract was prepared by boiling 50g of mung bean leaves in a litre of distilled water, and 50g of oatmeal agar was boiled in 1 litre of distilled water for 1h. Equal portions of the filtrates, 500ml each, were mixed and 20g of agar was dissolved in the mixture placed in a boiling water bath.

G V-8 agar (V-8A)

V-8 juice agar was prepared by dissolving 20g of agar and 4g of $CaCo_3$ in 300ml of V-8 juice and the mixture made up to 1 litre with distilled water.

3.3.2 Liquid media

A Cowpea leaf broth

200g of cowpea leaves was steamed in 1 litre of distilled water for 1h and filtered through two layers of cheese cloth; the pH of the filtrate after autoclaving was 6.2.

B V-8 juice broth

300cm$^3$ of V-8 juice plus 3g of $CaCo_3$ was added to 700cm$^3$ of distilled water. The mixture was autoclaved and the pH left at 7.0.

C Potato dextrose broth

200g of washed potatoes were boiled in 1 litre of water until soft and the broth was filtered through three layers of cheese cloth.

3.3.3 Plant nutrient solution (Long Ashton Solution)

Stock solutions of the compounds listed below were prepared by dissolving the stated weights in 1 litre of distilled water, and the solutions diluted 100 times when needed:

| | | | Weight (grams) |
|---|---|---|---|
| 1. | Potassium nitrate | $KNO_3$ | 20.2 |
| 2. | Calcium nitrate | $Ca(NO_3)_2$ | 65.6 |
| 3. | Sodium dihydrogen orthophosphate | $NaH_2PO_4$ | 20.8 |
| 4. | Magnesium sulphate | $MgSO_4.7H_2O$ | 36.9 |
| 5. | Iron ED.TA Ferric Sodium Salt | | 2.45 |
| 6. | Manganese sulphate | $MnSO_4.4H_2O$; | 0.223 |
| 7. | Cupric sulphate | $CuSO_4.5H_2O$; | 0.024 |
| 8. | Zinc sulphate | $ZnSO_4.7H_2O$; | 0.029 |
| 9. | Boric acid | $H_3BO_3$ | 0.186 |
| 10. | Ammonium molybdate $NH_4$ | $MO_7O_{24}.4H_{20}$ | 0.0035 |

## 3.4 Assessment of sporulation and morphology of conidia produced in culture

### 3.4.1 Sporulation from radially growing colonies

To evaluate the sporulation per unit area of radially growing colonies, five disks of mycelium were cut with a sterile 5mm cork borer from the periphery of the colonies. The disks were lifted with a flamed spatula and the excess agar cut from the disk with a scalpel. The remaining thin layers bearing the fungus were placed in specimen tubes in $2cm^3$ of sterile distilled water to which a drop of 0.1% lactophenol cotton blue had been added to stain the hyaline conidia for ease of identification and counting. The spores were shaken into a suspension or a rotamixer at high speed for one minute. Six counts were made for each suspension of spores and the average number of conidia formed by each isolate in the five media was calculated. The results of the counts were rated on a 0 - +++ scale, in which

0 = no conidia formed

\+ = sparse sporulation, with few conidia but numerous conidiophores (200 spores/$cm^2$)

++ = moderate sporulation 300-400 spores/$cm^2$

+++ = abundant sporulation > 500/ spores/$cm^2$.

### 3.4.2 Evaluation of sporulation by a whole-plate harvest technique

A suspension of mycelial fragments and/or conidia was prepared by macerating six 5mm agar disks cut from dark sites of 5-day-old colonies of the isolates on PDA and V8-A

for mycelial fragments and mycelium plus conidia respectively in 10cm$^3$ of distilled water. One millilitre of each suspension was spread over the surface of agar containing 150mg/l of streptomycin sulphate which was added to the medium when cooled to check bacterial growth. The inoculated plates were incubated under alternate periods of 12h light near UV and dark at 25 ± 2$^o$C for 4 or 5 days. Sporulation was evaluated by adding 5, 10 or 20cm$^3$ of sterile distilled water to each plate and with a sterile artist brush, the colonies were brushed gently to release the spores. The spores were counted in 5 x 0.1cm$^3$ samples using a haemacytometer. The average number of spores counted was multiplied by the appropriate number of each volume of distilled water used in washing the spores from the plates.

3.4.3 Determination of spore morphology.

Semi-permanent slide mounts of spores produced on agar media were prepared by mixing a drop of the spore suspension with a drop of 0.1% lactophenol cotton blue on the side. Each preparation was heated slightly and a cover slip placed on the drop. The slide preparations were allowed to equilibrate overnight before spore measurements were made.

## 3.5 Production of inoculum for pathogenicity tests

Sporulating cultures of most of the isolates were obtained after 3-5 days, by following the procedure outlined in Section 3.4.2. Better and sufficient numbers of conidia

of the isolates that tended to sporulate sparsely was obtained by repeated transfers of the few conidia produced to fresh plates of the same medium at 3-day incubation intervals until numerous conidia were present. The suspension was filtered through two layers of cheese cloth to remove mycelia and agar fragments. To prepare inoculum for inoculation, the desired concentration was obtained by dilution with sterile distilled water containing 0.01% of the surfactant Tween 20; (polyoxyethylene sorbitan monolaurate) to increase the wetting of leaves and even dispersion of conidia.

## 3.6 Inoculation of Plants

### 3.6.1 Leaf disk inoculation

Leaf disks 1cm in diameter were cut from the test plants with a leaf punch and placed in Petri dishes containing benzimidazole agar prepared according to the method of Williams and Owen (1975), by adding 15 parts of 2% Oxoid Ion agar $NO_2$, to 85 parts of 80ppm benzimidazole solution. Alternatively, the leaf disks were floated on sterile distilled water containing 50ppm of benzimidazole in 2 x 5 x 3cm clear polystyrene boxes; there was little movement of the leaf disks when six were placed in each box. The leaf disks were inoculated by placing 20ul droplet of a suspension of spores of known concentration in the centre of the disk. The polystyrene boxes with the leaf disks were incubated under light in clear plastic germinating trays lined with moist absorbent tissue (Kimwipe) to maintain a

high relative humidity.

3.6.2 Inoculation of detached leaflets

Detached whole leaflets were washed and floated on sterile distilled water containing 5% sucrose in Petri dishes. The leaflets were inoculated either by spraying a suspension of conidia on to the leaf surface or by placing drops of inoculum at marked points on the leaf surface from a hypodermic needle attached to an Agla micrometer syringe.

3.6.3 Inoculation of detached trifoliate leaves (method described by Melouk and Banks (1978)

The first trifoliate leaves of 4 to 6-week-old plants grown in the glasshouse were washed in a jet of distilled water and allowed to dry before they were detached by excising the petiole through the pulvinus. Individual leaf petioles were immersed in Long Ashton nutrient solution in 4 x 5cm plastic screw top vials through 2mm diameter holes bored in the tops. The nutrient solution was replaced at weekly intervals in preliminary experiments. In later experiments the detached leaves were rooted in sterile vermiculite watered with the nutrient solution.

3.6.4 Inoculation of tagged leaves on intact plants

Trifoliate leaves of similar size and age were marked with thin strips of masking tape around petioles. The marked leaves were inoculated by: (i) spraying with a fine spray each trifoliate leaf until run-off; (ii) streaking a

suspension of inocula onto each leaflet with a soft-bristled camel hairbrush or (iii) by placing 20µl droplet of inoculum on marked areas with an Agla micrometer syringe. Inoculated leaves were incubated in separate polythene bags to maintain a high relative humidity.

### 3.6.5 Inoculation by spraying whole plants

All leaves on 4 to 8-week-old plants at the flowering stage, were inoculated by spraying both leaf surfaces with a suspension of conidia by means of a hand atomiser until run-off. The inoculated plants were incubated in a polythene-enclosed chamber built on a metal frame. The inoculated plants were kept under distilled water mist by means of an automatic humidifier (Defensor 505, Atkiengessel Schaft, Zurich) fitted to a clock timer, and set to mist for 15 minutes at 4-hour intervals between 18.00 hrs and 0.600hrs. This maintained a high ambient relative humidity of 96-100% in the chamber and produced a film of moisture on the leaves.

## 3.7 Assessment of lesion development and disease severity

### 3.7.1 Classification of the type of symptom

According to Chupp (1953), '*Cercospora* spp. infect plants causing either distinct necrotic spots or no spots. The spots vary from a faint discolouration on the upper leaf surface, to definitely defined and characteristically marked lesions. When no leaf spots are visible, an effuse fruiting of the fungus ordinarily shows on the lower leaf surface. Rarely neither leaf spots nor effuse fruiting is present,

but the presence of the fungus is made evident by single or clustered groups of dark stromata. These may occur on one or both sides of the leaf as well as on petioles or tender stems of host.

Based on the above notes and preliminary observations of inoculation experiments, the reaction of test plants to inoculation with isolates of C. canescens was evaluated by examining the leaves under a binocular microscope and the type of lesion formed classified on a 0-5 scale, summarised below.

5; distinctly defined necrotic spot.

4; well defined 'green islands' but no necrosis.

3; a faint chlorotic discolouration on the adaxial surface

2; no distinct spots nor discolouration but effuse sporulation of the fungus in a moist chamber

1; no leaf spots nor effuse sporulation but scattered areas with single or clustered conidiophores

0; leaves apparently healthy.

3.7.2 Measurement of lesion size

The diameters of distinct circular to irregular lesions formed by C. canescens were measured along two axes at right angles and a mean calculated for each leaf spot. The area of lesions was determined by tracing on translucent paper

and estimated using graph paper.

3.7.3 Determination of sporulation from lesions

Leaflets inoculated with Cercospora spp. either showing distinct lesions or no lesions were incubated in Petri dish moist chambers for 3-5 days from 2-6 weeks after inoculation, to induce sporulation. The leaves were examined under a binocular dissecting microscope (x20) and the degree of sporulation rated on a four point scale 0 - +++ in which 0 = no sporulation, + = sparse; ++ = moderate and +++ abundant sporulation. In experiments with P. cruenta, in which marked areas were inoculated with droplets of a suspension of conidia, and in which discrete necrotic lesions were formed, sporulation per unit area was assessed by cutting out individual lesions and shaking the conidia into a suspension in distilled water containing 0.01% Tween 20 in specimen bottles on a "Rotamixer". The numbers of conidia were determined with a haemacytometer and the index of sporulation classified as follows;

| | | |
|---|---|---|
| 0 | No sporulation | |
| + | 100-200 conidia/$mm^2$ | sparse |
| ++ | 200-500 " " | moderate |
| +++ | 500-1000+ " " | abundant |

3.7.4 Assessment of severity of disease

The ratings for disease severity on inoculated plants were usually made 4 to 6 weeks after inoculation, and were based on; (i) the number of lesions per leaflet

(ii) area of the lesions (infected area)

(iii) sporulation index.

A disease severity scale of 0-5, was derived from the above parameters as follows:-

0 no infection

1 few small lesions 1-2mm with sparse sporulation

2 few medium sized lesions 3-5mm with moderate sporulation

3 many medium sized lesions 3-5mm with moderate sporulation

4 medium to large lesions with abundant sporulation

5 few large lesions 11-15mm wide with abundant sporulation

The overall reaction of a plant was classified as:

0 = Resistant

1-2 = Moderately susceptible

3-4 = Susceptible

5 = Highly susceptible

## 3.8 Microscopic and Histological techniques

### 3.8.1 Whole leaf clearing and staining methods

#### (a) Alcohol-Lactophenol-trypan blue - chloral hydrate

Whole leaf pieces were stained and cleared by the technique described by Shipton and Brown (1962) using trypan blue instead of cotton blue to stain the fungus. Alcohol-lactophenol trypan blue consisted of 1 part lactophenol

trypan blue and 2 parts of 95% alcohol. Lactophenol-trypan blue was prepared as follows:-

| | |
|---|---|
| Phenol | 10g |
| Glycerine | 10ml |
| Lactic acid | 10ml |
| trypan blue | 0.02g |
| Distilled water | 10ml |

The solution containing the leaf sections was brought to boiling and simmered for 1 minute; after the leaves had sank, the solution was brought to boiling again for 0.5min. The leaves were allowed to remain in the stain for approximately 48h at room temperature, removed, rinsed in water, and placed in chloral hydrate (5g chloral hydrate to 2ml water) for 2-3 days; and then mounted on a microscope slide in 50% glycerine.

(b) Clearing Staining Solution (Bruzzese and Hasan, 1983)

This method was used to mitigate the problem of dislodging conidia from the leaf surface during boiling and simmering associated with the method of Shipton and Brown (1962). The clearing-staining solution was prepared by sucessively adding the following compounds in the following proportions:

| | |
|---|---|
| 95% ethanol | 300ml |
| Chloroform | 150ml |
| 90% lactic acid | 125ml |
| phenol | 150g |
| Chloral hydrate | 450g |

Aniline blue 0.6g

Whole leaf pieces were cleared and stained by immersing them in 2-5ml of the clearing-staining solution in stoppered glass vials for 48h at laboratory temperature, 20-22°C. The leaf portions were then removed, placed for 12-24h in a concentrated chloral hydrate solution and finally rinsed rapidly in water. The cleared specimens were mounted in 50% glycerine containing a drop of lactophenol to preserve the specimens.

3.8.2 Assessment of infection (Observations of the fungus during infection)

(a) Spore germination on leaf surfaces

Samples of whole leaf pieces were cut with a leaf punch at 3h-intervals, following inoculation and placed on a microscope slide. Spore germination and growth of the germ tube, was observed by adding a drop of acid fuschin (for P. cruenta) or 0.1% trypan blue in lactic acid (for C. canescens) to the leaf disk and covered with a cover slip for microscopic examination. With a strong light source, the germinating conidia and mycelial growth appeared red, (for P. cruenta stained with acid fuschin) and blue/black (for C. canescens stained with trypan blue) on unstained green leaf tissue. The percentage of conidia that germinate, the numbers of germ tubes per conidium were assessed and the lengths measured with an ocular micrometer. A conidium was considered as germinated, if the germ tube was longer than 5µm, the average diameter of the conidium of

Cercospora spp.

(b) Observation of the fungus during leaf penetration

Penetration of host leaf by germ tubes, the development of the fungus in the host, and the host reaction to the presence of the fungus was observed on leaf samples taken at 24h intervals up to 168h (one week) after inoculation. Two leaflets were sampled for each cultivar - isolate combination at each time point, and cut into rectangular sections to fit on a microscope slide. The leaf pieces were cleared and stained in alcohol-lactophenol-trypan blue, chloral hydrate, or a clearing staining solution (Section 3.8.1). The numbers of germinated spores, germ tubes per conidium and the percentage of germ tubes growing towards and penetrating stomata were recorded.

(c) Observation of host reaction following penetration by the fungus

Samples of leaf tissue that included a lesion were cut with a 5mm leaf punch, cleared and stained (Section 3.8.1). The samples of whole leaf pieces, were taken at four visually different stages of lesion development.

(i) when lesion were barely detectable and appeared as chlorotic spots.

(ii) from chlorotic areas becoming dotted with necrotic spots (P. cruenta) or developed into distinct green islands (C. canescens).

(iii)when lesions had enlarged up to 1mm in diameter, and turned necrotic and

(iv) when the lesions were sporulating.

# 4. EXPERIMENTAL

## 4.1 Growth, Cultural Characteristics and Sporulation of Isolates of Cercospora spp.

### Introduction

Although Cercospora canescens has been recorded as economically important on cowpea, mung bean and bambarra groundnut, (Teyegaga and Clerk, 1972; Schneider, 1973; Mew et al 1975) there have been no studies to compare isolates of Cercospora spp. from the three hosts, with a view to investigating variability and physiologic specialization among the isolates. Also, it has been observed that symptoms of leaf spot caused by C. canescens on certain leguminous plants are not particularly characteristic and often are similar to those caused by other species of Cercospora. It was therefore necessary to examine the variability among single spore isolates of Cercospora spp. from leguminous hosts originating from different countries, to evaluate forms of physiologic specialization, and determine whether the isolates might represent different species.

Growth and cultural characteristics, including: colony morphology, growth rate, the variety of pigments produced in culture (namely; red, pink and green), and the presence or absence of zonation and radial folds in colonies grown on agar media, have been used to describe cultural variability and to establish interrelationships among some species of Cercospora (Berger and Hanson, 1962; La, 1963; Fajola,

1978a; El Gholl et al., 1981).

This first section describes experiments conducted to determine the cultural and morphological characteristics of isolates of Cercospora canescens from cowpea leaves obtained from Ghana, Tanzania and Zambia; and isolates from the leaves of four other legume species, lima bean, bambarra groundnut, mung bean and groundnut, obtained from Ghana, Zambia, The Philippines and Malawi respectively (Table 3.2).

### 4.1.1 Comparison of the growth, cultural characteristics and sporulation of isolates of Cercospora canescens from cowpea and lima bean

In preliminary experiments, the radial, linear growth and colony characteristics of seven isolates of Cercospora canescens from cowpea and three isolates from lima bean were compared.

(a) Radial growth and colony characteristics on agar media

Five media; potato dextrose (PDA); potato carrot (PCA); potato dextrose carrot (PDCA); cowpea leaf decoction (CLDA) and V-8 juice agar (V-8A)) which have been tested by other research workers for the growth of Cercospora spp. were investigated for their suitability for the growth and sporulation of the Cercospora isolates.

Colonies were started by placing 5mm-diameter agar-mycelium disks, cut from the margins of 1-week old cultures

of the isolates grown on potato dextrose agar (PDA), the second transfer after initial isolation, in the centre of 90mm Petri dishes containing $20cm^3$ of agar medium. The Petri dishes were arranged randomly with three replicates, for each isolate/medium combination, approximately 30cm under two white fluorescent tubes 40W, and one black tube (near UV), Philips TL40 at 25 $\pm$ 1$^{o}$C. The light sources were regulated by a 24-h time switch (Veener Auto Switch) to provide alternating periods of 12h dark and 12h white and near UV light. Radial growth of the colonies was determined by averaging two measurements at right angles to each other, ten days after incubation. The experiment was repeated once and the mean colony radius for each isolate on a particular medium calculated from the six readings in the two experiments.

The data were analysed by the analysis of variance test and significant differences between treatments were evaluated by Duncan's multiple range test.

The results presented in (Table 4.1.1) show that there were significant differences in colony diameters among the isolates on the five media investigated. Interactions between isolates and media were also significant, indicating that certain isolates grew better than others on certain media (Appendix Table 1). Separate comparisons were made for the growth of each isolate on the five media, and for the growth of all isolates on a particular medium to

determine which medium was best for each isolate. Considering the mean growth of the isolates in the five media they could be separated into six groups in each of which the size of the colonies after ten days' growth were not significantly different from each other (Table 4.1.1).

Isolate $KP_2$ grew the best on all five media with a mean colony radius of 34mm, followed by isolates $KP_1$ (33mm) and then IMI 306 (32mm). Two other groups in which the linear growth of the colonies were not significantly different comprised, isolates IMI 292, $ZB_1$, $KP_3$ and TZ, $ZB_2$, LB, leaving isolate $ZB_3$ which grew the least (27.9mm). Potato dextrose carrot agar (PDCA) supported more rapid radial growth of all the isolates followed by potato dextrose agar (PDA) and V-8 juice agar (V-8A) which did not differ significantly in this respect. Cowpea leaf decoction agar (CLDA) and potato carrot agar supported significantly less growth.

A comparison of the data for the growth of each isolate on the five media revealed that all isolates except the lima bean isolates LB and IMI 306 grew better on both PDCA and PDA. The lima bean isolates, however, grew best on PDA and V-8A. Comparisons of the ten isolates on each of the five media also showed significant differences on all media among the isolates. On potato dextrose carrot agar (PDCA), the linear growth of the cowpea isolates $KP_1$ and $KP_2$, was significantly different from the lima bean isolate (LB) but

Table 4.1.1 Comparison of radial, linear growth of cowpea and lima bean isolates of Cercospora canescens on five agar media.
Radial growth after 10 days at 25 + 1o$^{C}$ (mm)*

| Medium | Cowpea isolates | | | | | | | Lima bean isolates | | | Mean of isolate for each medium |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | KP1 | KP2 | KP3 | TZ | ZB1 | ZB2 | ZB3 | LB | IMI 292 | IMI 306 | |
| PDA | 34.8ab+ AB++ | 37.0a A | 27.6d C | 31.2bc A | 31.5bc BC | 31.7bc A | 28.5c AB | 30.3c A | 29.5c D | 34.7ab A | **31.6B** |
| PCA | 30.0b D | 29.0c D | 27.2ef C | 28.2cd B | 27.0ef D | 28.3cd B | 27.7de ABC | 27.8def C | 30.5b CD | 32.0a ABC | **28.8D** |
| CLDA | 32.3b C | 34.7a B | 31.8b B | 28.3cd B | 31.8b AB | 27.5d B | 27.0d BC | 29.3cd B | 31.8b BC | 30.2c C | **30.3C** |
| PDCA | 35.3a A | 36.3a A | 33.0ab A | 31.8ab A | 33.3ab A | 31.7ab A | 29.8ab A | 28.0b C | 32.8ab AB | 30.8ab BC | **32.3A** |
| V-8A | 33.7a B | 33.5ab C | 32.8ab AB | 28.8cd B | 31.2bc BC | 27.5ef B | 26.5f C | 30.2cd A | 33.8a A | 33.8a AB | **31.2B** |
| Mean of each isolate for all media | **33.2b** | **34.1a** | **30.5d** | **29.7e** | **31.0d** | **29.3e** | **27.9f** | **29.1e** | **31.1d** | **32.3c** | |

*Each value is the mean of six measurements in two experiments. + Values followed by the same lower case letter(s) in each row, and by the same upper case (subscript) letter(s) in each column are not significantly different by Duncan's New Multiple Range Test (P = 0.05).

not from the other cowpea isolates and from the lima bean isolates; IMI 292 and IMI 306. Growth of the three lima bean isolates was not significantly different from the cowpea isolates $KP_3$, TZ, $ZB_1$, $ZB_2$ and $ZB_3$. The differences in linear growth among the isolates after 10 days, were more prominent on PCA and CLDA, on which the grouping of the isolates based on colony radius was not possible compared to PDA and PDCA. But the differences in the means for medium and isolates are not much more than 4mm.

The cultural characteristics of the colonies of the isolates on the five media, including general appearance, colour of the colonies and pigmentation of the surrounding medium were recorded after 14 days. A summary of the observations is presented in Table 4.1.2(a) and (b) and Plate 6. The colonies formed by the isolates ranged from compact slightly raised colonies with little aerial mycelium (isolates $ZB_1$, $ZB_2$, and $ZB_3$ on PDA, PCA and V-8A) to effuse colonies with tufty aerial mycelium (isolates $KP_1$, $KP_2$, LB and 306 on all the media tested). Concentric rings of growth were formed by isolates: $KP_1$, $KP_2$, $ZB_1$, $ZB_2$, $ZB_3$ and LB on potato-dextrose agar, and by all the isolates on potato carrot agar (PCA), except that the rings of growth formed by isolates TZ, $ZB_1$ and $ZB_2$ were not so clear as those formed by the other seven isolates. On V-8 juice agar concentric rings were more prominent in colonies formed by isolates $KP_1$, $KP_2$, 292 and to some extent by isolate 306. In addition to concentric rings of growth, deep radiating

Table 4.1.2(a) Colony morphology of isolates of C. canescens from cowpea and lima bean on different media at $25_{o}C$.

| Medium | Isolate | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Cowpea | | | | | | | Lima bean | | |
| | KP1 | KP2 | KP3 | TZ | ZB1 | ZB2 | ZB3 | LB | 292 | 306 |
| PDA | Effuse | Effuse | Compact | Tufty | Compact | Tufty | Compact | Tufty | Compact | Effuse |
| PCA | Effuse | Effuse | Effuse | Tufty | Compact | Compact | Compact | Effuse | Effuce | Effuse |
| CLDA | Effuse | Tufty | Tufty | Tufty | Compact | Compact | Tufty | Effuse | Effuse | Effuse |
| PDCA | Effuse | Effuse | Compact | Tufty | Compact | Compact | Compact | Compact | Effuse | Effuse |
| V-8A | Effuse | Effuse | Compact | Tufty | Compact | Compact | Compact | Compact | Effuse | Effuse |

Table 4.1.2(b) Colour and extent of diffusion* of pigments produced by C. canescens isolates from cowpea and lima bean in the media.

| Medium | Isolate | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Cowpea | | | | | | | Lima bean | | |
| | KP1 | KP2 | KP3 | TZ | ZB1 | ZB2 | ZB3 | LB | 292 | 306 |
| PDA | Green$_{++}$ | Green$_{++}$ | Purple$_{+++}$ | Pink$_{++}$ | Pink$_{++}$ | Pink$_{+}$ | Green$_{+}$ | Purple$_{+++}$ | Pink$_{++}$ | Purple$^{+++}$ |
| PCA | Green$^{+}$ | Green$^{+}$ | Purple$_{++}$ | Pink$_{+}$ | Green$_{++}$ | Pink$_{+}$ | Pink$_{+}$ | Purple$_{++}$ | Purple$_{+++}$ | Purple$_{++}$ |
| CLDA | None | None | None | None | None | None | None | None | None | |
| PCDA | Green$_{+}$ | Green$_{+}$ | Purple$_{++}$ | Pink | Pink | Pink$_{+}$ | Green$_{+}$ | Purple$_{+++}$ | Purple$_{++}$ | Purple$_{+++}$ |
| V-8A | None | None | None | None | None | None | None | Purple$_{++}$ | Pink$_{++}$ | Purple$_{+}$ |

* Extents of diffusion of the pigment into the medium
\+ Traces at the periphery of the colony
++ Low yield with a diffusion zone of less than 2mm.
+++ Good yield, with diffusion of the pigment up to 1cm beyond the periphery of the colony into the medium.

PLATE 6

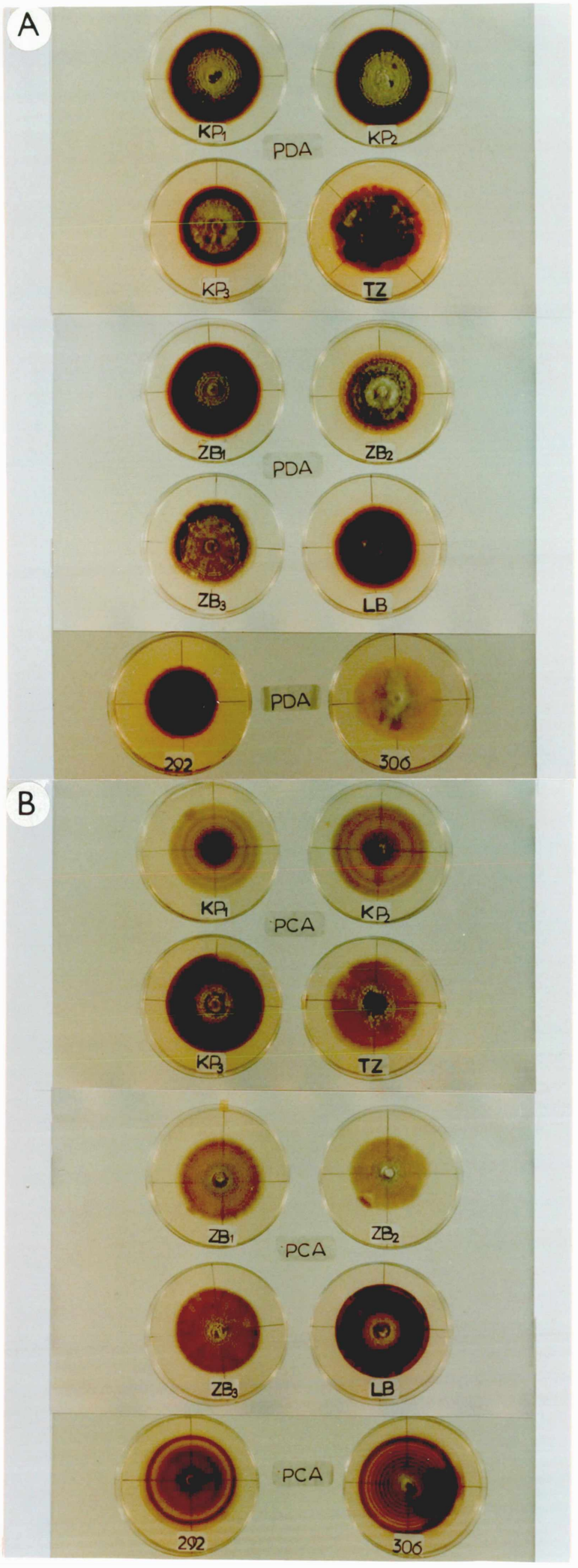
Colony characteristics of cowpea. (KP1, KP2, KP3, TZ, ZB1, ZB2 and ZB3) and lima bean (LB, 292 and 306) isolates of Cercospora canescens on:

A. Potato dextrose agar (PDA).

B. Potato carrot agar (PCA).

incubated under alternate periods of 12 hours fluorescent light plus near UV (black light) and 12 hour darkness at 25°C.

Plate 6


A
KP1
KP2
PDA
KP3
TZ
ZB1
ZB2
PDA
ZB3
LB
PDA
292
306
B
KP1
KP2
PCA
KP3
TZ
ZB1
ZB2
PCA
ZB3
LB
PCA
292
306

folds were formed by isolate $KP_3$ on V-8 juice agar (Plate 7c). The colour of the colonies, viewed from the top ranged from olive grey to dark grey towards the centre and olivaceous towards the edges. When viewed from the bottom of the plates, the colonies were either olivaceous, dull green, brown or black. The marginal pigmentation of the colonies and the surrounding medium for all isolates was either yellowish glaucous green or pink to dark purple (Plate 7A). Pigmentation of the colonies and surrounding medium was more prominent on potato-dextrose agar (PDA), potato dextrose carrot agar (PDCA) and potato carrot agar (PCA) than on V-8 juice agar (V-8A) with no pigmentation in the surrounding medium on cowpea leaf decoction agar (CLDA). The cowpea isolates $KP_1$ and $KP_2$ produced a glaucous green pigment on potato-dextrose agar, potato-dextrose carrot agar and potato carrot agar. The marginal pigmentation of the colonies and the surrounding medium of the cowpea isolates $KP_3$, TZ, $ZB_1$ and the lima bean isolates LB, 306 and 292 was a pink purple to deep purple. The purple pigment produced by isolates $KP_3$, LB, 292 and 306, diffused to varying extents into the surrounding medium (Plate 6). Isolates $ZB_2$ and $ZB_3$ produced an olivaceous green marginal pigmentation on potato dextrose and potato carrot agar on initial isolation, but when cultures of the isolates grown on potato dextrose agar (PDA) were incubated in the light over a two-week period, sectors of the colonies formed either an amber or pink pigment (Plate 7B). The colonies formed by isolate TZ, on the other hand, produced a pink pigment on some

PLATE 7

Pigmentation and sectoring of C. canescens isolates in culture.

A. Cultures of cowpea isolates KP3 and KP2, showing the production of a pink and green pigment respectively on potato dextrose agar (PDA).

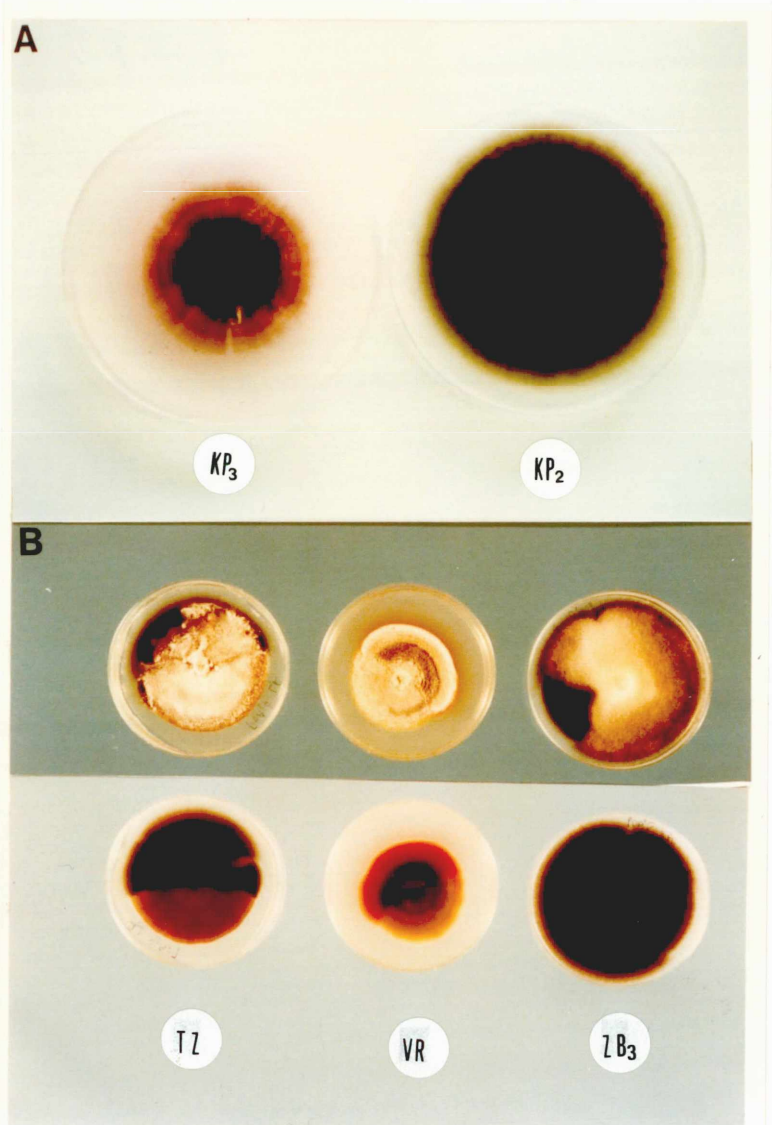
B. Three-week-old cultures of C. canescens isolates on potato dextrose agar (PDA), showing the development of sectors varying in the type of aerial mycelium and pigmentation released into the medium-

TZ - cowpea isolate from Tanzania.

VR - mung bean isolate from the Philippines.

ZB3 - Cowpea isolate from Zambia.

C. Two-week-old culture of cowpea isolate KP3 of C. canescens on V-8 juice agar, showing deep radial folds.



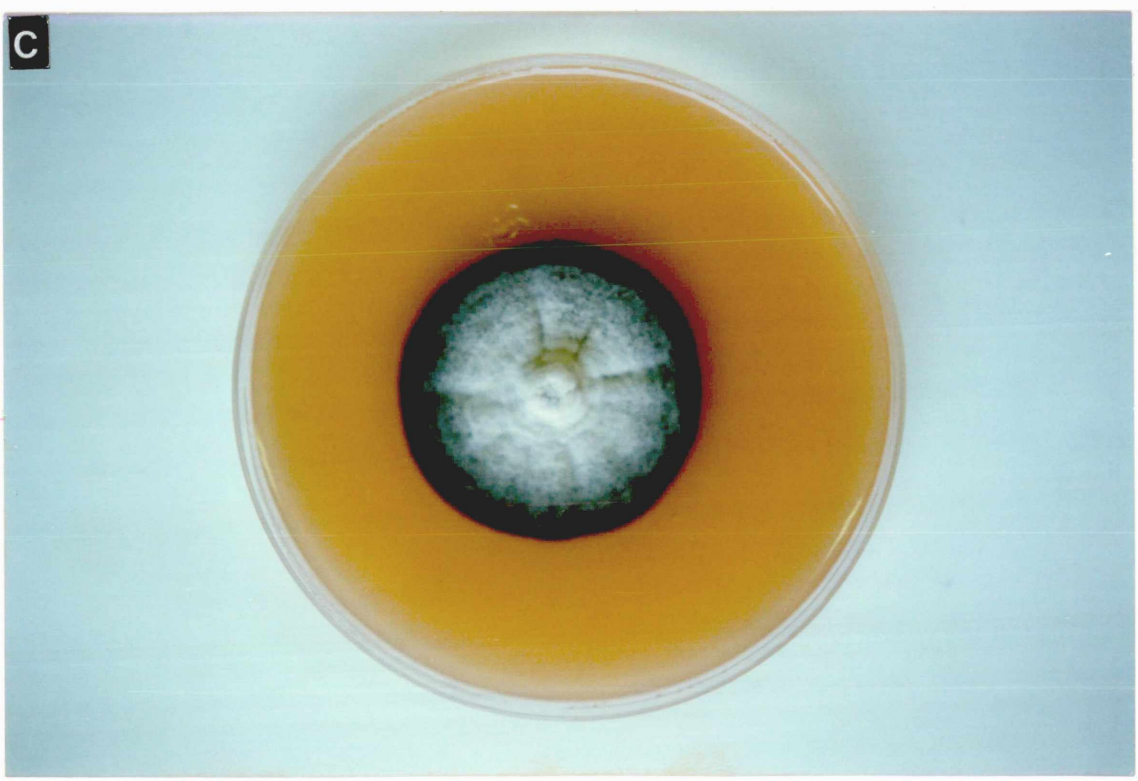




Plate 7

plates of PDA but not on others. Isolate 306 also failed to produce a pink pigment on some replicate plates when grown on PDA under light.

Sporulation of the isolates in colonies started with mycelial agar disks was determined first, by a visual rating under a binocular dissecting microscope and then by counting the numbers of conidia formed per unit area (Section 3.4.1). The observations showed that most spores formed around the periphery of the colonies. The combined results of the estimates of sporulation, are summarised in Table 4.1.3. The cowpea isolates $KP_1$ and $KP_2$ produced relatively more conidia compared to the other six isolates on all media except on PDA on which no conidia were produced by any of the isolates. The lima bean isolates 292 and 306 did not produce conidia on any of the five media. The cowpea isolate TZ produced moderate numbers of conidia on cowpea leaf decoction agar (CLDA) and V-8 juice agar (V8-A) but not on potato dextrose carrot agar (PDCA). The other cowpea isolates $ZB_1$, $ZB_2$ and $ZB_3$ all of which were isolated from infected leaves obtained from Zambia, produced only very few conidia, but numerous conidiophores were observed scattered in the colonies formed on PDCA, CLDA and V-8 Agar. The lima bean isolate LB however, produced few conidia on V-8 agar and PDCA but a moderate number of conidia was formed on PCA and CLDA. The results indicate that the isolates differed in their ability to sporulate under the conditions in which the cultures were incubated. Also, the type of medium

Table 4.1.3 Sporulation in cultures of C. canescens isolates from cowpea and lima bean started with mycelium-agar.

| Medium | Sporulation score* after 10 days at $25_oC$ | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Isolate | | | | | | | | | |
| | Cowpea | | | | | | | Lima bean | | |
| | KP1 | KP2 | KP3 | TZ | ZB1 | ZB2 | ZB3 | LB | 292 | 306 |
| PDA | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| PCA | + | + | + | + | + | + | + | ++ | | |
| CLDA | +++ | +++ | + | ++ | + | + | + | ++ | 0 | 0 |
| PDCA | ++ | +++ | + | + | + | + | + | + | 0 | 0 |
| V-8A | +++ | +++ | + | ++ | + | + | + | + | 0 | 0 |

* 0 = No sporulation
\+ = Sparse sporulation with few conidia but numerous conidiophores
++ = Moderate, with 300-400 spores/$cm_2$.
+++ = Abundant sporulation > 500 spores/$cm_2$

influenced sporulation of the isolates to varying extents.

(b) Comparison of the sporulation of the isolates on multi-point inoculated agar plates.

The preceding observations made on the sporulation of the isolates from single point inoculation with mycelial plugs indicated that relatively few conidia were formed on the five media used. Several workers have, however, reported that for most species of Cercospora, if mycelial fragments and/or a suspension of conidia are spread or streaked over the surface of soft agar, many minute colonies are formed and this greatly increases sporulation in culture. Nevertheless, a medium found to be suitable for one species may not induce the sporulation of other species (Section 2.3). To obtain sufficient conidia for use as inocula in later experiments on pathogenic variability among the isolates, it was necessary to investigate the medium and incubation conditions that supported good sporulation of each of the isolates and thereby also determine the relative abilities of the isolates to sporulate in culture under optimal conditions.

Based on the results of the previous experiments (Section 1.1a) five media, V-8 juice supplemented with either 1.2 or 2% agar (V-8A), cowpea leaf docoction supplemented with 2% agar (CLDA), potato-dextrose carrot agar (PDCA) and potato carrot agar (PCA) were investigated using the procedures described in Section 3.4.2, to compare

sporulation of the seven cowpea isolates and the lima bean isolate (LB), leaving out the two lima bean isolates IMI 292 and IMI306, that failed to produce conidia in the previous experiment. Five replicate plates of each medium were inoculated with either mycelial fragments or mycelial fragments plus conidia and the treatments were randomized and incubated under alternating periods of 12h near UV and white light and 12h darkness. Five separate spore counts were made from each plate using a haemacytometer and a mean for the five replicate plates for each type of inoculum per medium calculated (Appendix Table 2). The results obtained shows that increases in the numbers of conidia were obtained when both mycelial fragments and conidia was used as inoculum.

Separate comparisons of the mean numbers of spores produced by the isolates on the five media (Tables 4.1.4a and 4.1.4b) revealed that the cowpea isolates $KP_1$ and $KP_2$ produced a significantly higher number of spores compared to the other cowpea isolates except for isolate TZ which produced a similar number of conidia when mycelial fragments or conidia was used as inoculum.

Generally, the numbers of conidia produced on cowpea leaf decoction agar (CLDA) and 1.2% V8 juice agar did not differ significantly.

Table 4.1.4(a). Sporulation of C. canescens isolates from cowpea and lima bean (mycelial fragments used as inoculum).

| Isolate code | Mean number of spores ($x10_5$ per plate)* on | | | | | |
|---|---|---|---|---|---|---|
| | PCA | CLDA | PDCA | 1.2% V-8A | 2% V-8A | Mean of (5 media) |
| KP1 | 2.0 | 2.1 | 1.7 | 3.3 | 3.2 | 2.5 a+ |
| KP2 | 2.1 | 3.2 | 1.6 | 3.6 | 2.7 | 2.6 a |
| KP3 | 0.8 | 0.8 | 1.7 | 1.0 | 0.9 | 1.0 b |
| TZ | 1.1 | 2.1 | 1.4 | 1.6 | 1.2 | 1.5 b |
| ZB1 | 1.5 | 1.6 | 1.3 | 1.6 | 1.0 | 1.4 b |
| ZB2 | 1.4 | 0.9 | 1.5 | 2.2 | 1.3 | 1.5 b |
| ZB3 | 1.0 | 1.0 | 1.0 | 1.4 | 1.0 | 1.1 b |
| LB | 0.6 | 1.2 | 1.9 | 1.0 | 1.2 | 1.2 b |
| Means of 8 isolates | 1.3c+ | 1.6 ab | 1.5 ab | 2.0 a | 1.5 ab | |

* The values are means of 10 counts

\+ Fisher's least significant difference; means followed by the same letter(s) are not significantly different at P = 0.05.

Analysis of Variance Table

| Source | Df | SS | MS | F |
|---|---|---|---|---|
| Isolate | 7 | 13.276 | 1.8965 | 8.3 |
| Medium | 4 | 1.784 | 0.44600 | 1.96 |
| Isolate x medium | 28 | 6.3680 | 0.2743 | |
| | 39 | 21.428 | | |

LSD values, for comparisons Medium = 0.49
Isolate = 0.60

Table 4.1.4(b). Sporulation of *C. canescens* isolates from cowpea and lima bean (conidia plus mycelial fragments used as inoculum).

| Isolate code | Mean number of spores ($x10^5$ per plate)* on | | | | | Mean of (5 media) |
|---|---|---|---|---|---|---|
| | PCA | CLDA | PDCA | 1.2% V-8A | 2% V-8A | |
| $KP_1$ | 3.5 | 4.6 | 3.6 | 4.7 | 4.3 | 4.1 b |
| $KP_2$ | 3.9 | 5.3 | 4.1 | 6.0 | 5.8 | 5.0 a |
| $KP_3$ | 1.6 | 2.3 | 2.4 | 2.6 | 2.2 | 2.2 d |
| TZ | 2.4 | 5.0 | 3.4 | 4.8 | 3.6 | 3.8 b |
| $ZB_1$ | 2.3 | 3.9 | 2.7 | 3.5 | 3.0 | 3.1 c |
| $ZB_2$ | 2.3 | 2.9 | 2.1 | 2.3 | 2.3 | 2.4 d |
| $ZB_3$ | 2.0 | 3.2 | 2.0 | 2.0 | 2.0 | 2.2 d |
| LB | 2.3 | 3.9 | 2.7 | 3.5 | 3.1 | 3.1 c |
| Mean of 8 isolates | 2.5d+ | 3.9 a | 2.9 cd | 3.7 ab | 3.3 bc | |

* The values are means of 10 counts

\+ Means followed by the same letter(s) are not significantly different at P = 0.05. Fisher's least significant difference.

Analysis of Variance Table

| Source | Df | SS | MS | F |
|---|---|---|---|---|
| Isolate | 7 | 35.8 | 5.116 | 28.68** |
| Medium | 4 | 9.8935 | 2.4734 | 13.87** |
| Isolate x medium | 28 | 4.9945 | 0.178 | |
| | 39 | 50.7 | | |

LSD values for comparisons Medium = 0.43
Isolate = 0.55

(c) Spore morphology

The lengths and the largest widths of fifty conidia formed on V-8 juice agar were measured with an ocular micrometer at x150 and 600 respectively in two separate determinations of twenty five (25) conidia each. The conidia were washed from three plates with sterile distilled water and mixed before aliquots were taken on microscope slides for spore measurements. A comparison of the mean spore lengths (Table 4.1.5) of the isolates shows that the average conidial length does not differ significantly among the cowpea isolates; $KP_1$, $ZB_2$, and TZ, and between isolates $ZB_1$ and $KP_2$. But the lengths of the other cowpea isolates; $ZB_3$ and $KP_3$ were less and similar to that of the lima bean isolate LB. There was however, a wide range of spore lengths for each of the isolates.

(d) The effects of medium composition and light on colony morphology and pigmentation

The observations made on the growth of the seven cowpea and three lima bean isolates of C. canescens on agar media (Section 1.1(a) showed that the marginal pigmentation of the colonies and the surrounding medium was either pink/purple or glaucous to olivaceous green (Plate 7A). Although many species of Cercospora with hyaline, acicular conidia have been reported as producing a pink/purple pigment usually referred to as cercosporin, isolates of certain species have failed to produce the pigment in culture. (Balis and Payne, 1971; Lynch and Geoghegan 1979). The production of

Table 4.1.5 Variation in the size of conidia of isolates of C. canescens from cowpea and lima bean produced in culture on V-8 juice agar after 5 days at 25°C.

| Isolate | Length µm | | Width µm |
|---|---|---|---|
| | Range | Mean* | |
| $KP_1$ | 90-300 | 193a+ | 2.4 - 4.5 |
| $KP_2$ | 60-290 | 170bc | 2.5 - 5.0 |
| $KP_3$ | 80-270 | 153c | 2.5 - 4.0 |
| TZ | 120-280 | 182ab | 2.5 - 4.0 |
| $ZB_1$ | 100-260 | 189ab | 2.5 - 3.5 |
| $ZB_2$ | 100-300 | 204a | 2.5 - 4.0 |
| $ZB_3$ | 75-230 | 155c | 2.5 - 5.0 |
| LB | 100-300 | 159c | 2.8 - 5.0 |

* Mean of 50 conidia

+ Common letters indicate no significant differences among values. Separation by Duncan's multiple range test ($P < 0.05$).

cercosporin by some strains is apparently influenced by the cultural conditions, the most important being the type of medium and light (Lynch and Geoghegan, 1979). In view of the important role that cercosporin is thought to play in disease development (Section 2.4.2), the potential of the Cercospora isolates from cowpea and lima bean to produce the pigment in culture was investigated by growing the isolates on agar plates incubated under different light regimes.

Sub-cultures of the isolates from sectors varying in aerial mycelium were made by transferring mycelium-agar-disks to Petri dishes containing $20cm^3$ of potato dextrose agar (PDA) or potato carrot agar (PCA). Inocula were taken from cultures of the isolates that had been sub-cultured once, twice and three times, before storage, because earlier work with Cercospora beticola by Lynch (1975) suggested that isolates that had not produced cercosporin in initial experiments were induced to produce stable amounts of the pigment with continuous sub-culturing. Three replicate plates of each isolate per medium, were incubated under three treatments of light regimes; continuous light, alternate 12h dark/light, and continuous darkness. The plates receiving continuous and alternate light/dark treatments were randomized and arranged on separate shelves under two 40w-cool white fluorescent tubes mounted 30cm above the surface of the cultures in a controlled temperature room maintained at 25 $\pm$ $2^{o}C$. The light intensity at the surfaces of the cultures was about 110 lux,

and was measured with a battery operated light meter which recorded the light units in foot candles, and converted to lux units using a standard curve. The alternate periods of dark and light were regulated by a 24h time switch (Venner Auto Switch), set to illuminate the cultures from 06.00 - 18.00h daily. The cultures receiving continuous darkness treatment were placed in opaque plastic boxes. The cultures incubated in continuous light were observed daily from 3 days after incubation and a final assessment of pink-purple pigment production was made 10 days after inoculation, and was based on a score scale 0-3, where 0 = no pigment production, 1 = very small traces of the pigment at the periphery of the cultures; 2 = low yields with a diffusion zone of <2mm; and 3 = good yield with diffusion of the pigment up to 1cm beyond the periphery of the colony.

Separate scores were given for each isolation x inoculum x type of light treatment (Appendix Table 5). The totals for the scores of each isolate were calculated for the two media, three light regimes and for the three types of inocula (Tables 4.1.6 a, b and c).

A comparison of the scores for the isolates on the two media (Table 4.1.6a) indicates that the type of medium had very little effect on the degree of pink-purple pigment productions, except for isolate $ZB_1$ which produced more pigment on potato dextrose agar (PDA) than on potato carrot agar (PCA).

Table 4.1.6 Pink-purple pigment production by isolates of C. canescens from cowpea and lima bean

(a) Effect of medium

| Medium | Isolate | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $KP_1$ | $KP_2$ | $KP_3$ | TZ | $ZB_1$ | $ZB_2$ | $ZB_3$ | LB | 292 | 306 |
| PDA | 0 | 0 | 24* | 14 | 16 | 1 | 2 | 22 | 16 | 14 |
| PCA | 0 | 0 | 22 | 13 | 3 | 4 | 4 | 16 | 12 | 18 |

* Each figure is the total for the scores for three light treatments and three types on inocula.

(b) Effect of light/dark regimes

| Light Regime | Isolate | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $KP_1$ | $KP_2$ | $KP_3$ | TZ | $ZB_1$ | $ZB_2$ | $ZB_3$ | LB | 292 | 306 |
| CL | 0 | 0 | 15* | 9 | 7 | 0 | 0 | 13 | 12 | 12 |
| CD | 0 | 0 | 13 | 6 | 6 | 1 | 6 | 7 | 4 | 3 |
| L/D | 0 | 0 | 18 | 12 | 6 | 4 | 0 | 18 | 12 | 17 |

* Each figure is the total for the scores for both PDA and PCA. The light regimes were:- CL = continuous light, CD = continuous darkness L/D = 12h alternate light and darkness.

(c) Effect of the type of inoculum

| Inoculum Source | Isolate | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $KP_1$ | $KP_2$ | $KP_3$ | TZ | $ZB_1$ | $ZB_2$ | $ZB_3$ | LB | 292 | 306 |
| I | 0 | 0 | 17* | 10 | 7 | 2 | 2 | 13 | 10 | 12 |
| II | 0 | 0 | 15 | 6 | 7 | 1 | 2 | 13 | 9 | 10 |
| III | 0 | 0 | 14 | 8 | 5 | 2 | 2 | 12 | 9 | 9 |

* Each figure is the total for the scores obtained for both PDA and PCA exposed to the three regimes of light.
The inocula were from cultures that had been transferred
I = once;
II = two to three times;
III = up to five times

There was an interaction between the isolates and the different regimes of light treatments. The effect of light regime was noticeable with cultures of isolate $ZB_3$ which did not produce the pigment when incubated in continuous light or alternate light/dark periods but produced traces of the pigment when the cultures were incubated in the dark (Table 4.1.6b and Plate 8b). Isolate $ZB_2$ also produced no pink-purple pigment when incubated in continuous light; it produced traces of the pigment in continuous darkness, and when cultures of the isolate were incubated initially in the dark and exposed for five to six hours of light daily, diffusible amounts of pink-purple pigment were produced in alternate concentric bands (Plate 9). There was very little effect of the light regimes on the production of the pigment by the cowpea isolates $KP_3$, TZ and $ZB_1$ and the lima bean isolates, which produced diffusible amounts of the pigments.

(e) Growth and mycelial interactions of cowpea and lima bean isolates of Cercospora canescens.

The results of the experiments described in the preceding sections have shown that there is a wide range of variation among the isolates in the cultural and morphological characteristics that were compared. Interrelationships between isolates of Cercospora species with hyaline acicular conidia have been based on growth rates and their ability to produce a purple pigment in culture (Roy, 1982), sporulation and dimensions of

PLATE 8

Effects of medium composition and light on the colony characteristics and pigmentation of cowpea and lima bean isolates of C. canescens grown on potato dextrose agar (PDA) A and B and on potato carrot agar (PCA) C; incubated in continuous darkness (D) and alternate periods of 12 h light (near UV + white light) and darkness (L/D).

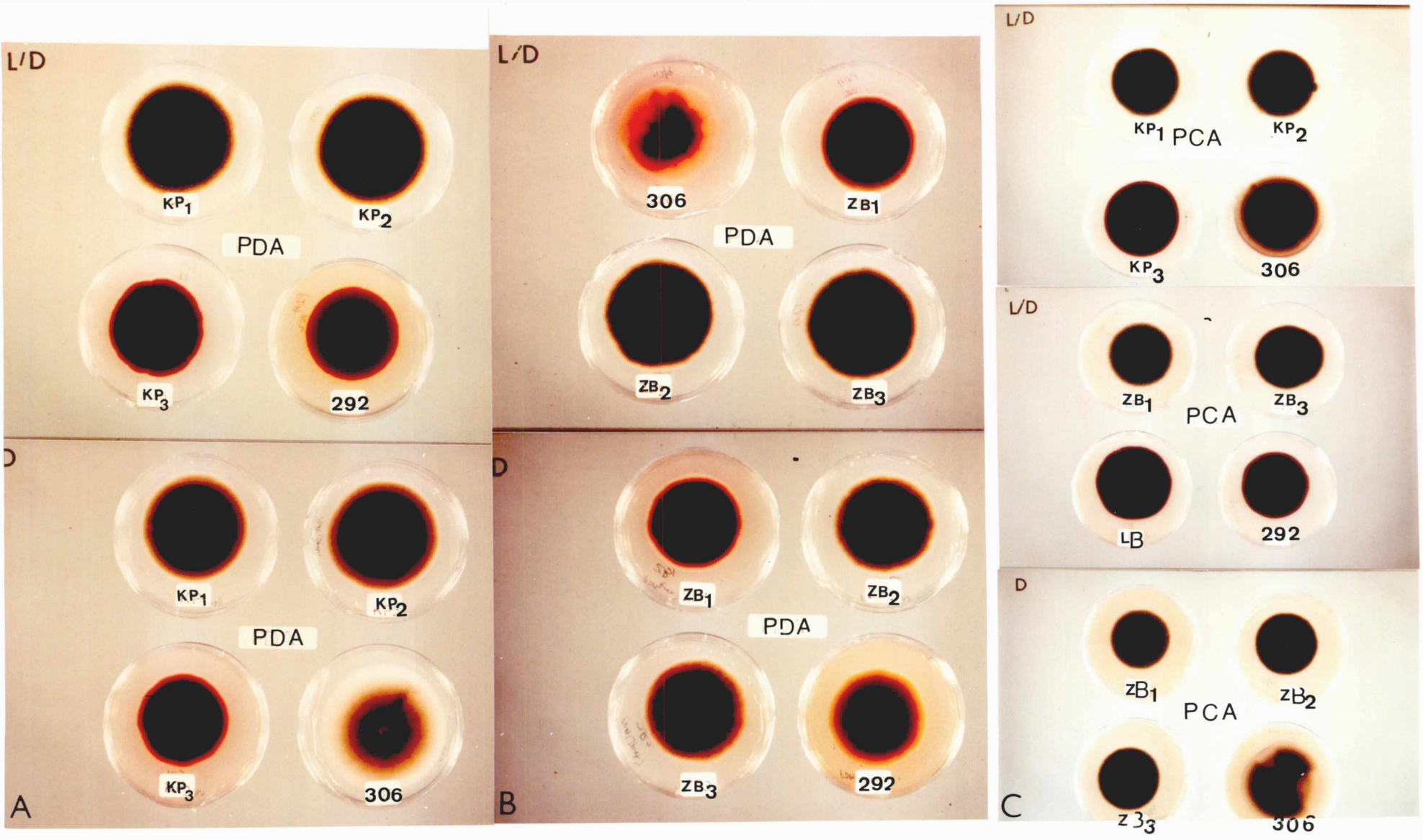

L/D
KP1
KP2
PDA
KP3
292
D
KP1
KP2
PDA
KP3
306
A
L/D
306
ZB1
PDA
ZB2
ZB3
D
ZB1
ZB2
PDA
ZB3
292
B
L/D
KP1 PCA
KP2
KP3
306
L/D
ZB1
ZB3
PCA
LB
292
D
ZB1
ZB2
PCA
Z B3
306
C

Plate 8

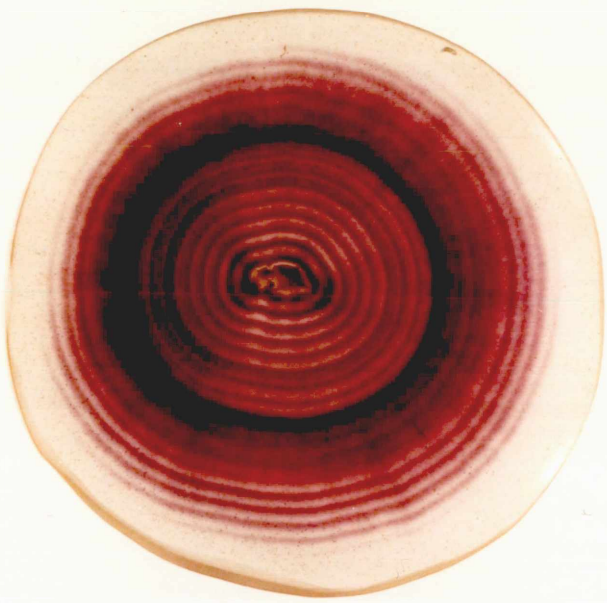


PLATE 9: Dried agar culture of cowpea isolate ZB2 of Cercospora canescens, grown on potato dextrose agar and incubated in the dark (wrapped in aluminium foil) with daily exposures of 5 to 6 h under three rows of white fluorescent light, showing the induction of concentric rings of pink/purple pigment production in response to light.

conidiophores and conidia (Fajola, 1978a) and by using similarity in protein patterns obtained by gel electrophoresis (Peterson and Latch, 1969; Macri et al., 1979). There have however, been no reports on experiments that have examined the type of reaction which occurs between mycelia of isolates of Cercospora spp. The type of reaction which occurs between mycelia of fungal isolates is probably an indication of their genetic relationship. Intermingling of hyphae without incompatibility and accompanied by anastomoses suggests a close relationship, whereas the growth of one hypha around another is considered as an antagonistic reaction which appears as a white zone to the unaided eye due to excessive branching of the mycelium (Wong and Willetts, 1975). The following experiments were conducted to explore the relationships between the isolates of Cercospora from cowpea and lima bean based on the interaction between their mycelia in co-inoculation studies, and to evaluate the practical application of the procedure in grouping the isolates.

In preliminary experiments, six isolates, $KP_1$, $KP_2$, $KP_3$ and TZ from cowpea, and isolates 292 and 306 from lima bean, were grown in separate columns in a 6 x 6 grid pattern on 12mm square dishes (Sterilin products) containing $40cm^3$ of potato dextrose agar (PDA). The colonies were started with 3mm mycelium-agar disks cut from the periphery of 1-week-old cultures of the isolates growing on PDA, and placed 1.5cm apart (Plate 10A). The cultures were incubated in

triplicate at 25 ± 2°C in the dark until the colonies were large enough to meet. The mycelial reactions in the regions of association were examined macroscopically and confirmed by microscopic examination to determine if the mycelia intermingled or showed some mutual antagonism, which was indicated by the formation of a white zone when viewed from the top of the cultures. This was observed to be the result of excessive branching of the hyphal tips of one or both of the colonies (Plate 12C).

The results of the preliminary experiments (Plate 10A) showed that after 10 days growth the mycelium of colonies formed by the cowpea isolates $KP_1$, $KP_2$ and $KP_3$ had grown and intermingled within colonies of the same isolates and between the isolates when examined microscopically. The colonies formed by the cowpea isolate TZ and the lima bean isolates 292 and 306 on the other hand, appeared to have ceased to grow so that there was a clear gap between the colonies within an isolate and between isolates. Microscopic examination of the cultures however, indicated that there was an apparent loose intermingling between the mycelia at the periphery of the colonies within the lima bean isolate 306. A second experiment (Plates 10B, 10C) was set up to investigate further the mycelial interactions between the cowpea isolates $KP_1$ and TZ, examined in the preceding experiment, and three other isolates from cowpea; $ZB_1$, $ZB_2$, $ZB_3$ obtained from Zambia, and a lima bean isolate, LB obtained from Ghana. The mycelial interactions were

PLATE 10

Growth and mycelial interactions between colonies of cowpea and lima bean isolates of Cercospora canescens grown on potato dextrose agar (PDA) and V-8 juice agar (V-8A).

PLATE 11

Mycelial interactions between colonies of isolates of C. canescens from cowpea and lima bean grown in three separate combinations.

A. Alternate columns of colonies of isolates that consistently produced a pink/purple pigment ZB1, LB, TZ and KP3) and isolates that produced an olivaceous to dark green pigment (ZB2, ZB3, KP1, KP2).

B. Diagonally arranged colonies of the isolates that produced an olivaceous to dark green pigment.

C. Diagonally arranged colonies of the isolates that produced a pink/purple pigment.

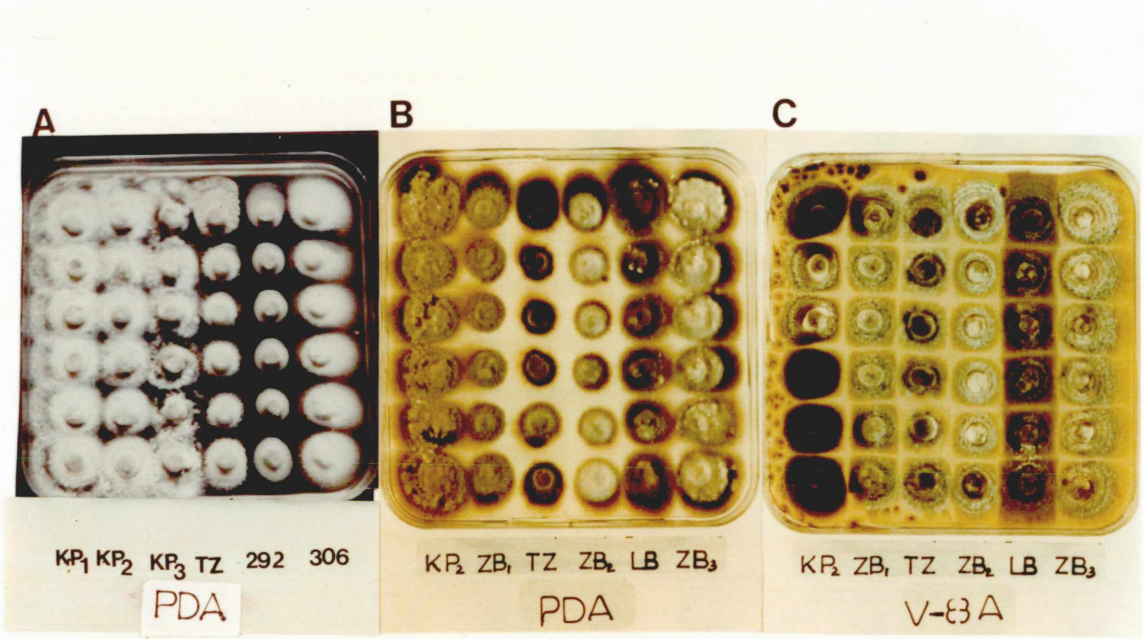



PLATE 10

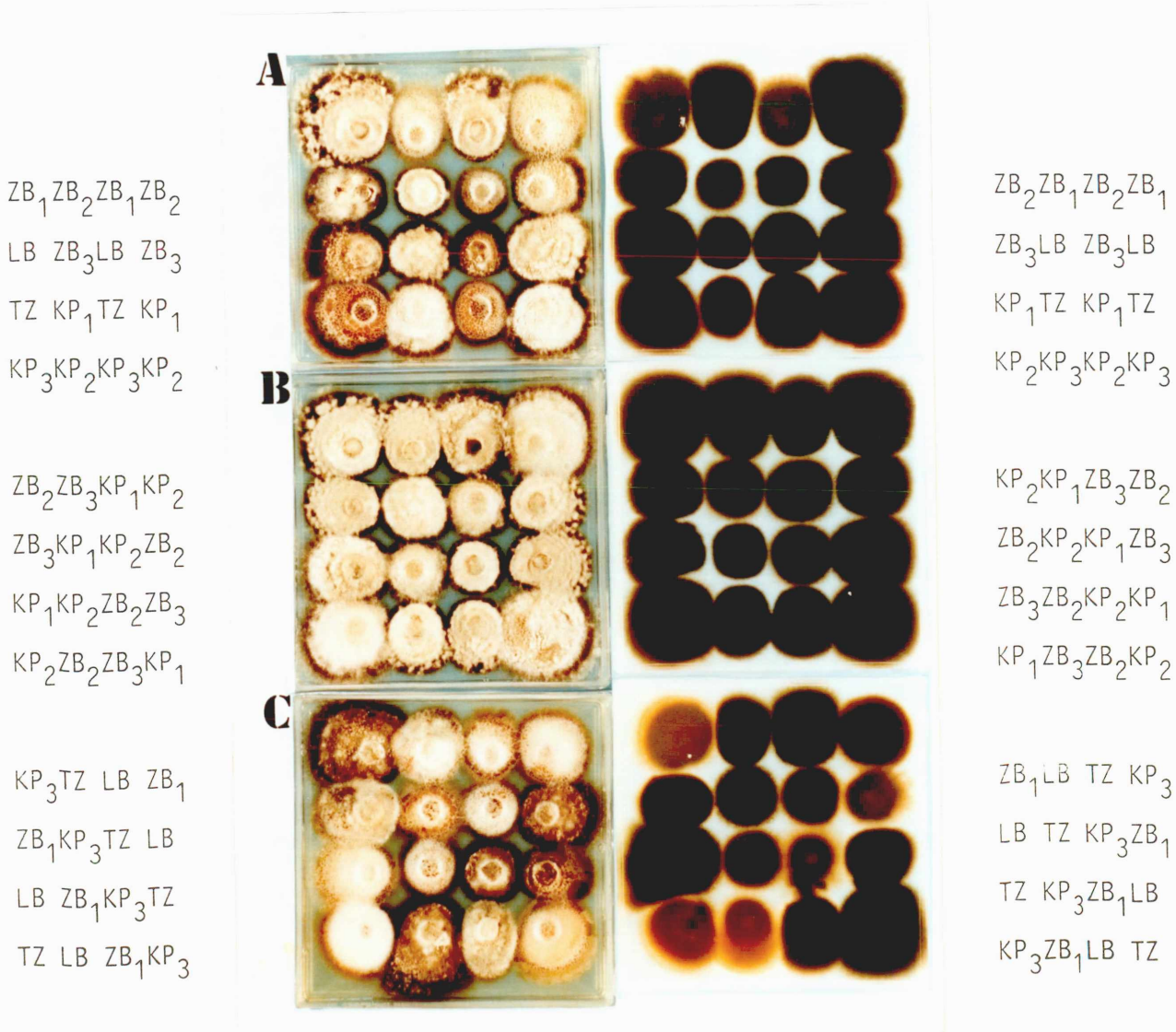



PLATE 11

investigated on PDA and V-8 juice agar. The colonies formed by the isolates (Plate 10B and C) $KP_2$ and TZ, were similar to those observed in the previous experiment. The colonies of isolate $KP_2$ intermingled freely whereas there was only a loose intermingling in colonies formed by isolate TZ. Free intermingling was also observed in colonies formed by the lima bean isolate LB and the cowpea isolate $ZB_3$. A striking difference in the morphology and general appearance of the colonies formed by all the isolates was observed on V-8 juice agar. The colonies particularly of isolates $ZB_1$ and LB appeared to have stopped growing radially towards the column of colonies of the opposing isolate, leaving clear gaps between the colonies. There were clear gaps between the colonies within an isolate too but these appeared indistinct compared to those formed between isolates. Microscopic examination of the colonies, from the reverse of the plates showed that the colonies had intermingled freely in isolates LB, $ZB_3$ and $ZB_1$ and loosely in isolates $KP_2$, TZ, and $ZB_1$ and $ZB_2$. The results of the two experiments therefore indicate no form of incompatibility between the isolates based on intermingling of their mycelia.

In a third experiment (Plate 11 ) further observations were made on the interaction between the isolates by selecting eight of the isolates, $ZB_1$, LB, TZ, $KP_3$ and $KP_1$, $KP_2$, $ZB_2$, $ZB_3$, on the basis of the pigmentation they produced in colonies on PDA (Section 1.1.1a). The isolates were grown in three separate combinations, to determine if

the isolates could be further grouped on the basis of mycelial interactions. In the first set of combination, the isolates were grouped into two; those producing a pink/purple pigment on PDA (isolates; $ZB_1$, LB, TZ and $KP_3$) were grown in alternate columns with isolates producing glaucous to dark-green pigmentation (isolates $KP_1$, $KP_2$, $ZB_2$ and $ZB_3$) (Plate 11A) in the second combination, the isolates that produced green pigment were grown so that colonies of the same isolate appeared diagonal to each other. This was done to examine if similar isolates would grow and intermingle diagonally, (Plate 11B). In a third treatment only the isolates producing a pink/purple pigment were grown in a pattern similar to the second treatment (Plate 11C). The colonies were started with 3mm mycelium-agar disks placed 2.0cm apart in a 4 x 4 grid pattern on 100cm square dishes (Sterilin) containing potato dextrose agar. After 2 weeks growth under alternate light/dark regimes most of the colonies had grown and met. The results (Plate 11), shows that there was an intermingling of mycelium irrespective of whether they formed a pink pigment or green pigment. In treatment A, the colonies formed by the lima bean isolate LB, and the cowpea isolate $ZB_3$ only intermingled loosely and this was not apparent to the unaided eye. The colonies formed by the other isolates, however, intermingled freely. There was a much more free intermingling between colonies producing similar pigments (Plate 11B and C).

The colours of the colonies formed by the isolates

PLATE 12

Photomicrographs of the mycelial interactions in the regions of association between colonies of C. canescens isolates on agar medium (PDA).

A. Loose intermingling (L) of hyphae at the periphery of colonies of the isolates that appear as gaps in the regions of association.

B. Free intermingling and anastomosis of hyphae (I).

C. Excessive branching of the hyphal tips of colonies, indicative of an antagonistic, incompatible reaction (A) which appear as white raised lines (See PLATE 16c).

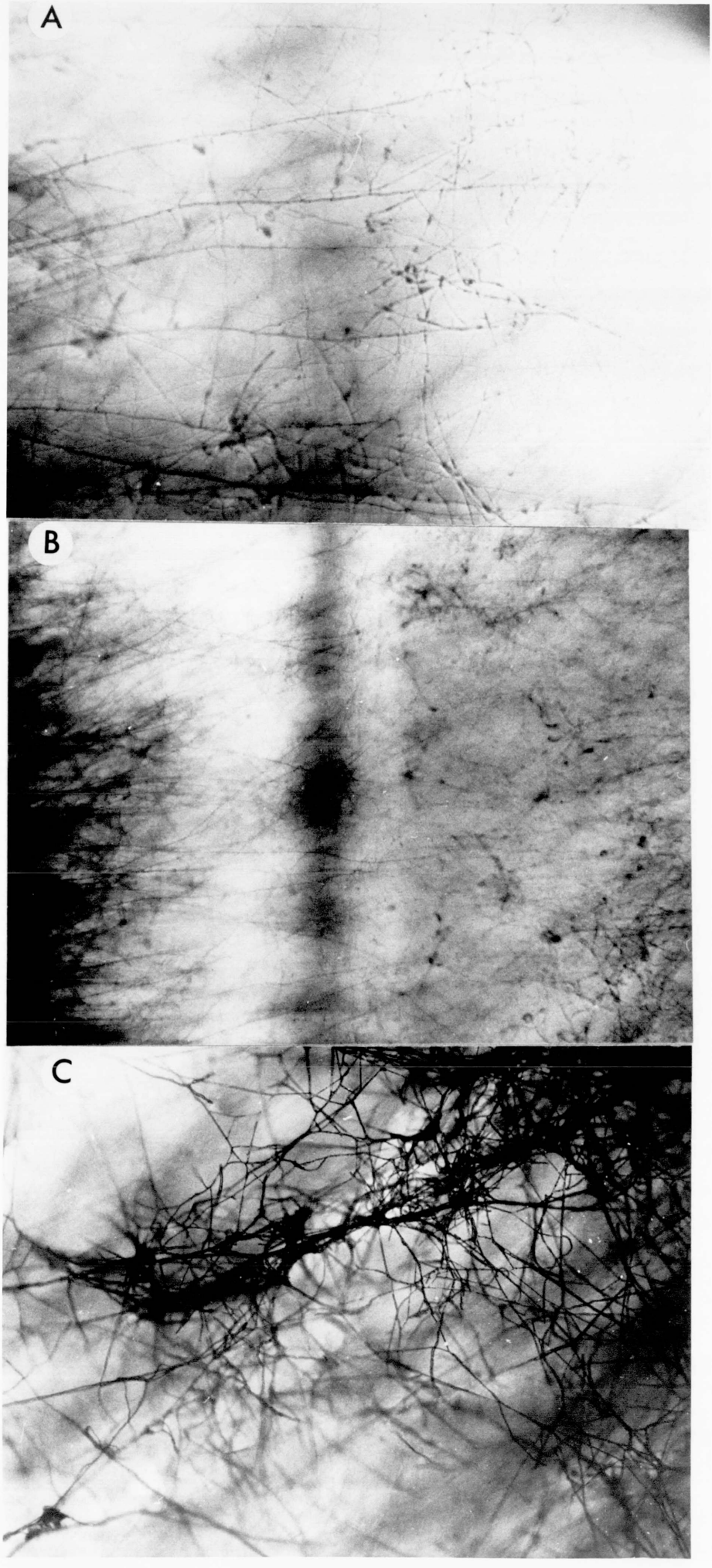



Plate 12

however were different, as observed in previous experiments, and were not generally altered. The pigmentation of the colonies were also not as distinct as in the previous experiments. The results thus indicate that there was no incompatibility reactions among the isolates examined at least when based on whether or not mycelia intermingled.

(f) Growth of cowpea and lima bean isolates of Cercospora canescens in liquid media

The results of the experiments that compared the linear growth on agar plates of isolates of Cercospora spp. (Section 1.1a) indicated small but significant differences between colony radii. To determine whether the observed differences were related to increases in mycelial mass, the growth of the 7 cowpea isolates in three liquid media were compared with the lima bean isolate LB, leaving out the other two lima bean isolates 306 and 292, (because the sub-cultures of the isolates that had been stored for some time began to sector frequently on further transfers).

The media, potato dextrose broth, cowpea leaf decoction and V-8 juice broth, were prepared as described in Section 3.3.2. Fifty millilitres of each medium was distributed in 150cm$^3$ Erlenmeyer flasks and inoculated with one 5mm mycelium-agar disk cut from the periphery of 1-week-old cultures of the isolates grown on PDA. The treatments were incubated in triplicate on a reciprocal shaker (Baird and Tatlock, M.330/0025) in a controlled temperature room (25 ±

$2^{o}C$) for 14 days. The dry weights of mycelial mass produced by the isolates differed significantly on the three media (Table 4.1.7). Isolates $KP_1$ and $KP_2$ produced more mycelial mass compared to the other five cowpea isolates ($KP_3$, TZ, $ZB_1$, $ZB_2$ and $ZB_3$) on all the liquid media, but did not differ significantly from the lima bean isolate (LB) on cowpea leaf decoction. In general, on the three media, isolate $KP_1$ produced more mycelium followed next by isolates $KP_2$ and $ZB_1$. Isolate $KP_3$ produced the least amount of mycelium. V-8 juice broth supported the best growth of the isolates in liquid media and the smallest growth was observed on cowpea leaf decoction. Ranked comparisons of the growth of the isolates on agar and liquid medium, using the Spearman's Rank-order Correlation coefficient (Appendix Table 7) indicates that there was an association, but not strong, between the growth of the isolates on agar and liquid medium when potato dextrose was used as the growth medium ($r_s = 0.67$, $P < 0.05$). But there was no such significant correlation when V-8 juice and cowpea leaf decoction were used as growth media.

Isolates $KP_1$, $KP_2$, TZ and $ZB_1$ grew well in both liquid and agar media in all the media tested, and the growth of isolate $KP_3$ was equally good in both potato dextrose broth and on potato dextrose agar. On the other hand the growth of isolate $KP_3$ in cowpea leaf decoction was relatively lower compared to its growth on cowpea leaf decoction agar. Conversely, the lima bean isolate (LB) grew better in the

Table 4.1.7 Growth of cowpea and lima bean isolates of Cercospora canescens in liquid media. Mean dry weight of mycelium (mg)*

| Isolates | Potato dextrose broth | Cowpea Leaf decoction | V-8 Juice broth | Means of weight for Isolate |
|---|---|---|---|---|
| $KP_1$ | 243.0a | 212.7a | 215.0ab | 223.6a |
| $KP_2$ | 233.3ab | 214.0a | 201.3bc | 216.2b |
| $KP_3$ | 136.7e | 133.7d | 186.3d | 152.2e |
| TZ | 194.7d | 128.0d | 191.7cd | 171.4d |
| $ZB_1$ | 208.0cd | 190.0b | 228.3a | 208.8bc |
| $ZB_2$ | 137.7e | 163.7c | 212.0bc | 171.1d |
| $ZB_3$ | 141.0e | 168.0c | 192.7cd | 167.2d |
| LB | 219.7bc | 202.7ab | 191.7d | 204.7c |
| Means of weight for media | 189.3b | 176.6c | 202.3a | |

* Each value is the mean of three replicates. Values in each column and the bottom row (means of weight for media) followed by similar letters are not significantly different from each other as determined by Duncan's multiple range test at the 5% level of significance.

liquid media than agar media; and isolate $ZB_3$ grew best on agar media compared to the liquid media.

### 4.1.2 Comparisons of the growth and cultural characteristics of isolates of C. canescens from five species of legumes

The results of the experiments that compared the growth and cultural characteristics of isolates of C. canescens from cowpea and lima bean (Section 4.1.1) shows that there were some differences in the morphology, pigmentation and small, but statistically significant, differences in radial growth among the ten isolates studied. The following experiments compared two of the cowpea isolates, ($KP_2$ and TZ) and the lima bean isolate (LB) with isolates of C. canescens from mung bean (VR) bambarra groundnut (VS) and groundnut (AH), isolated from infected leaves obtained from the sources listed in Table 3.4. Comparisons of the isolates were made by repeating the experiments described in Section 4.1.1, with some modification of the experimental procedures where indicated.

#### (a) Growth and cultural characteristics on agar media

In addition to the five media tested in Section 4.1.1a, mung bean leaf extract plus oat meal agar (MOA) and cowpea leaf decoction plus potato dextrose agar (CLPDA) see Section 3.3.1 for composition, were included. Four replicate plates of each medium were inoculated with agar-mycelium disks and

incubated as previously described. Increases in radial growth of the colonies was measured at 7, 10 and 14 days after incubation. A comparison of the growth of the isolates after the first week, shows that there were small but significant differences in the increase of the colony sizes (Table 4.1.8). The colonies formed by the isolates from cowpea ($KP_2$, TZ) and lima bean (LB) had grown slightly more radially compared to the isolates from bambarra groundnut (VS) and groundnut (AH). The colonies formed by the mung bean isolate (VR) on the other hand were very small and compact. Generally the isolates grew better on potato dextrose carrot agar, and there was a significant isolate x medium interaction. The extent of increases in radial growth of the isolates during the first and second week's growth differed with respect to the kind of medium. A comparison of the mean increases for the two periods (Table 4.1.9) confirmed that the cowpea ($KP_2$, TZ) and lima bean (LB) isolates grew relatively faster radially compared with the isolates from mung bean (VR), bambarra groundnut (VS) and groundnut (AH). To determine the rates at which the isolates grew, the mean increments in colony diameters between the 7th and 10th day and the increases between the 10th and 14th day were compared. The results (Table 4.1.10) show a significant isolate x medium x time interaction for the rates of growth. The overall results however, indicates that the differences were relatively small with a maximum range of 11-13mm in three days.

Table 4.1.8

Comparison of the radial growth of isolates of C. canescens from five legume species on 7 agar media after 7 days growth at 25 + 1°C.

| Medium | Mean radial growth (mm)* Isolate | | | | | | Mean |
|---|---|---|---|---|---|---|---|
| | $KP_2$ | TZ | LB | VR | VS | AH | of 5 Isolates |
| PDA | 28.0 | 30.0 | 25.0 | 18.3 | 23.8 | 23.5 | 24.8 b |
| PCA | 28.5 | 27.5 | 23.5 | 19.8 | 21.8 | 24.8 | 24.3 b |
| PDCA | 33.0 | 27.5 | 27.3 | 20.8 | 24.3 | 28.5 | 26.9 a |
| V-8A | 24.8 | 24.5 | 22.3 | 19.5 | 19.0 | 20.0 | 21.7 c |
| CLDA | 27.5 | 26.8 | 21.8 | 19.5 | 18.5 | 20.0 | 22.4 c |
| CLPDA | 25.8 | 22.8 | 21.0 | 20.5 | 18.8 | 18.8 | 21.3 c |
| MOA | 23.3 | 23.0 | 20.8 | 18.8 | 18.3 | 20.5 | 20.8 c |
| Mean of 7 Media | 27.3 a+ | 26.0 b | 23.0 c | 19.6 e | 20.6 de | 22.3 cd | |

* Mean of 4 replicates

\+ Means followed by the same letter(s) are not significantly different (according to Fisher's least significant test P = 0.05).

**Table 4.1.9**

Comparison of the radial growth of isolates of C. canescens from five legume species on 7 agar media after 14 days growth at 25 + 1°C.

| Medium | Mean radial growth (mm)* Isolate | | | | | | Mean |
|---|---|---|---|---|---|---|---|
| | $KP_2$ | TZ | LB | VR | VS | AH | of 5 Isolates |
| PDA | 23.7 | 24.3 | 23.0 | 17.4 | 23.1 | 24.4 | 22.6 e |
| PCA | 25.4 | 23.8 | 23.5 | 23.0 | 22.3 | 22.5 | 23.4 d |
| PDCA | 27.5 | 27.4 | 25.3 | 20.1 | 23.6 | 24.1 | 24.7 a |
| V-8A | 26.9 | 25.8 | 25.6 | 23.0 | 20.6 | 21.8 | 23.9 c |
| CLDA | 27.5 | 27.5 | 25.5 | 21.0 | 21.3 | 23.0 | 24.3 bc |
| CLPDA | 27.4 | 24.3 | 23.3 | 19.1 | 20.5 | 20.8 | 22.5 e |
| MOA | 27.4 | 27.5 | 26.4 | 20.1 | 21.3 | 23.6 | 24.4 b |
| Mean of 7 Media | 26.5 a | 25.8 b | 24.6 c | 20.5 f | 21.8 e | 22.9 d | |

* Mean of the increases in radial growth during the first and second weeks

\+ Means followed by the same letter(s) are not significantly different (according to Fisher's least significant test P = 0.05).

**Table 4.1.10**

Growth rates of isolates of C. canescens from five legume species on 7 media

| Isolate | Mean increment in radial growth mm/3 days* Medium | | | | | | | Mean of 7 media |
|---|---|---|---|---|---|---|---|---|
| | PDA | PCA | PDCA | V-8A | CLDA | CLPDA | MOA | |
| $KP_2$ | 9.6 | 11.1 | 10.9 | 14.5 | 13.8 | 14.1 | 15.8 | 12.9 a |
| TZ | 9.3 | 10.1 | 11.1 | 13.5 | 14.1 | 14.5 | 16.0 | 12.7 a |
| LB | 10.5 | 11.8 | 11.6 | 14.5 | 14.9 | 12.8 | 16.0 | 13.1 a |
| VR | 8.3 | 13.0 | 10.1 | 13.0 | 11.3 | 8.9 | 10.9 | 10.7 c |
| VS | 11.0 | 11.4 | 11.1 | 11.3 | 12.0 | 11.3 | 12.1 | 11.5 b |
| AH | 12.6 | 9.9 | 9.9 | 11.6 | 12.9 | 11.4 | 13.6 | 11.7 b |
| Mean of 5 Isolates+ | 10.2 e | 11.2 d | 10.8 d | 13.1 b | 13.2 b | 12.2 c | 14.0 a | |

* means of the rates of growth during 7-10th and 10-14 days

\+ Means followed by the same letter(s) are not significantly different. (Fisher's least significant difference test)

The morphology and other cultural characteristics of five of the isolates, $KP_2$, VR, VS, AH and LB were compared on potato dextrose agar, (PDA) potato carrot agar (PCA), potato dextrose carrot agar (PDCA) and V-8 juice agar (V-8A) leaving out cowpea leaf decoction (CLPDA) and mung bean leaf juice plus oat meal agar (MOA) because of the opacity of these media. Colony morphology, colours of the colonies from the top of the plates and the colours of the pigmentation of the surrounding medium when viewed from the reverse of the plates differed considerably among the isolates on all the media (Plate 13). The colour and topography of the colonies formed by an isolate also varied slightly with the kind of medium. The colonies formed by the isolates ranged from the very compact dark grey to black colonies formed by the mung bean (VR) and bambarra groundnut (VS) isolates to the effuse, felty light grey to olivaceous grey colonies formed by the cowpea isolate ($KP_2$) and the groundnut isolate (AH). The mung bean (VR) and lima bean (LB) isolates produced a pink purple pigment similar to that produced by the lima bean isolates (LB, 292 and 306) and the cowpea isolates ($KP_3$ and TZ) studied in Section 4.1.1, on all the four media tested. The extent of production and diffusion of the pigment was however most prominent on potato dextrose carrot agar. The cowpea ($KP_2$), bambarra groundnut (VS) and groundnut (AH) isolates on the other hand produced traces of a green to amber pigment at the periphery of the colonies.

PLATE 13

Variations in colour and colony morphology of isolates of Cercospora canescens from five legume species* cultured on potato dextrose agar (PDA), potato carrot agar (PCA), potato dextrose carrot agar (PDCA) and V-8 juice agar (V-8A) at 25°C.

A. View of colonies from the top to show the variation in topography and general appearance of the colonies.

B. Reverse of the colonies showing the variation in the colour of the pigmentation released into the surrounding medium.

* Isolates from:

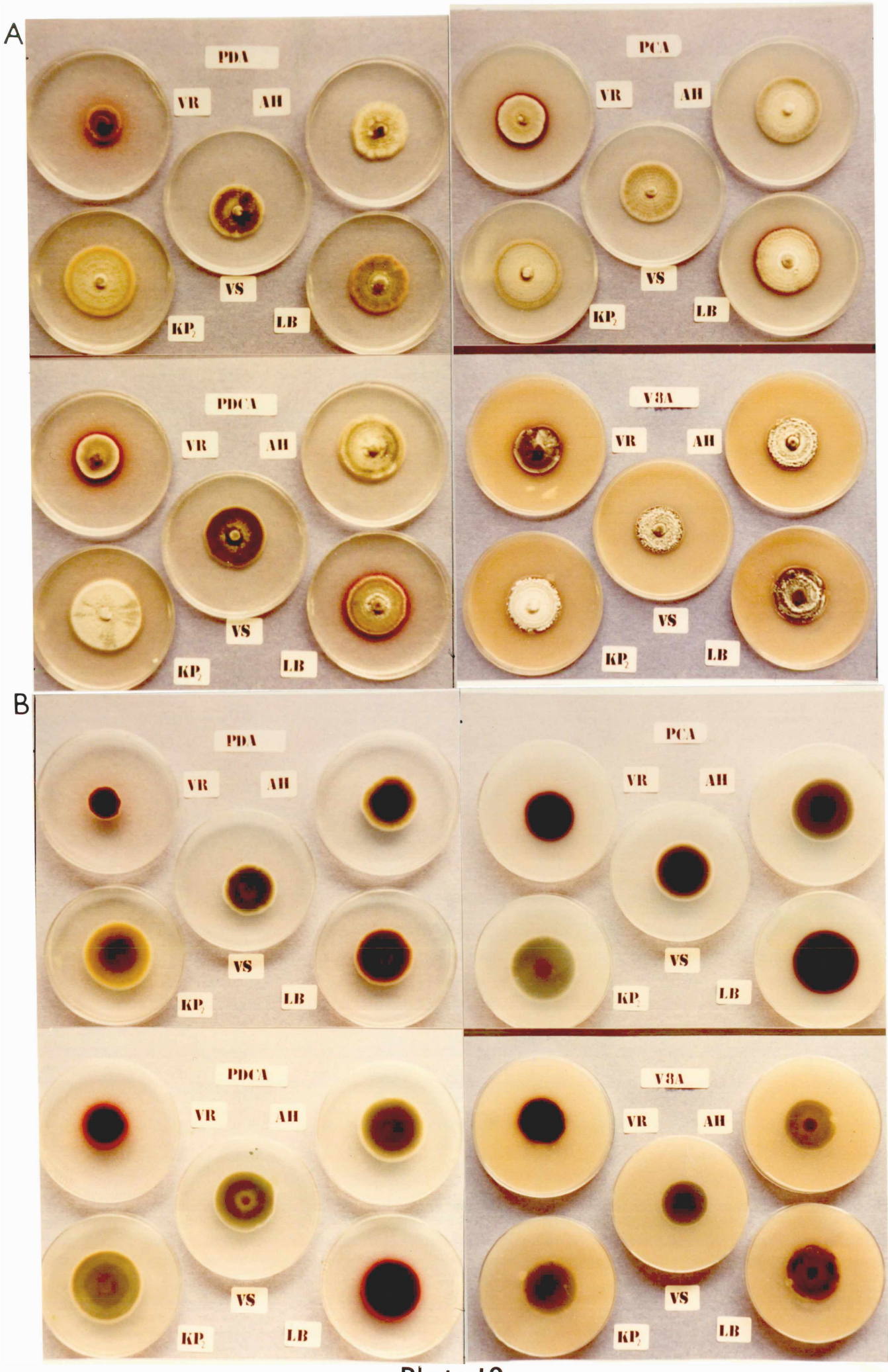
mung bean (Vigna radiata) = VR

groundnut (Arachis hypogaea) = AH

cowpea (Vigna unguiculata) = $KP_2$

lima bean (Phaseolus lunatus) = LB

bambarra groundnut (Voandzeia subterranea) = VS


A
PDA
VR
AH
VS
KP2
LB
PCA
VR
AH
VS
KP2
LB
PDCA
VR
AH
VS
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LB
V8A
VR
AH
VS
KP2
LB
B
PDA
VR
AH
VS
KP2
LB
PCA
VR
AH
VS
KP2
LB
PDCA
VR
AH
VS
KP2
LB
V8A
VR
AH
VS
KP2
LB

Plate 13

(b) Sporulation of the isolates on three media

Sporulation by the isolates was compared on V-8 juice agar (V-8A), cowpea leaf decoction agar supplemented with potato dextrose agar (CLPDA), and mung bean leaf extract plus oatmeal agar (MOA). Petri dishes containing $20cm^3$ of agar medium were inoculated by spreading a suspension of conidia plus mycelial fragments over the surface as described in Section 3.4.2. The conidia produced were washed from the plates and the numbers estimated with a haemacytometer. The experiment was repeated once, and the results were combined for an analysis of variance test. The cowpea isolate ($KP_2$) produced significantly higher number of spores, $5 \times 10^5$/plate (mean of the spores produced in the three media) and the least number of spores was produced by the mung bean isolate (VR) (Table 4.1.11). The overall results show that there was only a small but significant difference in the numbers of conidia produced on cowpea leaf decoction plus PDA and V-8 juice or mung bean leaf juice plus oatmeal agar. Among the isolates, the mung bean (VR), bambarra groundnut (VS) and groundnut (AH) isolates sporulated better on mung bean leaf juice plus oatmeal agar, whereas the isolates from lima bean (LB) and cowpea ($KP_2$ and TZ) sporulated slightly better on either V-8 juice agar or cowpea leaf decoction plus PDA (CLPDA).

To obtain sufficient numbers of conidia for inoculation, the mung bean isolate (VR) was grown on mung bean leaf extract plus oatmeal agar and the other isolates

**Table 4.1.11**

Comparison of the sporulating abilities of isolates of C. canescens from 5 legume species on 3 agar media

| Isolate Code | Spores per plate ($x10^5$)* on Medium† V-8A | CLPDA | MOA | Mean of three media |
|---|---|---|---|---|
| VR | 2.2 cC** | 2.5 cB | 3.3 bcA | 2.7 e |
| VS | 2.8 cB | 3.9 bA | 3.3 bcA | 3.3 d |
| AH | 4.5 bA | 4.7 aA | 4.8 aA | 4.7 a |
| LB | 4.0 bA | 4.1 bA | 2.9 cB | 3.7 c |
| $KP_2$ | 5.2 aA | 4.9 a AB | 4.8 aB | 5.0 a |
| TZ | 4.0 bB | 4.8 aA | 3.7 bB | 4.2 b |
| Mean of six isolates | 3.8 B | 4.1 A | 3.8 B | 3.9 |

* Means of 10 subsamples in two experiments (spore washes of 10 mls per plate)

\+ V-8A = V-8 juice agar
CLPDA = Cowpea leaf decoction plus oxoid PDA
MOA = Mungbean leaf juice plus oatmeal agar.

** Values followed by the same lower case letters in each column, and by the same upper case letters in each row, are not significantly different. $P < 0.05$ Duncan's multiple range test.

on V-8 juice agar and/or cowpea leaf decoction plus PDA.

(c) Morphology and dimensions of conidiophores and conidia

The morphology and dimensions of the conidia produced by the isolates on infected leaves of their respective hosts and of those produced in culture on cowpea leaf decoction plus PDA (CLPDA) and V-8 juice agar (V-8A) were compared including an isolate of Cercospora beticola from sugar beet (see Table 3.4 for source). The lengths and widths of thirty conidia formed on each of the three substrates and the lengths and widths of thirty conidiophores formed on infected host leaves were measured with an ocular eye piece. There was a considerable variation in the range of the lengths of the conidia produced by the isolates on all the three substrates, but only small differences in the widths of the conidia (Figure 4.1.1). The cowpea isolate (TZ) formed relatively longer conidia compared to the other isolates but there were no significant differences in the lengths of the conidia formed by isolates from bambarra groundnut (VS), and cowpea ($KP_2$) and between the latter and the isolates from groundnut (AH), lima bean (LB) and the isolate of C. beticola (Figure 4.1.2). The conidia produced by the isolates on infected host leaves were generally longer than those produced in culture and between the two agar media, the conidia formed on V-8 agar were slightly longer but not significantly (Figure 4.1.3).

The dimensions of the conidiophores also varied between

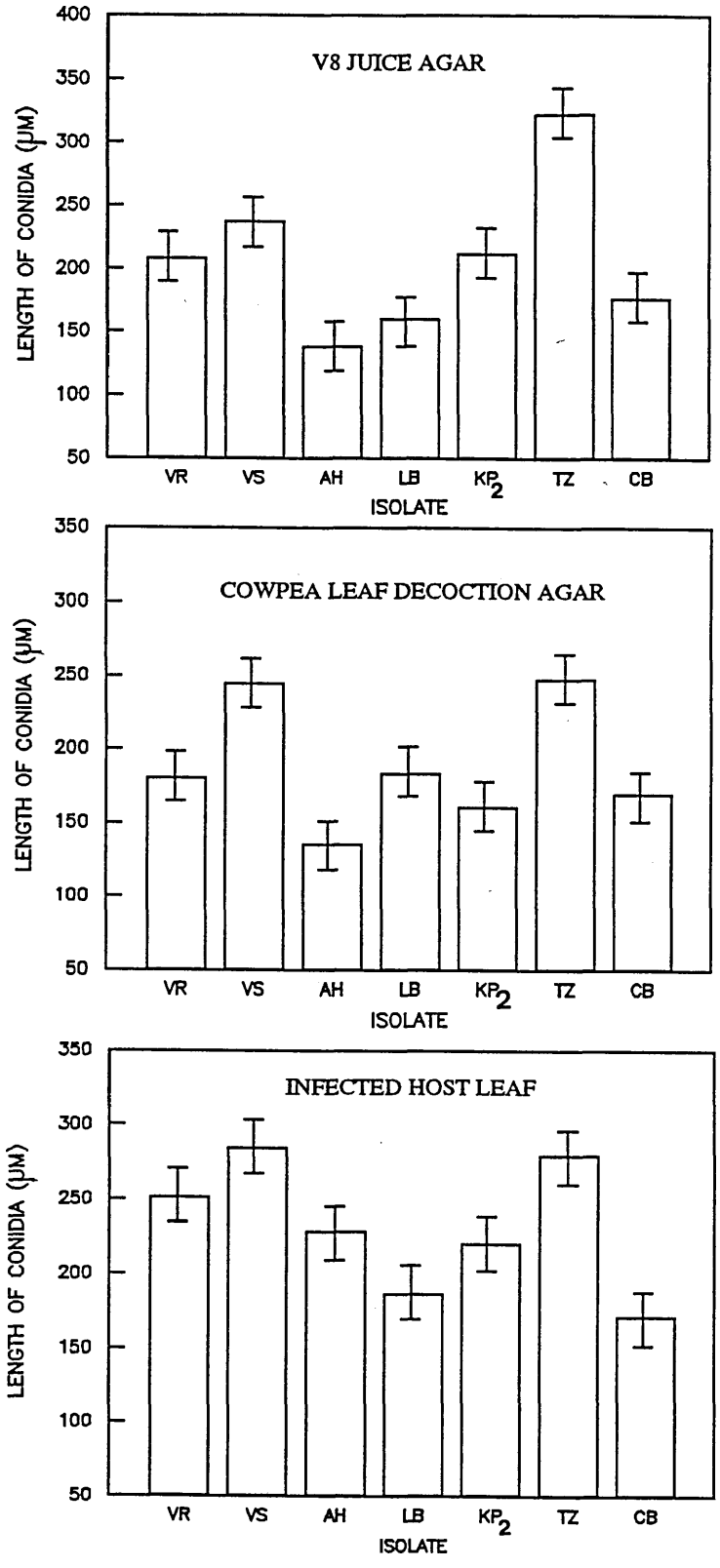



Figure 4.1.1. Comparison of conidia length of Cercospora isolates on three substrates. Vertical bars are LSD (0.05) values.

VR = Mungbean VS = Bambarra groundnut AH = Groundnut
$KP_2$= Cowpea ex Ghana TZ = Cowpea ex Tanzania
CB = Cercospora beticola

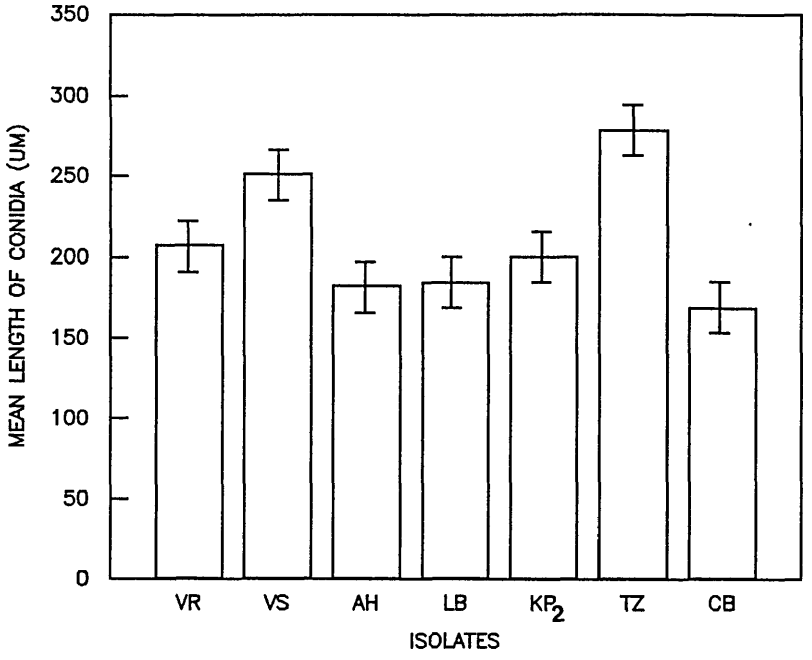



Figure 4.1.2. Variation in the lengths of conidia formed by the Cercospora isolates on all three media. Vertical bars indicate LSD values at 5% level.

VR = Mungbean VS = Bambarra groundnut AH = Groundnut
$KP_2$= Cowpea ex Ghana TZ = Cowpea ex Tanzania
CB = Cercospora beticola

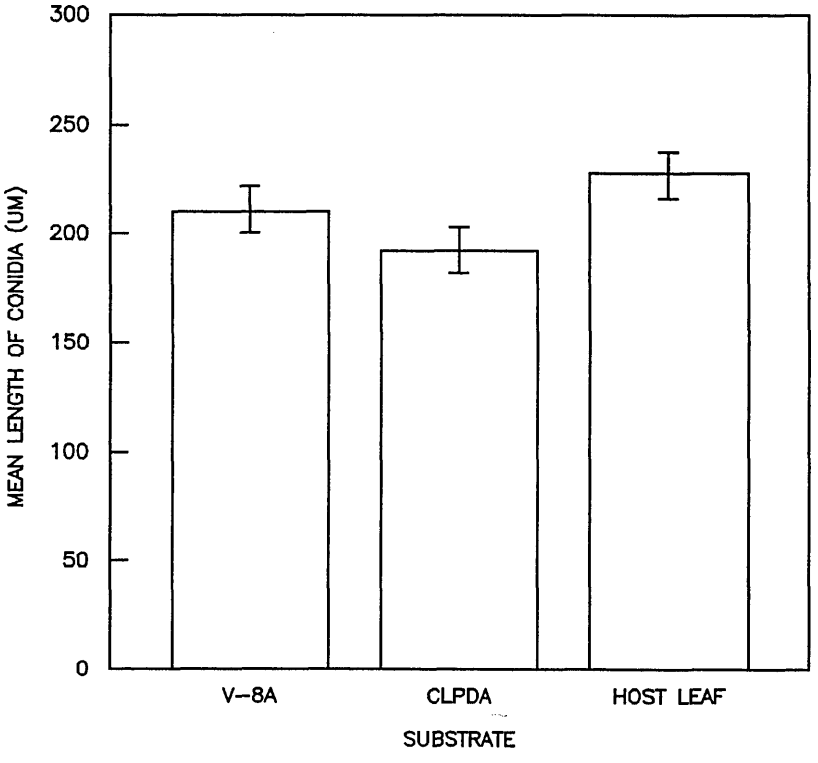



Figure 4.1.3. Lengths of conidia formed in three substrates.

the isolates of C. canescens and the conidiophores produced on infected groundnut leaves averaged the longest (mean of 848 ± 50 μ). The shortest conidiophores were produced by the lima bean isolate (LB) (mean of 301 ± 73 μ). The overall lengths of the conidiophores produced on infected leguminous host averaged longer those observed for the isolate of C. beticola. The morphology of the conidiophores differed among the isolates with respect to the density, compactness and the degree of geniculation on leaves incubated in Petri dish moist chambers, (Plates 14 and 15). The isolates from cowpea ($KP_2$ and TZ), groundnut (AH) and the mung bean isolate (VR) formed dense fascicles of conidiophores (10-30 stalks) whereas the fascicles of conidiophores formed by the bambarra groundnut isolate (VS) and the lima bean isolate (LB) were very sparse, consisting of only a few stalks, 2-9. Apart from being dense, the conidiophores of the cowpea isolates ($KP_2$ and TZ) tended to be more compact, but they were slightly divergent in those produced by the groundnut (AH), the mung bean (VR) and the bambarra groundnut (VS) isolates. Stromata formed by the isolates were relatively small in all the isolates except for the bambarra groundnut isolate (VS) in which stromata were very small and almost absent. Generally there was no branching of conidiophores except on some prepared slides of the groundnut isolate (AH) on which branching and catenulation were observed (Plate 15, AH). The colour of the conidiophores formed by all the isoaltes were pale to medium brown uniform in width (5-6.25 μ), ending in rounded apices with prominent conidial scars.

PLATE 14

Photomicrographs of the bases of conidiophores of isolates of C. canescens on infected host leaves showing the density, compactness, divergence and the relative sizes of stromata.

VR = mung bean isolate from The Philippines

VS = bambarra groundnut isolate from Zambia

AH = groundnut isolate from Malawi

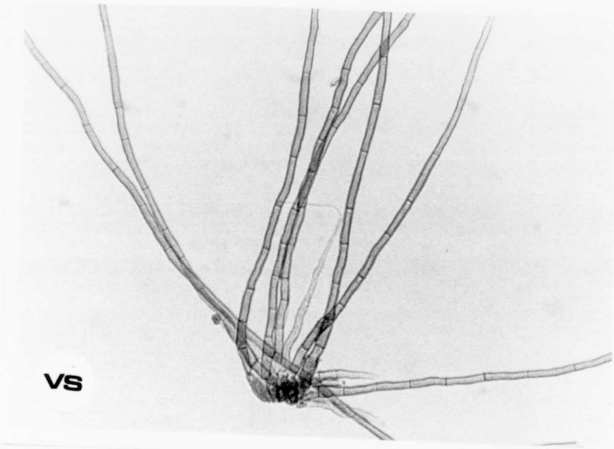
LB = lima bean isolate from Ghana

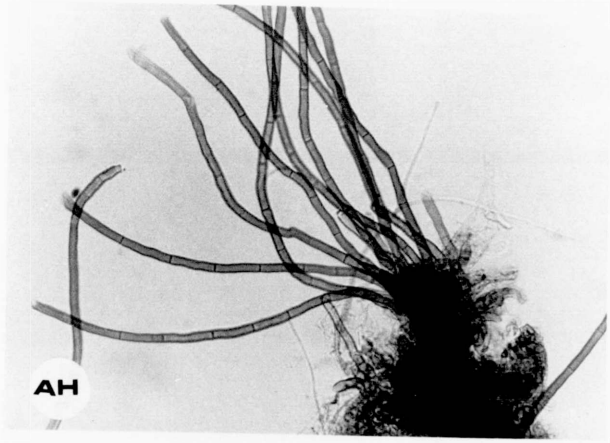
$KP_2$ = cowpea isolate from Ghana

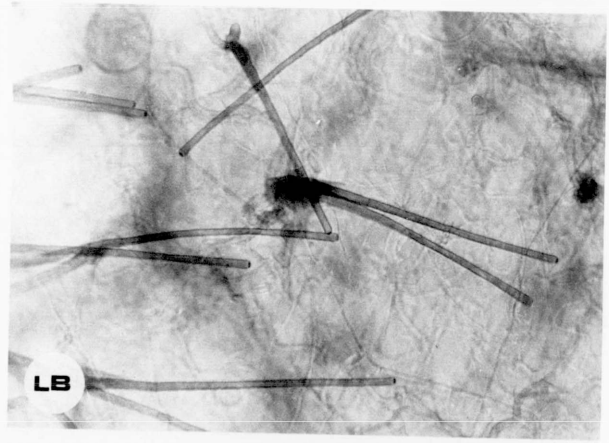
TZ = cowpea isolate from Tanzania

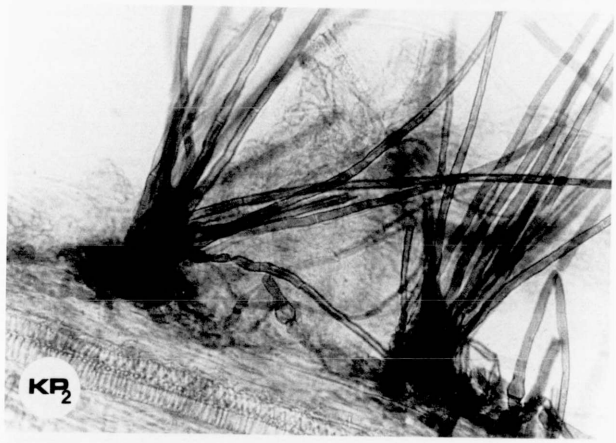
CBsb = Cercospora beticola, from sugarbeet (cv Julia)

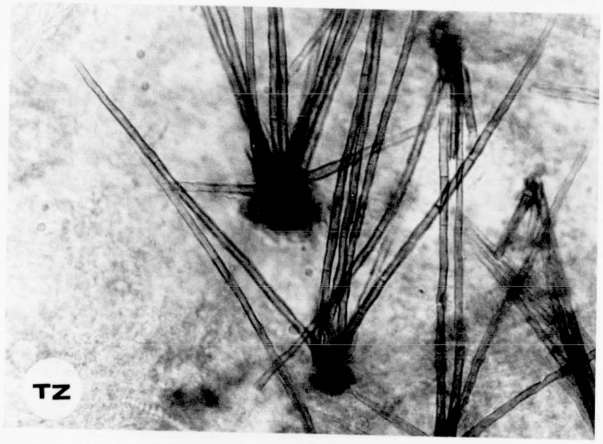
CBvu = Cercospora beticola from glasshouse infected cowpea cv Amantin (see Plate 23).

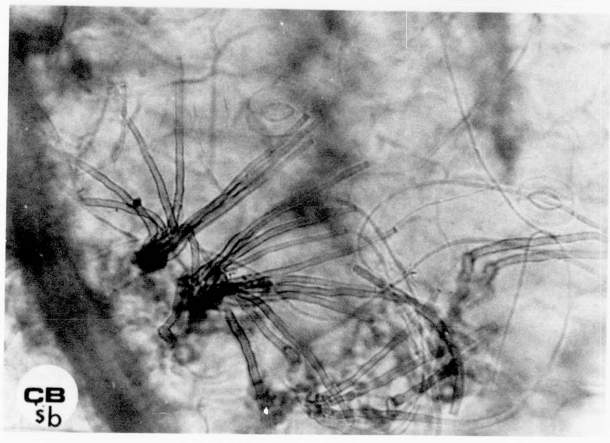



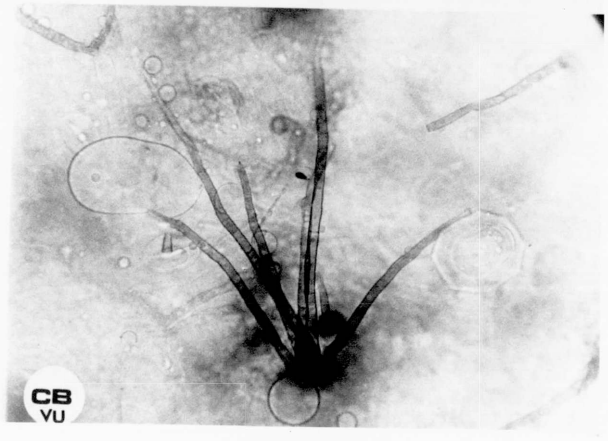




Plate 14

PLATE 15

Photomicrographs of the tips of conidiophores of C. canescens isolates showing rounded apices (slightly swollen in lima bean isolate (LB)) with prominent conidial scars.

Note branching (br) and caterpulation (ct) of some conidiophores formed by the groundnut isolate (AH).

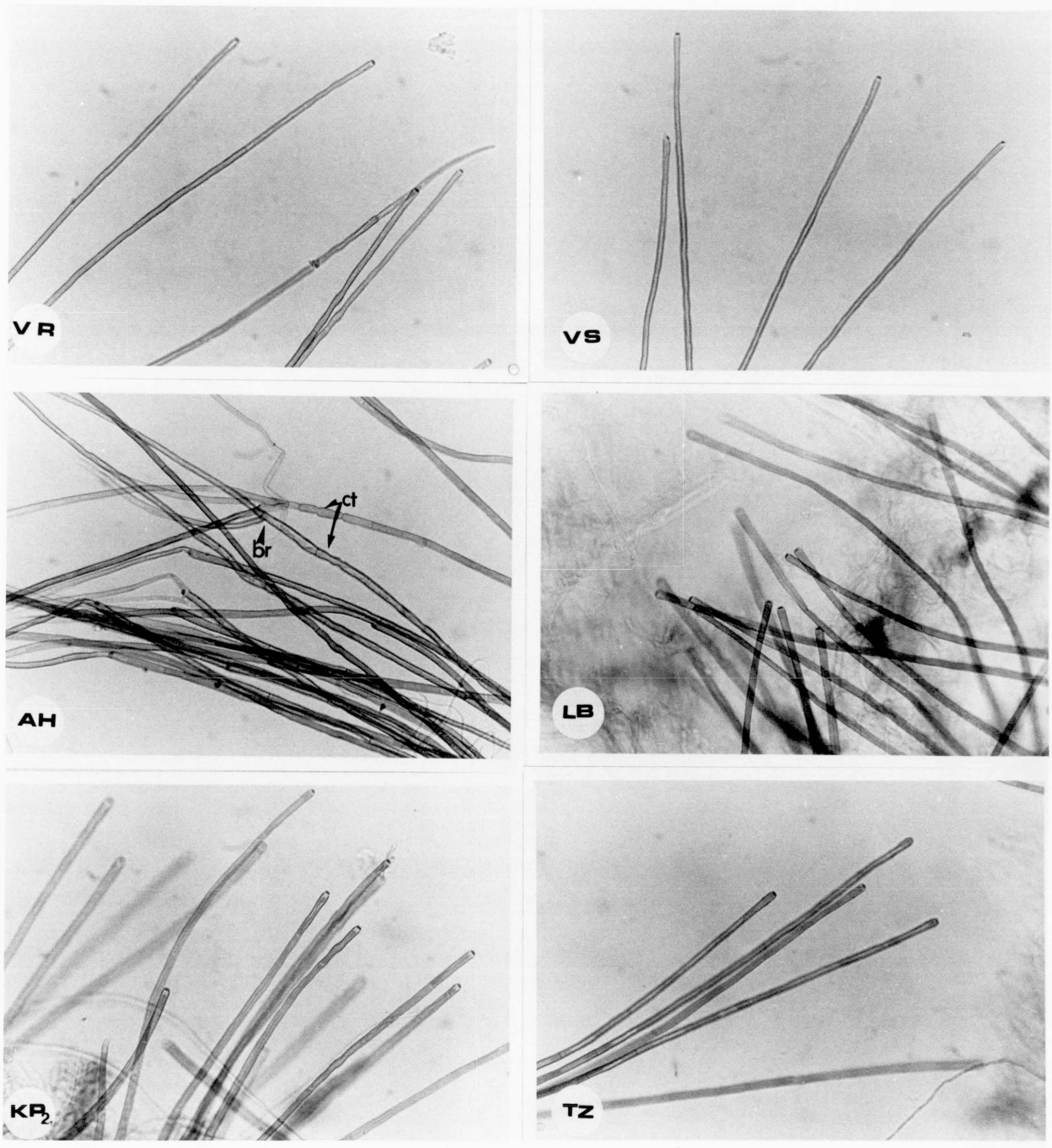



Plate 15

The tips of the conidiophores formed by the lima bean isolate (LB) however tended to be slightly swollen.

(d) Mycelial interactions between isolates of C. canescens from five legume species including an isolate of Cercospora beticola

The interaction between mycelia of the isolates of C. canescens and with C. beticola was investigated on potato dextrose agar (PDA). In a preliminary experiment, (Plate 16A), six colonies, each of similar isolates, were grown in columns as was done in Section 4.1.1.e, to determine if the colonies intermingled to produce a uniform colony. The isolates of C. canescens from cowpea ($KP_2$) and lima bean (LB) were included as controls. The cultures were incubated in the dark for two weeks before they were examined. The colonies formed by the cowpea ($KP_2$) and lima bean (LB) isolates intermingled freely within each column as was observed in the previous experiment. The colonies of the isolate of Cercospora beticola also intermingled but this was not apparent to the unaided eye. Colonies of the isolates from mung bean (VR), bambarra groundnut (VS), and groundnut (AH), on the other hand intermingled loosely and this was only visible under the microscope.

In a second experiment the colonies of similar isolates were arranged diagonally (Plate 16B) to determine if a pattern of intermingling, reflecting their interrelationships would emerge. In contrast to what was

PLATE 16

Mycelial interactions between isolates of C.canescens from cowpea ($KP_2$), mungbean (VR), groundnut (AH), lima bean (LB), bambarra groundnut (VS) and an isolate of Cercospora beticola (CB) from sugar beet grown on potato dextrose agar (PDA).

A. Colonies of the isolates grown in columns to determine if colonies of similar isolates intermingled.

B. Diagonal arrangement of similar colonies.

| | | | | | |
|---|---|---|---|---|---|
| CB | VS | LB | AH | VR | $KP_2$ |
| $KP_2$ | CB | VS | LB | AH | VR |
| VR | $KP_2$ | CB | VS | LB | AH |
| AH | VR | $KP_2$ | CB | VS | LB |
| LB | AH | VR | $KP_2$ | CB | VS |
| VS | LB | AH | VR | $KP_2$ | CB |

C. Colonies of the six isolates of Cercospora and a morphological variant of the cowpea isolate $KP_2{}^1$, to show the formation of thick white lines, representing an incompatible reaction between opposing colonies (excessive branching of hyphal tips).

D. Reverse of the colonies in C.

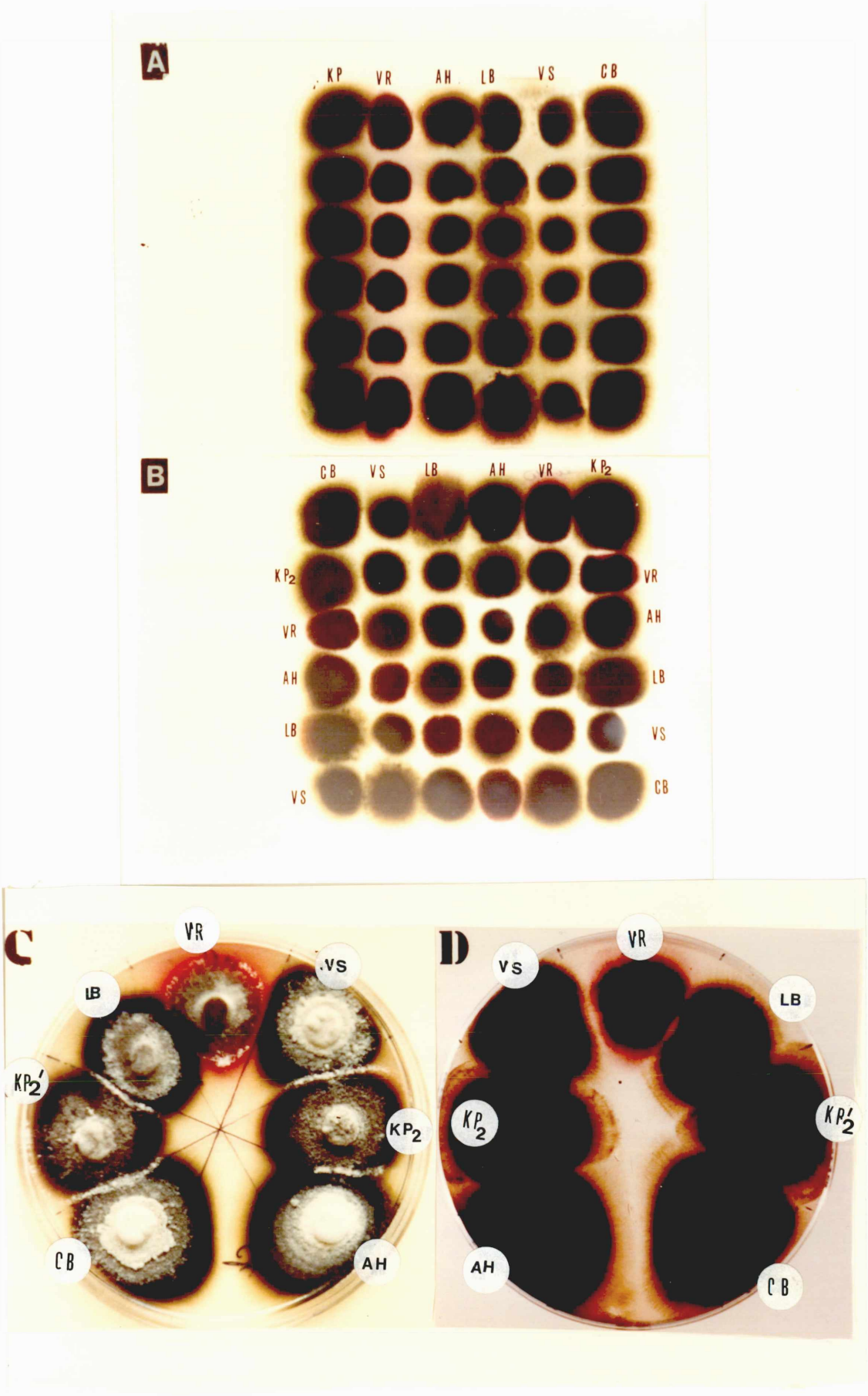

A
KP
VR
AH
LB
VS
CB
B
CB
VS
LB
AH
VR
KP2
KP2
VR
AH
LB
VS
CB
C
VR
VS
LB
KP2'
KP2
CB
AH
D
VR
VS
LB
KP2
KP2'
AH
CB

Plate 16

observed when colonies of the isolates were arranged in columns (Plate 16A), the colonies of similar isolates arranged diagonally failed to intermingle. Generally the type of reaction which occurred between opposing colonies was inconsistent. Some colonies of the cowpea isolate ($KP_2$) intermingled diagonally on some plates but appeared to have stopped growing on others.

To confirm the practicality of using the procedure in establishing the interrelationships between the isolates of Cercospora, a third experiment was done, in which each of the isolates was grown (on potato dextrose agar (PDA) in 9.0cm Petri plates) in an alternate arrangement of an isolate with each of the other five isolates, (Fig. 4.1.4). There were three replicates for each treatment and the plates were incubated in the dark at 25°C for two weeks. The results are summarised in Table 4.1.12.

An antagonistic reaction, (white lines) representing a region of excessive branching of hyphal tips of the opposing colonies around each other, was observed between the colonies of the cowpea isolate ($KP_2$) and the isolates from lima bean (LB), groundnut (AH), bambarra groundnut (VS) and with Cercospora beticola, (Plate 16C and D).

The cowpea isolate ($KP_2$) intermingled only with the

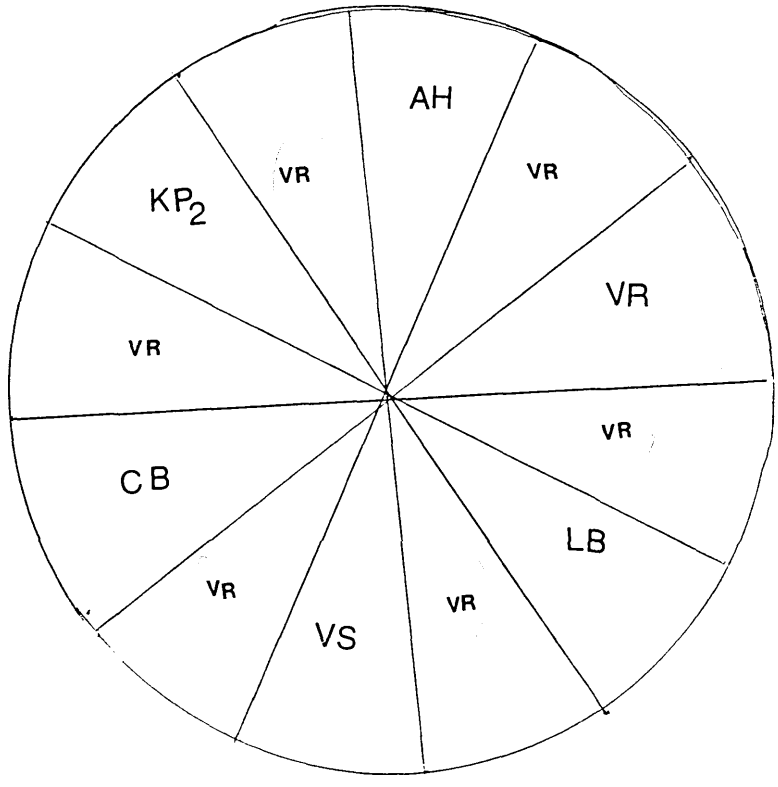



Figure 4.1.4 Alternate arrangement of colonies of mung bean (VR) isolate of Cercospora, with colonies of five other isolates to determine the type of reaction between mycelia of opposing colonies.

Table 4.1.12

Mycelial interactions between isolates of *C. canescens* from five leguminous hosts and an isolate of *Cercospora beticola*

| Isolate | Observed reaction* with isolate from | | | | | |
|---|---|---|---|---|---|---|
| | Cowpea ($KP_2$) | Mungbean (VR) | Groundnut (AH) | Lima bean (LB) | bambarra (VS) | C. beticola (CB) |
| Cowpea ($KP_2$) | I | - | - | - | - | - |
| Mungbean (VR) | L | L | - | - | - | - |
| Groundnut (AH) | A | L | L | - | - | - |
| lima bean (LB) | A | L | L | I | - | - |
| bambarra (VS) | A | L | L | L | L | - |
| *C. beticola* (CB) | A | G | G | G | G | I |

* I = free intermingling
L = loose intermingling
A = excessive branching of hyphal tips around each other
G = clear gaps between opposing colonies

isolate from mung bean (VR). The isolates from lima bean (LB), bambarra groundnut (VS), mung bean (VR) and groundnut (AH), intermingled either loosely or freely. There were however, clear gaps between the colonies of C. beticola and the isolates of C. canescens from mung bean (VR), groundnut (AH), and lima bean (LB). The reaction between C. beticola and the isolate from bambarra groundnut (VS) was not clearly defined. The mycelia of the two isolates intermingled loosely in the treatment in which a colony of the bambarra groundnut isolate (VS), was grown between two colonies of C. beticola (CB), but in the treatment in which C. beticola (CB) was grown between two colonies of the bambarra groundnut isolate (VS), there were gaps between the colonies of the two isolates.

### 4.1.3 Growth, colony characteristics and sporulation of two isolates of Pseudocercospora cruenta

Radial growth and sporulation of two isolates of P. cruenta; Pckp and AC 5114 (see Table 3.4) was studied on the five agar media tested with C. canescens isolates. Petri dishes containing 20cm$^3$ of medium were inoculated with five replicates for each medium. The cultures were incubated under alternate periods of 12h darkness followed by 12h white and near UV light, (a combination of 2 white fluorescent tubes 40w and 1 black tube). The increases in colony diameters on the five media were measured after 10 days. The experiment was repeated once, and because the results were similar, they were combined for an analysis of

variance test. The colonies formed by the two isolates on all the five media were very compact with very little aerial mycelium (Plate 17), compared with the colonies formed by isolates of C. canescens (see Sections 4.1.1 and 4.1.2). There were small but significant differences in the radial growth of the isolates on the five media. Growth of both isolates was slightly faster on potato dextrose agar (PDA) and least on potato carrot agar (PCA) and leaf decoction agar (CLDA) (Table 4.1.13). Sporulation of the radially growing colonies was assessed daily as described in Section 3.4.1. Very few conidia were produced on PDA and PDCA, but moderate sporulation of both isolates was observed on V-8 agar and cowpea leaf dedoction (CLDA). The sporulating ability of the isolates was also assessed with multi-point inoculation, on V-8 agar, potato dextrose carrot agar (PDCA) and cowpea leaf decoction agar (CLDA). Better sporulation of the isolates was observed with multipoint inoculation on cowpea leaf decoction agar (CLDA) c. $6.7 \times 10^5$/ml (mean of two isolates (Table 4.1.14).

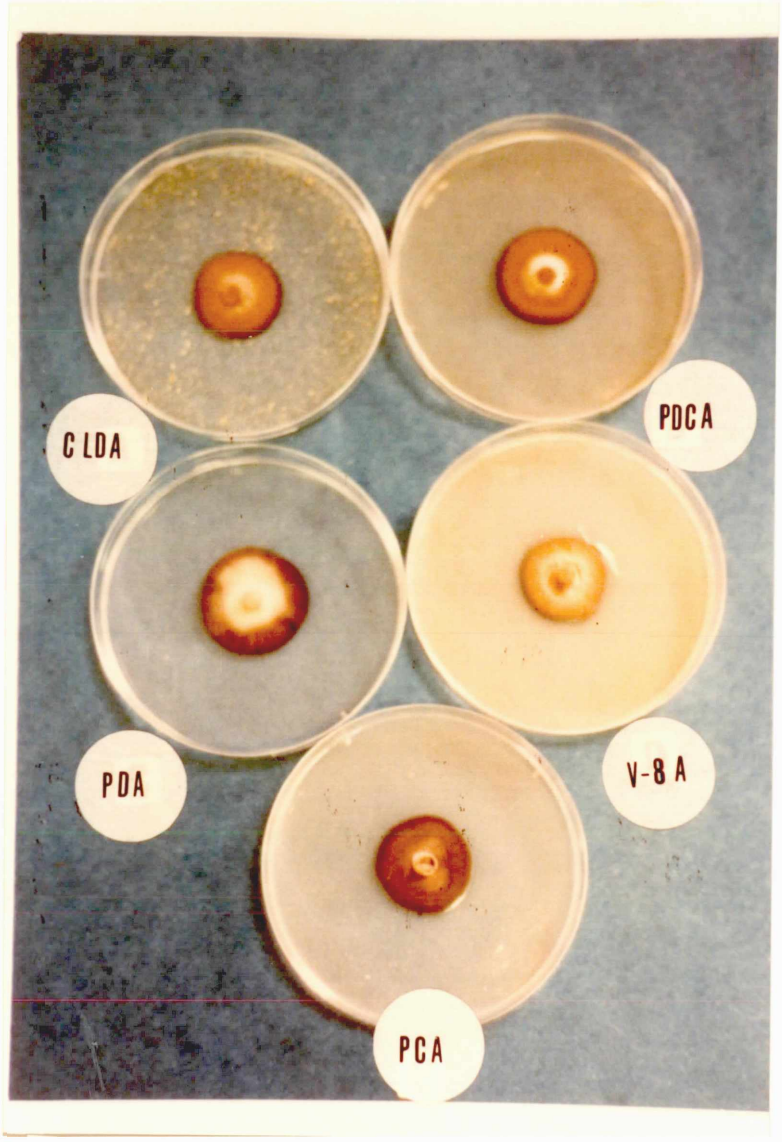



PLATE 17. Colony characteristics of cowpea (cv. caloona) isolate of Pseudocercospora cruenta on five media

CLDA = cowpea leaf decoction agar

PDCA = potato:dextrose carrot agar

PDA = potato dextrose agar

V-8A = V-8 juice agar

PCA = potato carrot agar

Table 4.1.13

Mycelial growth* of P. cruenta on different media.

| | Mean colony diameter after 10 days (mm) | | |
|---|---|---|---|
| Medium | Pc kp | AC5114 | Mean of 2 isolates |
| PDA | 13.2 | 12.4 | 12.8 a** |
| PCA | 10.6 | 10.4 | 10.5 cd |
| PDCA | 11.8 | 11.8 | 11.8 b |
| V-8A | 10.6 | 11.4 | 11.0 c |
| CLDA | 9.3 | 10.7 | 10.0 d |
| Mean of 5 media | 11.1 | 11.3 | 11.2 |

* Mean of 10 measurements

** Means followed by common letters are not significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

Table 4.1.14

Sporulation of P. cruenta isolates in culture

| Medium | Single point inoculation (relative numbers)* | | Multi-point inoculation (x $10^5$ spores/ml)* | |
|---|---|---|---|---|
| | Pc kp | AC5114 | Pc kp | AC5114 |
| PDA | Sparse | Sparse | - | - |
| PCA | None | None | - | - |
| PDCA | Sparse | Sparse | 1.9 | 1.5 |
| V-8A | Moderate | Abundant | 4.9 | 3.1 |
| CLDA | Abundant | Abundant | 7.1 | 6.2 |

* - Not tested
Means of numbers of conidia in 10 sub-samples

## 4.2 Pathogenicity and host range of isolates of Cercospora canescens and Pseudocercospora cruenta

### Introduction

Both C. canescens and P. cruenta have been recorded as having a wide host range (see Section 2.2.4). However, it appears that most of the reports are of disease incidence in various countries and only a few are original research reports of experiments carried out to establish the identity, pathogenicity and the host range of the species involved. The results of experiments that have examined the pathogenicity of P. cruenta have varied (Chandrasekaran and Rangaswami, 1960; Verma and Patel, 1969; Amin et al., 1976; Vakili, 1977). It is not clear whether these variations are caused by diverse ecological conditons or by pathogenic variability. The experiments reported in this section were done firstly to determine the pathogenicity and cross-infectivity of isolates of C. canescens from different species of legumes and secondly to investigate the pathogenicity of other species of Cercospora to selected legume species, and to determine if species of Cercospora with hyaline acicular conidia have similar host ranges. Thirdly, and in view of the relative importance of P. cruenta when compared to C. canescens the reaction of selected cultivars of cowpea of diverse origin was investigated.

### 4.2.1 Pathogenicity tests of cowpea and lima bean isolates of C. canescens

Experiment 1: Inoculation of 4-6 week-old plants

In a preliminary experiment, the pathogenicity of seven (7) isolates from cowpea and one (1) isolate from lima bean (Table 4.1.4a) was tested on ten (10) cultivars of cowpea and two of lima bean. Inocula (containing $10^4$ conidia $ml^-$) were sprayed onto both leaf surfaces of four plants of each cultivar, (4 to 6 weeks old) in the glasshouse. The plants were incubated in a mist chamber for five days before they were removed to the open glasshouse bench maintained at 25 ± 5°C and a relative humidity of 50-80% during the day. No distinct macroscopic lesions were observed on any of the inoculated plants (isolate x cultivar combinations) after two weeks incubation.

Experiment 2: Inoculation of mature flowering plants (8-12 weeks old)

The preceding experiment was repeated by inoculating 8-12 week-old plants with a higher concentration of inoculum, 2 x $10^4$ conidia $ml^-$, and the plants incubated as previously described. Two weeks after inoculation, no leaf spots were observed; however six weeks after inoculation when the lower leaves on most of the cowpea cultivars were senescing, fungal sporulation was observed on the abaxial leaf surfaces of cowpea cultivars Amantin and Adua Ayera inoculated with conidia of isolates TZ, $ZB_1$ and $ZB_2$. The observations suggested that the isolates were either only weakly

pathogenic or that lesion development had been affected by the incubation environment.

Experiment 3: Inoculation of tagged intact leaves and detached trifoliate leaves by drop inoculation

The pathogenicity of the eight isolates was tested further by inoculating tagged leaves on intact plants as well as detached leaves rooted in sterile vermiculite (see Section 3.6.3) in two separate experiments. Marked areas on both leaf surfaces (five 10mm-diameter circles on each of six leaflets per treatment) were inoculated with 20µl droplets of inocula containing 2 x $10^4$ conidia $ml^{-}$. Inocula were allowed to dry briefly and the plants were incubated in a mist chamber for five days. The reaction of the plants was assessed 21 to 28 days after inoculation, and was based on the score scale described in Section 3.7.1. The results are summarised in Table 4.2.1. The most common reaction observed was the development of a dark green area of infected tissue in otherwise chlorotic leaves, similar to green islands (Plate 18A). The cowpea isolate TZ, isolated from infected leaves of cowpea obtained from Tanzania, caused distinct necrotic spots four weeks after inoculation on cowpea cultivars; Amantin Caloona and Ife brown, and on the two lima bean cultivars. The cowpea isolates from leaves obtained from Zambia ($ZB_1$, $ZB_2$ and $ZB_3$) also caused distinct necrotic lesions on cowpea cv. Ife brown and the lima bean cultivars. The cowpea isolates, $KP_1$ and $KP_2$

caused green island-type of lesions but the cowpea isolate $KP_3$ and the lima bean isolate (LB), induced faint chlorotic lesions.

Experiment 4: Inoculation tests with two types of inocula.

The results of the experiments described above showed that lesion development and the general symptoms of infection caused by the isolates of C. canescens tested, were atypical compared to those observed in the field (see Plate 2). In view of the fact that other investigators have observed under glasshouse conditions, the typical necrotic lesions seen in the field, the results in part suggest that the isolates might have become less virulent after culture on agar media. To investigate this the virulence of conidia of four cowpea isolates ($KP_2$, TZ, $ZB_1$ and $ZB_2$, and the lima bean isolate (LB)) obtained from infected host leaves were compared with those obtained from culture on V-8 juice agar. Two leaves of similar size and age (8-12 week-old) on each of three plants of cowpea cv. Amantin were spray-inoculated with inocula of the isolates ($2 \times 10^4$ conidia $ml^{-}$) from the two sources until thoroughly wet. The plants were incubated in a draped polythene hood chamber for 24h and then removed to a mist chamber with alternate periods of mist and dry periods for five days before they were returned to the glasshouse bench. The plants were examined daily for lesion development and the final reaction of the plants was recorded 35 days after inoculation. The virulence of the two types of inocula was evaluated on the basis of the

Table 4.2.1

Comparative virulence among single spore isolates of C. canescens from cowpea (7) and lima bean (1).

| Plant species/cultivar | Reaction of test plants to inoculation with isolates from Cowpea | | | | | | | Lima bean |
|---|---|---|---|---|---|---|---|---|
| | $KP_1$ | $KP_2$ | $KP_3$ | TZ | $ZB_1$ | $ZB_2$ | $ZB_3$ | LB |
| Cowpea | | | | | | | | |
| Amantin | 4* | 4 | 3 | 5 | 4 | 4 | 4 | 4 |
| Adua ayera | 4 | 4 | 2 | 5 | 4 | 4 | 4 | 4 |
| Caloona | 4 | 4 | 2 | 5 | 4 | 4 | 5 | 3 |
| IT 82D 889 | 4 | 4 | 3 | 4 | 4 | 4 | 3 | 3 |
| IT 82D 885 | 4 | 4 | 3 | 4 | 4 | 4 | 3 | 3 |
| IT 82D 875 | 3 | 4 | 3 | 4 | 4 | 4 | 3 | 3 |
| Muliana | 4 | 4 | 3 | 4 | 5 | 4 | 3 | 3 |
| New Era Grey | 3 | 3 | 3 | 4 | 4 | 4 | 3 | 3 |
| Ife Brown | 3 | 3 | 3 | 5 | 5 | 5 | 5 | 3 |
| TVX 3236 | 4 | 4 | 3 | 4 | 3 | 4 | 4 | 3 |
| Lima bean | | | | | | | | |
| Brown | 3 | 3 | 3 | 5 | 5 | 5 | 5 | 4 |
| White | 3 | 3 | 3 | 5 | 5 | 5 | 5 | 4 |

* A score was assigned for the most consistent reaction on five marked areas per leaflet on each of two trifoliate leaves (30x10mm-circles) in which;
0 = leaves apparently healthy
1 = no lesions, but sparse sporulation
2 = no distinct lesions but moderate sporulation of fungus.
3 = faint chlorosis
4 = green islands
5 = distinct necrotic spots


PLATE 18

Types of lesions caused by isolates of C. canescens on cowpea in the glasshouse.

A. Areas of green tissue in otherwise chlorotic leaves ('green islands') formed on 4 week-old leaves of cowpea cv. Amantin inoculated with cowpea isolate $KP_2$

B. Infected areas of 'green islands' developed into dark sporulating lesions on cowpea cvs. Caloona, Adua ayera, and Caloona inoculated with cowpea isolates $ZB_1$, $ZB_2$ and $ZB_3$ (left to right) respectively.

C. Characteristic reddish brown lesion on cowpea cv. Amantin spray inoculated with conidia of isolate TZ obtained from infected leaves (Photograph taken 6 weeks after inoculation.

**Table 4.2.2**
Comparisons of the relative virulence on cowpea cv. Amantin of conidia of isolates of C. canescens obtained from infected host leaves or from cultures on agar media.

| (a) Isolate code. | | Incubation period* (days to first macroscopic lesions) with inoculum obtained from V-8 agar | Infected leaves | Mean of Isolate |
|---|---|---|---|---|
| Cowpea | $KP_2$ | 26.5 | 23.0 | 24.8 a** |
| | TZ | 21.3 | 17.5 | 19.4 c |
| | $ZB_1$ | 24.7 | 23.0 | 23.8 a |
| | $ZB_3$ | 22.7 | 21.0 | 21.8 b |
| Lima bean | LB | 25.7 | 22.2 | 23.9 a |
| Mean of substrate | | 24.2 a | 21.3 b | |

* Each figure is the mean of six observations
** Values followed by the same letters are not significantly different according to the least significant test (P = 0.05).

| (b) Isolate code. | | Mean disease severity score* V-8 agar | Infected leaves | Mean of Isolate |
|---|---|---|---|---|
| Cowpea | $KP_2$ | 2.3 | 2.5 | 2.4 |
| | TZ | 4.7 | 5.0 | 4.9 |
| | $ZB_1$ | 2.5 | 3.0 | 2.8 |
| | $ZB_3$ | 2.7 | 3.7 | 3.2 |
| Lima bean | LB | 3.3 | 3.8 | 3.6 |
| Mean of substrate | | 3.1 | 3.6 | |

* Each value is the mean of six scores based on a scale 0-5, in which 0 = no lesions, and 5 many lesions.

incubation period (number of days to when first lesions were observed) and the type/relative density of the lesions formed. The results summarised in Table 4.2.2 shows that the time taken for the appearance of lesions with inocula obtained from infected host leaves for all the isolates was slightly faster compared to inoculum produced in culture on agar plates and there were only slight differences in the appearance of lesions among the isolates. More lesions were also produced by all the isolates with inocula obtained from infected host leaves. Statistical comparison of the mean ranks for disease severity (Appendix Table 9 confirmed that there was a higher rate of infection with inocula obtained from infected host leaves). Among the isolates, the cowpea isolate (TZ) was the most virulent of the five compared. The cowpea isolate TZ, caused numerous distinct necrotic lesions after 17 to 21 days incubation and there was abundant sporulation from the lesions 4-5 weeks after inoculation (Plate 18C).

### 4.2.2 Pathogenicity and cross-infection of C. canescens isolates from five legume species

The pathogenicity of isolates of C. canescens from cowpea (TZ), lima bean (LB), bambarra groundnut (VS), groundnut (AH) and mung bean (VR) was studied partly because of the differences in their cultural characteristics on agar media (see Section 4.1.2).

Experiment 1: Pathogenicity of the isolates

Virulence and cross-infectivity (by which is meant the infection of one legume species by an isolate obtained from another species) of the isolates was tested on eight legumes. Two cultivars each of cowpea, lima bean, mung bean, bambarra groundnut, groundnut, and one cultivar each of soya bean, pigeon pea and winged bean were tested. Three leaves on each of three plants of the legume species were inoculated by spraying both leaf surfaces with inocula containing 2 to 3 x $10^4$ conidia $ml^-$ from infected host leaves. The inoculated plants were incubated in a mist chamber for 5 days and subsequently placed on glasshouse benches at 25-30$^oC$ (the experiments were done during April to August 1987). The plants were observed daily and a final assessment of the reaction of the plants, based on the type of lesions and their relative numbers was made after six weeks.

The results summarised in Table 4.2.3, shows significant cross-infection of the eight legume species. As would be expected, the isolates were more aggressive on hosts from which they were isolated (Plate 19). Unexpectedly, the isolate from groundnut (AH), failed to infect the two cultivars of groundnut tested. But, surprisingly, cowpea was highly susceptible and lima bean and winged bean were moderately susceptible. Each of the five isolates was pathogenic on cowpea, but differed in respect of the incubation period for the development of

Table 4.2.3 Reaction of selected legume species to inoculation with isolates of C. canescens from five legume species.

| Legume species/cv. | Disease rating/scores* for inoculation with isolates from Cowpea TZ | Lima bean (LB) | Mung bean (VR) | bambarra (VS) | groundnut (AH) |
|---|---|---|---|---|---|
| Cowpea: Amantin | 4.6 | 3.6 | 3.1 | 3.7 | 4.8 |
| Ife Brown | 4.3 | 3.1 | 3.7 | 3.1 | 4.2 |
| Lima bean: White | 4.3 | 4.0 | 3.0 | 3.8 | 2.9 |
| Brown | 4.5 | 3.4 | 2.8 | 4.0 | 3.1 |
| Mung bean: MG50-10A | 0 | 0 | 4.6 | 4.1 | 0 |
| CES 2F-1 | 0 | 0 | 4.0 | 4.0 | 0 |
| bambarra: Mbawa | 0 | 0 | 3.7 | 4.5 | 0 |
| bean cv. 18 | 0 | 0 | 3.9 | 5.0 | 0 |
| Groundnut: TMV2 | 0 | 0 | 2.7 | 0 | 0 |
| Chitemba | 0 | 0 | 3.0 | 0 | 0 |
| Soya bean: 'Bragg' | 0 | 0 | 0 | 0 | 0 |
| Pigeon pea (not known) | 3.8 | 3.4 | 2.0 | 3.0 | 0 |
| Winged bean (Ex Kade) | 3.4 | 3.6 | 3.3 | 3.0 | 0 |
| | (3/7) | (3/7) | (6/7) | (5/7) | (2/7)** |

* Each value is the mean score for three trifoliate leaves based on an assessment of the type of lesion produced and their relative numbers, on a scale 0-5 in which 0 = no apparent lesions; 1 = few chlorotic lesions; 2 = few green-island type of lesions; 3 = large green islands 4 = necrotic lesions; 5 = large necrotic lesions.

** Proportions of legumes cross-infected.

**PLATE 19**

Symptoms of infection by host-isolates of C. canescens on leaves of:

A. Cowpea cv. Amantin

B. Mung bean cv. MG 50-10A

C. Bambarra groundnut cv. Mbawa

Spray-inoculated with conidia obtained from infected leaves of their respective hosts in the glasshouse. (Photographs taken 6 weeks after inoculation).

**PLATE 20**

Reddish brown coalescing lesions produced on cowpea cv. Amantin, spray-inoculated with conidia of groundnut isolate (AH) of C. canescens. (Photograph taken 6 weeks after inoculation).

PLATE 19

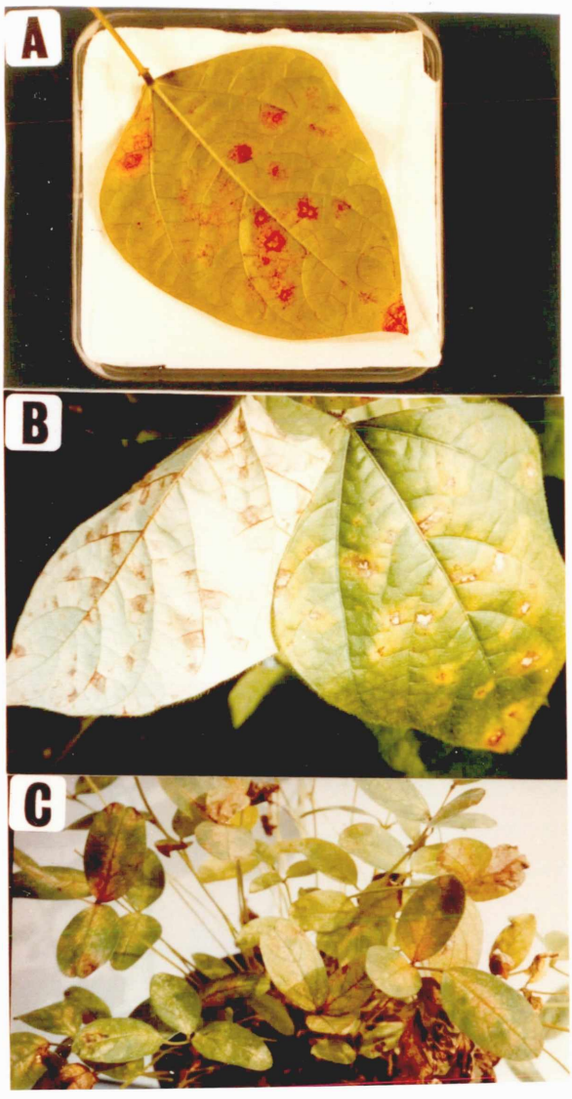
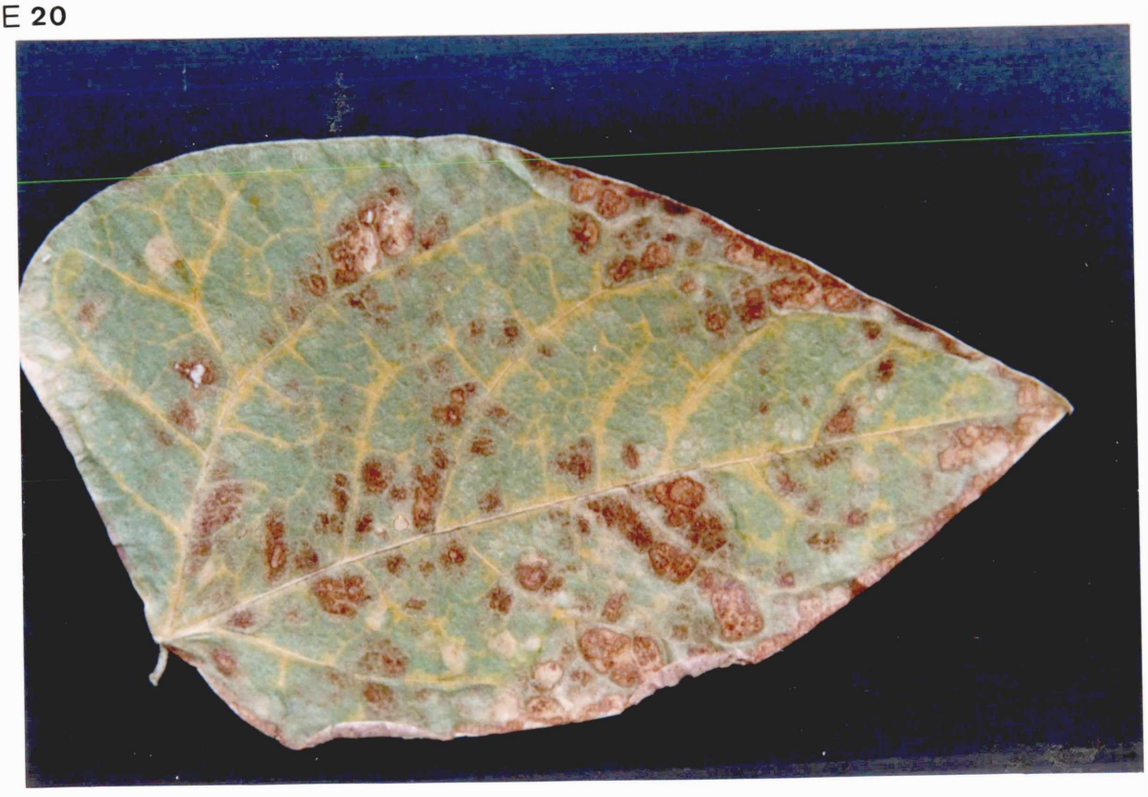



PLATE 20



symptoms and virulence (type of lesions formed). The cowpea (TZ) and groundnut (AH) isolates infected both cultivars of cowpea causing distinct reddish brown lesions after 4 weeks (Plate 19A and Plate 20 respectively). The isolates from lima bean (LB), mung bean (VR), and bambarra groundnut (VS) on the other hand induced areas of 'green islands' which later developed into sporulating lesions. All the isolates infected winged bean producing green islands and all isolates except the groundnut isolate (AH), also infected pigeon pea.

The mung bean isolate (VR) infected all the legume species tested except soya bean on which none of the isolates was pathogenic (Plate 21). The extent of cross-infection was greater than expected.

Experiment 2: Determination of variability of virulence

To determine the variability of virulence among the isolates, the reaction of cowpea cultivar Amantin was tested by inoculating the five isolates simultaneously to marked areas of the same leaflet to enhance homogeneity. Six 10mm-circles were marked on each leaflet of ten intact trifoliate leaves (180 circles) of 8 week-old plants. In a preliminary test, each of the five isolates was inoculated separately to one trifoliate leaf each (18 circles) by spreading $0.05cm^3$ of inoculum over each circle and one leaf was treated as a control by spreading $0.05cm^3$ of tween 20 over the circles. In a second treatment, each of the five

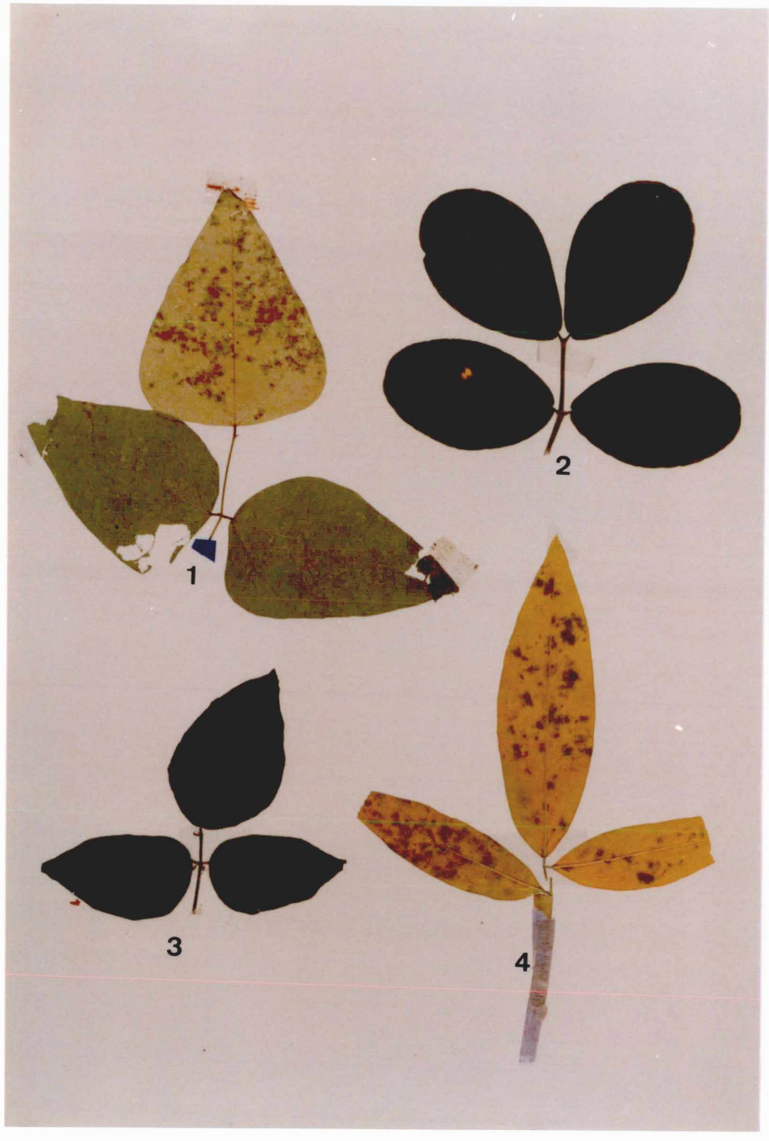



**PLATE 21**

Lesions caused by the mung bean isolate (VR) of C. canescens on

1. Winged bean ex. Kade Ghana
*2. groundnut cv. TMV2
3. mung bean cv. MG 50-10A
4. Pigeon pea

* The lesions formed on groundnut were observed 6 weeks after drop-inoculation.

isolates was inoculated to a different circle on each leaflet of the four remaining leaves (12 x 10mm-circles per isolate) and the sixth circle on each leaflet was treated as a control. The plants were incubated as previously described. Two weeks after inoculation the lesions formed by an isolate in all the circles of a leaf were similar. In the second treatment, the lesions caused by similar isolates inoculated to circles on different leaflets were also similar except that the time taken for the lesions to appear varied slightly. But there was little evidence that the isolates had induced resistance or susceptibility to one another as they developed in the leaflets.

### 4.2.3 Pathogenicity of C. canescens isolates to selected non-leguminous plant species

Cross-infectivity of isolates of C. canescens to plants in other genera was tested on six plant species, on which species of Cercospora with hyaline acicular conidia have been recorded (see Table 3.3). Three plants of each species (8-12 weeks old) were inoculated by spraying both leaf surfaces of all leaves with inocula containing 2 to 3 x $10^4$ conidia $ml^{-}$. The plants were incubated for 5 days in a mist chamber and then placed on a glasshouse bench. An assessment of the reaction of the plants was made four weeks after inoculation. The results in Table 4.2.4 shows that all the isolates were pathogenic to Okra (Hibiscus esculentus) but the cowpea isolate (TZ) was the most virulent of the five isolates, causing numerous angular-

Table 4.2.4

Pathogenicity of isolates of C. canescens to selected non-leguminous plants.

| Plant species | Disease reaction score* for isolate from Cowpea TZ | Lima bean LB | Mung bean VR | bambarra VS | groundnut AH |
|---|---|---|---|---|---|
| Sugar beet cv. Julia | 2 | 0 | 2 | 2 | 2 |
| Celery cv. Cutting | 2 | 1 | 2 | 2 | 1 |
| Tomato cv. Wosowoso | 0 | 0 | 0 | 0 | 0 |
| Aubergine cv. Black Beauty | 1 | 1 | 1 | 1 | 1 |
| Okra cv. Local | 3 | 1 | 2 | 1 | 1 |
| Cotton cv. not known | 0 | 0 | 0 | 0 | 0 |

* The assessment of disease reaction was based on the type of lesions and the relative amounts of sporulation on a scale 0-3 in which
0 = no apparent infection; 1 = very few chlorotic lesions; 2 = indistinct lesions with sparse sporulation; 3 = necrotic lesions with moderate to abundant sporulation.

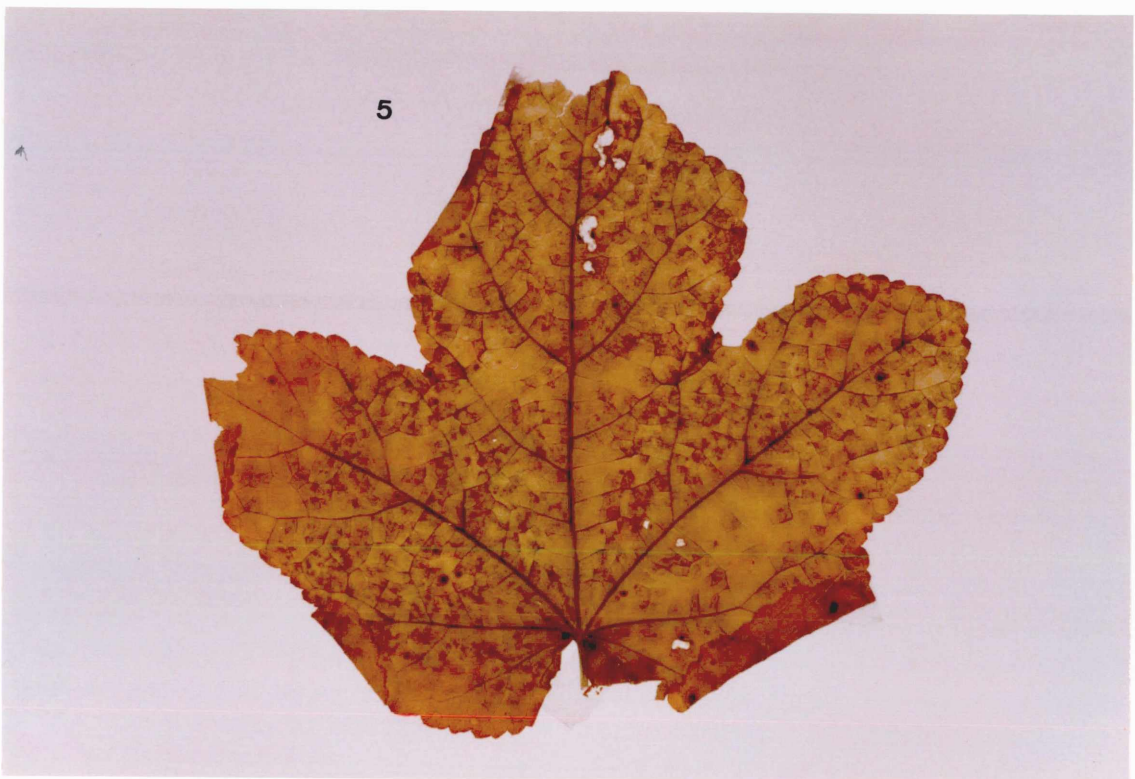



**PLATE 22**

Sporulating angular lesions on okra (Hibiscus esculentus) caused by cowpea isolate TZ of C. canescens (Photograph taken 6 weeks after inoculation).

shaped lesions with abundant sporulation (Plate 22). On sugar beet all the isolates except the lima bean isolate (LB) caused small circular straw coloured lesions but without the characteristic purple margins associated with Cercospora beticola infections in the field. The isolates from cowpea (TZ), mung bean (VR), and bambarra groundnut (VS), caused indistinct lesions with moderate sporulation on celery but the isolates from groundnut (AH) and lima bean caused few chlorotic lesions. Chlorotic lesions were also formed on aubergine by all the five isolates of C. canescens. None of the isolates was pathogenic to cotton or tomato although the conidia did germinate on the surfaces of leaves of these plants.

### 4.2.4 Pathogenicity of Cercospora beticola to leguminous plant species

The results of the experiments described in Sections 4.2.2 and 4.2.3 indicate that isolates of C. canescens from leguminous plants could cross-infect other legume species as well as plants in other genera. It therefore supports in part, the view that species of Cercospora with hyaline acicular conidia may represent forms of the type C. apii (Ellis, 1971). To investigate this, the ability of C. beticola to infect leguminous plants was tested. Three plants each of two cultivars of cowpea (cvs. 'Amantin' and 'Muliana') and one each of mung bean (MG 50-10A) lima bean (Henderson bush brown), common bean (Ex-Malawi) and bambarra groundnut (Mbawa) and five plants of sugar beet cv. Julia as

an inoculated control, were tested. The plants were inoculated when they were 8-12 weeks old as a group by spraying inoculum ($2 \times 10^4$ conidia $ml^{-}$) in 0.05% Tween 20 on both surfaces of all leaves to run off. One plant of each species/cultivar was sprayed with 0.05% Tween 20 as uninoculated controls. The plants were incubated under alternate night mist and day time drying for 5 days and were subsequently returned to the glasshouse and were examined daily for lesion development. A final assessment of the reaction of the plants was made three weeks after incubation. The experiment was repeated once and the observations are summarised in Table 4.2.5. C. beticola infected and caused lesions in all the legume species tested. On cowpea cv. Amantin, many circular to irregular lesions 3-5mm wide, began to develop 10-12 days after inoculation. These developed straw coloured centres and were bordered by a purple halo, typical of C. beticola infections of sugar beet in the field (Plate 23). The sugar beet plants which were controls, developed numerous circular lesions up to 2mm wide and were scattered over the leaf lamina. Although the centre of the lesions on sugar beet turned tan to straw coloured, most of the lesions were bordered by a band of green tissue which later developed into indistinct purple margins on the older leaves. C. beticola was, therefore, as or more virulent to cowpea than to sugar beet, an unexpected result. The lesions that formed on the other legumes; lima bean, common bean, and mung bean were comparatively very few and were not bordered

Table 4.2.5

Reaction of selected leguminous plants to inoculation with Cercospora beticola.

| Plant species/cultivar | | Lesions | |
|---|---|---|---|
| | | Type | Sporulation |
| 1. | Cowpea cv. Amantin | Many 20-30/leaflet circular to irregular 3-5mm wide and bordered by purple margins | Abundant |
| 2. | Cowpea cv. Muliana | Few irregular lesions 2-3mm wide | Moderate |
| 3. | Lima bean cv. Henderson bush (brown) | Few (1-10 per leaflet) irregular lesions 2-3mm wide. | Sparse |
| 4. | Common bean Ex. Malawi | Few 3-15 per leaflet | Sparse |
| 5. | Mung bean cv. MG 50-10A | Few reddish brown irregular 2-3mm | Sparse |
| 6. | Sugar beet cv. Julia (Control) | Numerous (21-100 per leaf) circular lesions 1-2mm and bordered green margins which later developed into straw coloured centres. | Abundant |

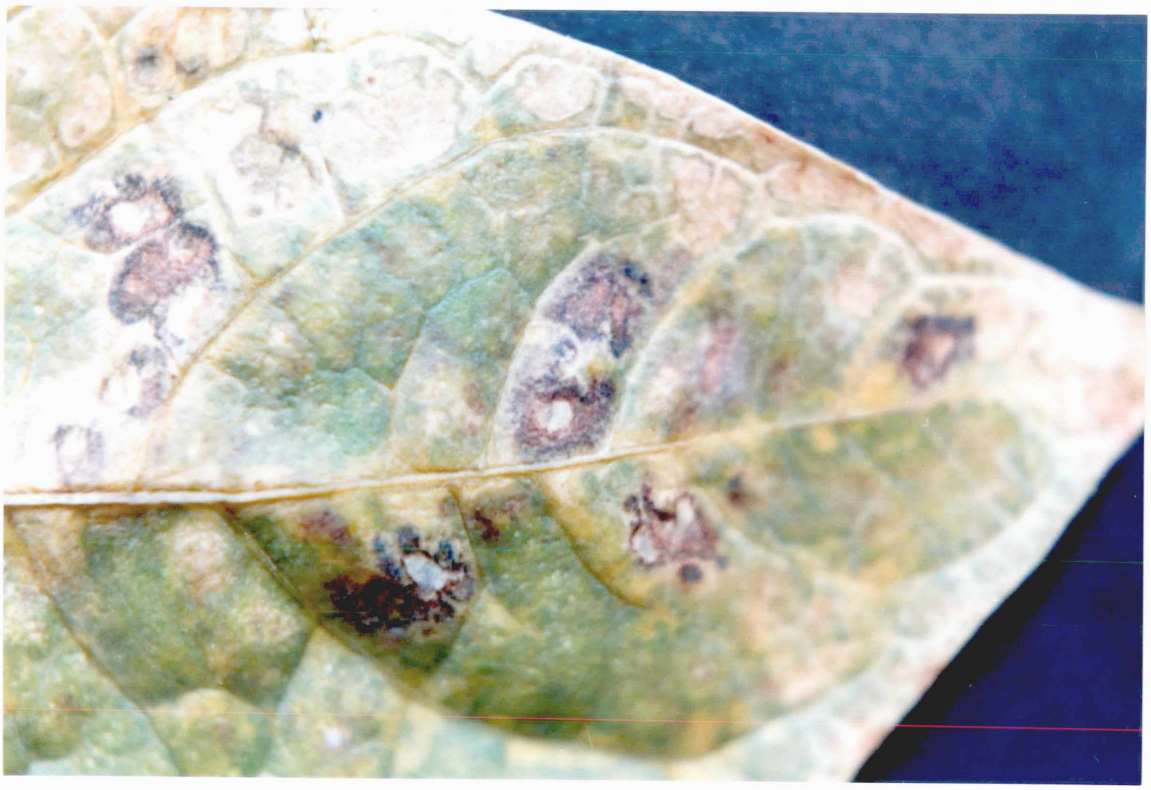


**PLATE 23**

Portion of the adaxial leaf surface of cowpea cv. Amantin enlarged 2 x to show clearly, leaf spots with straw-coloured centres surrounded by a purple halo, caused by Cercospora beticola isolate K897 ex ICI.

by distinct purple margins as was observed on cowpea. All infections were verified by placing surface sterilized samples of infected leaves with lesions in Petri dish moist chambers. Typical conidiophores and conidia were formed abundantly on cowpea cv. Amantin and on the sugar beet leaves (Plate 14 $CB_{vu}$ and $CB_{sb}$ respectively); 2 to 4 days after incubation. Very sparse sporulation was observed on some of the older lesions on mung bean, common bean and lima bean.

### 4.2.5 Pathogenicity of Pseudocercospora cruenta to cultivars of cowpea of diverse origin

The pathogenicity of a cowpea isolate of P. cruenta (PcKp) and the reaction of twenty selected cultivars of cowpea from a collection of samples obtained from Ghana, Nigeria, Zambia, Malawi, Puerto Rico and South Carolina (USA) was determined.

#### Experiment 1: Reaction of cultivars to inoculation

In a preliminary experiment all leaves on two plants of each cultivar (6-8 weeks old) were inoculated by spraying both surfaces of leaves with inoculum containing 2 x $10^4$ conidia $ml^-$ in 0.05 Tween 20. The inoculated plants were incubated under alternate night mist and day time drying for 3 days in a mist chamber before they were removed to a glasshouse bench. This was done to mitigate the problems of inconsistencies in lesion development. Apparently under

Table 4.2.6

Variations in the type of lesions formed on cowpea cultivars inoculated with P. cruenta

| | Type of Lesion | Cultivar showing the lesion |
|---|---|---|
| 1. | Small to medium (2-3mm diameter) irregular to angular shaped lesions bordered by a band of green tissue. 2-3mm wide within chlorotic tissue. | Amantin [1]* 2-4 weeks old leaves |
| 2. | Large necrotic lesions 8-15mm wide surrounded by a band of green tissue. | 8-12 week old leaves of cv. Amantin and on all leaves of cvs. Ife brown and TVX 3236 |
| 3. | Medium 3-5mm wide rusty to reddish brown lesions | Caroni, Adua ayera [4], Selection 8, 1603, Sudan and New Era. |
| 4. | Numerous angular-shaped lesions | Caloona [3] |
| 5. | Distinct light tan lesions 2-3mm wide | IT 82D 885 [5] IT 82D 875, IT 82D 889. |
| 6. | Numerous small lesions 1-2mm wide and of a hypersensitive type | Muliana [6] Colossus, Colossus 80, Ala 963.8. |

* Refers to Figures in Plate 24.

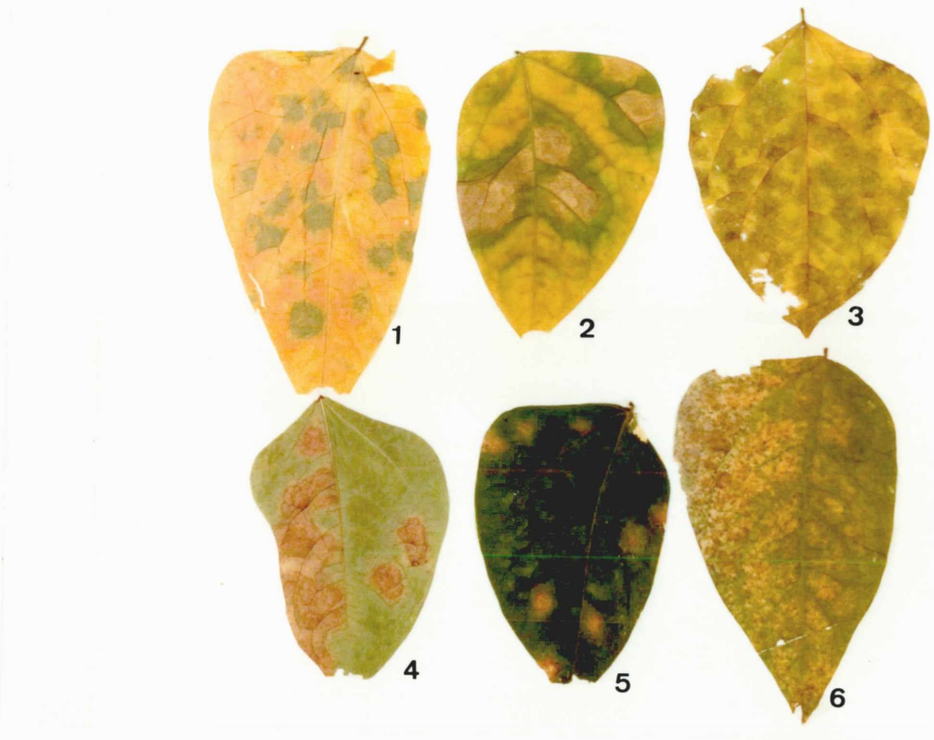



**PLATE 24**

Types of lesion formed on different cultivars of cowpea inoculated with P. cruenta, 14 to 21 days after inoculation.

1. cv. Amantin
2. cv. Ife brown
3. cv. Caloona
4. cv. Adua ayera
5. cv. IT 82D 885
6. cv. Muliana

continuous misting, germ tubes grew extramatrically without penetrating stomata due to the prolonged presence of free water. Four weeks after incubation of the plants under diurnal misting six types of reactions were identified based on the form and appearance of the lesions. These are summarised in Table 4.2.6 and illustrated in Plate 24.

Experiment 2: Effect of leaf age on disease severity

The experiment described above revealed that there were differences in the size of the lesions (necrotic area) and also of the extent of the band of green tissue that surrounded the lesions, on leaves at different stages of maturity on some cultivars. This was particularly evident on cultivars Amantin and Ife brown and to a lesser extent on the remaining cultivars. The effects of leaf age on the susceptibility (severity of the expression of symptoms) was therefore studied on leaves of three age categories I = 2-3 week old; II = 4-6 week old and III = 8-12 week old subtending a flowering peduncle. The leaves of different ages were tagged separately on plants of five cultivars of cowpea (Amantin, Ife brown, Caloona, IT82D885 and Muliana). Three trifoliate leaves of each age category for a cultivar were spray-inoculated with $2 \times 10^4$ conidia $ml^-$ in 0.05% Tween 20, and two leaves of each cultivar were sprayed with 0.05% Tween 20 as controls. The plants were incubated as previously described under mist for five days and then removed to the open glasshouse bench at $25 \pm 5^oC$. The incubation period (the number of days after inoculation when

the first symptoms were observed) was recorded, and the size of ten largest lesions on each of three leaves was measured 30 days after inoculation. The lesions were traced on translucent paper and the area assessed by square counting using graph paper. The experiment was repeated twice and the means for the incubation periods and lesion sizes calculated for each age category (Table 4.2.7). Macroscopic lesions were evident on all leaves between 11 and 19 days after inoculation. There were only small differences in the incubation peiods for leaves at different stages of maturity for all cultivars except Muliana for which incubation periods were 6 days shorter for the more mature leaves (8-12 week old) than for the younger leaves (2-3 week old). Differences in incubation periods were more prominent for leaves of different cultivars. The mean lesion size did differ significantly among the leaves of the three age categories increasing with the age of the leaves for each cultivar. These increases were however only significant for the leaves of different ages on cultivars Ife brown and Amantin. The largest lesions developed on the more mature leaves (8-12 week old). The lesion sizes were much bigger on leaves of cowpea cultivar Ife brown (range 68-164mm$^2$) followed by lesions formed on cultivar Amantin (range 25-122mm$^2$). The lesions formed on cv. Caloona were similar in range to those formed on cv. Amantin and those formed on cv. IT82D ranged between 14 to 64mm$^2$. The smallest lesions (1-4mm$^2$) were formed on cv. Muliana but these were numerous.

Table 4.2.7 Effects of leaf age and cultivar on the susceptibility and severity of symptoms in cowpea cultivars infected by P. cruenta.

| Leaf age Category (wks) | | Cowpea cultivars | | | | |
|---|---|---|---|---|---|---|
| | | Amantin | Caloona | Ife brown | IT 82D 885 | Muliana |
| (a) Incubation period (days)* | I (2-3) | 13.5±0.6 | 14.5±0.3 | 14.2±0.4 | 18.1±0.4 | 19.0±0.2 |
| | II (4-6) | 11.7±0.5 | 12.5±0.3 | 12.3±0.3 | 15.5±0.3 | 18.2±0.4 |
| | III (8-12) | 11.0±0.2 | 11.2±0.3 | 11.8±0.4 | 15.0±0.6 | 13.1±0.3 |
| (b) Lesion size ($mm^2$)** | I | 40.8±2.3 | 45.5±2.3 | 101.3±3.4 | 27.4±1.5 | 2.1±0.1 |
| | II | 63.5±2.0 | 65.1±2.1 | 115.2±3.3 | 38.7±1.8 | 2.6±0.2 |
| | III | 100.0±2.5 | 73.3±1.9 | 134.2±3.5 | 44.4±1.4 | 2.7±0.2 |

* Each value is the mean ± standard error for the assessment for the time taken for the lesions to appear on three trifoliate leaves each, in three experiments (9 trifoliate leaves)

** The lesion size is based on the mean area of thirty lesions (10 largest lesions on each trifoliate leaf in three experiments).

Experiment 3: Assessment of disease reactions on mature leaves of cowpea inoculated with P. cruenta

On the basis of the results obtained from the preceeding experiments, the reaction of the twenty cultivars studied in experiment 1 were tested using leaves that were 8-12 weeks old and all of which subtended a flowering peduncle. Five trifoliate leaves were each inoculated with $2 \times 10^4$ conidia $ml^-$ in 0.05% Tween 20 and incubated as previously described. The assessment of disease reaction of the cultivars based on the numbers of lesion per leaflet and size of the lesions was made four weeks (30 days) after inoculation (Table 4.2.8). Four cultivars, Amantin, Ife brown, TVX 3236 and Caloona were found to be highly susceptible producing medium to large lesions with abundant sporulation, while four others, Muliana, Colossus, Colossus 80 and TARS 48 were found to be resistant producing very small lesions with very little or no sporulation. The remaining twelve cultivars produced either few or many lesions with moderate sporulation and were rated as moderately susceptible or susceptible based on the sporulation index (see Section 3.7.4).

Table 4.2.8 Disease reaction of 20 cultivars of cowpea of diverse origin to P. cruenta.

| Cultivar | Lesions no/leaflet* | Lesions Size mm²** | Sporulation Index*** | Disease severity Rating+ |
|---|---|---|---|---|
| Adua ayera | 12.5 | 66.9 | ++ | S |
| Amantin | 10.7 | 90.6 | +++ | HS |
| Caroni | 11.8 | 67.6 | ++ | S |
| Caloona | 20.4 | 83.0 | +++ | HS |
| Ife Brown | 5.3 | 125.7 | +++ | HS |
| TVX 3236 | 10.5 | 92.1 | +++ | S |
| IT 82D 875 | 9.5 | 51.7 | ++ | S |
| IT 82D 885 | 10.8 | 44.5 | ++ | S |
| IT 82D 889 | 10.4 | 52.6 | ++ | S |
| Muliana | 19 | 3.8 | 0 | R |
| New Era | 12.2 | 62.7 | ++ | S |
| Colossus | 3.1 | 18.6 | + | R |
| Colossus 80 | 1.3 | 4.1 | 0 | R |
| Ala. 963.8 | 4.4 | 14.5 | + | MS |
| TARS 36 | 1.1 | 8.8 | + | MS |
| TARS 48 | 1.9 | 7.3 | 0 | R |
| TARS 62 | 1.3 | 4.9 | + | MS |
| Sudan | 11.3 | 26.5 | ++ | S |
| Sel 8 | 8.7 | 58.6 | ++ | S |
| 1603 | 11.4 | 20.7 | ++ | S |

* Mean of 30 leaflets (5 trifoliate leaves on each of 2 plants);
** Estimated from five (5) lesions per leaflet to the nearest mm.
*** 0 = no sporulation; + = sparse; ++ = moderate; +++ = profuse.
\+ HS = Highly susceptible; MS = moderately susceptible; S = susceptible; R = resistant.

LSD P = 0.05 No. of lesions/leaflet = 1.2
Lesion size mm² = 5.3

## 4.3 Investigation of the nature of host pathogen relationships of cowpea and Cercospora spp.

The results of the experiments described in Section 4.2, revealed that there was a rather long period between inoculation and development of macroscopically visible lesions caused by isolates of Cercospora spp. Schneider and Sinclair (1975) suggested that the relatively late development of Cercospora leaf spot on cowpea, relates to biochemical changes which affect spore germination and germ tube growth. In many other Cercospora-host interactions however, the slow rate of disease development has been attributed to the moisture relations of the plant (Goos and Tschirch, 1963; Solel and Minz, 1971).

Apart from earlier observations made by Latham (1934) on the life history of P. cruenta on cowpea, and a report on the effects of inhibition of conidial germination and germ tube growth of C. canescens by Schneider and Sinclair (1975), there has been little or no further research reported on the behaviour of either C. canescens or P. cruenta during the infection process. The experiments described in this section studied the infection of cowpea leaves by Cercospora spp. with a view elucidating the method of penetration and infection and then to determine the phases in the infection process at which pathogen virulence and host resistance are expressed.

4.3.1 Observations on the initial stages of infection of cowpea leaves by Cercospora spp.

In preliminary experiments leaf disks of cowpea cv. Amantin, susceptible to both C. canescens and P. cruenta on benzimidazole agar were inoculated with a suspension of conidia containing $10^4$ spores $ml^-$ as described in Section 3.6.1. Samples of leaf disks were removed at 6h intervals and examined microscopically (see Section 3.8). Conidia germinated well (>90% after 24h) on both leaf surfaces. The germ tubes then grew at considerable length and randomly over the leaf surface, with some passing near stomata without penetrating. Considerable difficulty was experienced in locating germ tubes that penetrated stomata up to 72h after inoculation. The experiment was repeated by floating the leaf disks on benzimidazole solution (50ppm) or 5% sucrose solution. Conidia germinated readily as previously observed followed by an extensive growth and branching of hyphae but no penetration was observed after 48h. The leaf disks turned yellow after three days. Therefore in subsequent experiments intact leaves were inoculated either by brushing with or spraying a conidial suspension of $2 \times 10^4$ $ml^-$ on both surfaces of leaves which were incubated in a mist chamber. Samples of the leaf disks were examined at 3h intervals up to 24h after inoculation. Additional samples were examined at weekly intervals up to 3 weeks after inoculation, after clearing and staining. The sequence of events leading to the infection and sporulation of the pathogens were deduced from the series of leaf pieces

using the techniques described in Section 3.8.

(a) Cercospora canescens

The conidia of C. canescens began germinating on both leaf surfaces 3 to 4h after inoculation and germ tubes generally appeared at the [illegible] ends first. By 48h after inoculation many cells of the multicellular conidia had germinated and germ tubes with intercalary branching grew for considerable lengths, but only a few side branches were observed to penetrate stomata (Plate 25, Fig. 1). Very rarely, short germ tubes penetating stomata were observed. Two weeks after inoculation, when chlorotic spots had appeared on inoculated leaves, conidiophores were observed on the walls of some epidermal cells. There was no evidence of penetration into mesophyll cells. The growth of conidiophores was observed to be more profuse from cells in the mid-rib region three weeks after inoculation; by which time distinct 'green islands' had formed on the leaves (Plate 25, Fig. 2-7). In a few instances when necrotic spots had developed (21-28 days after inoculation) conidiophores were observed to emerge from stomata (Plate 25, Fig. 8). Many leaf samples were examined but it was not possible to characterize clearly the pattern of growth into cells adjacent to those penetrated with the methods employed for the study.

## PLATE 25

Photomicrographs of the infection of cowpea (cv. Amantin) by C. canescens (Isolate TZ).

Fig. 1. A germinated conidium with several germ tubes and mycelial branches with a short side branch (arrowed) penetrating a stoma (48h after incubation).

Fig. 2. Mature conidiophores growing out from epidermal cell walls.

Fig. 3. A conidiophore with a developing conidium (slightly out of focus - arrowed) growing from epidermal cell wall).

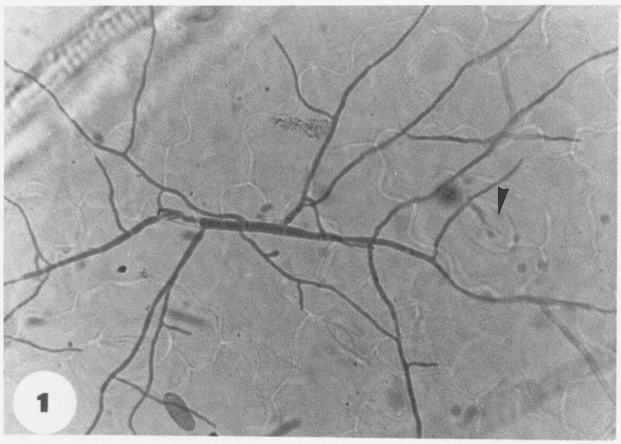
Fig. 4. Branched hyphae forming a stroma with developing conidiophores in subepidermal cells.

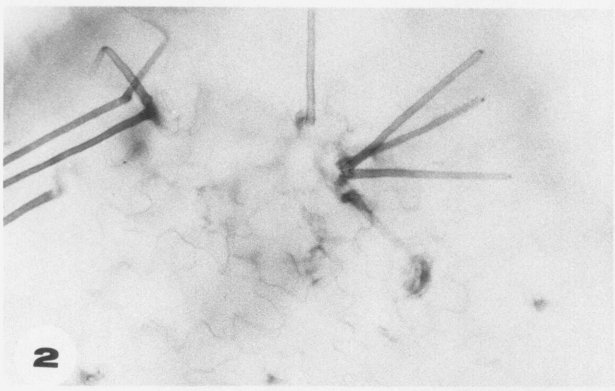
Fig. 5. Fascicle of conidiophores growing on epidermal surface (magnified).

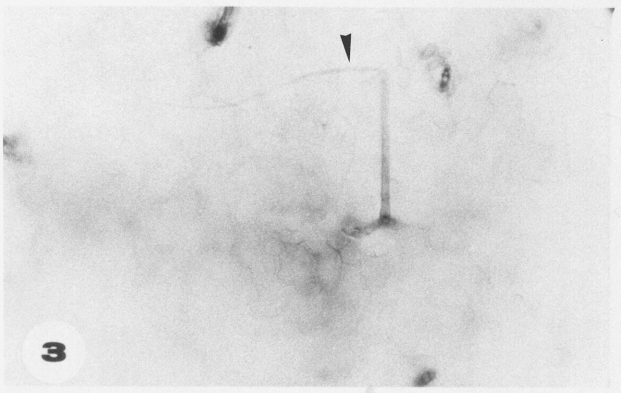
Fig. 6. Fascicles of conidiophores growing from midrib region.

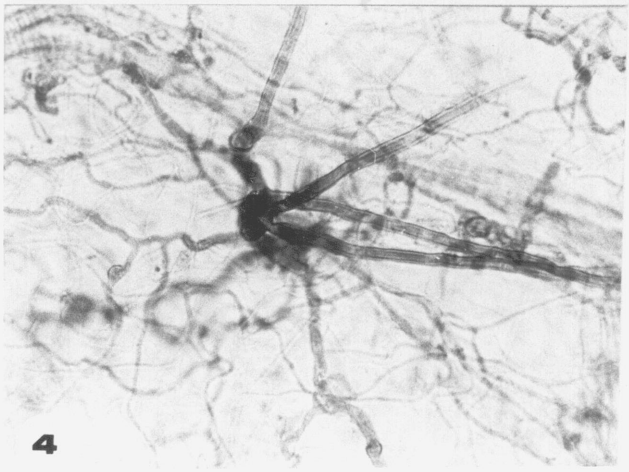
Fig. 7. High power view x1000 of (6) to show basal cells of conidiophores.

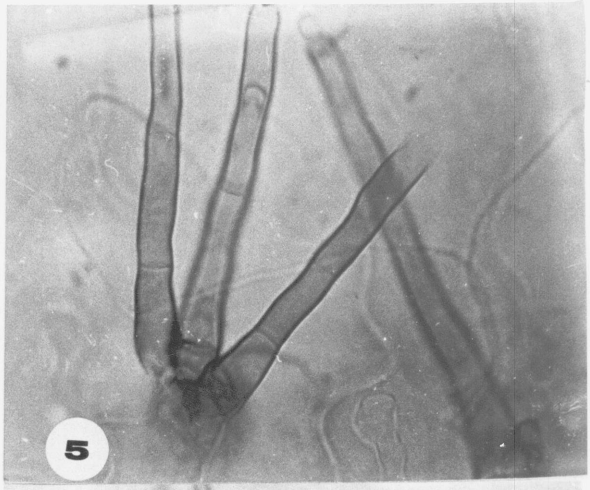
Fig. 8. Conidiophores emerging from a stoma.

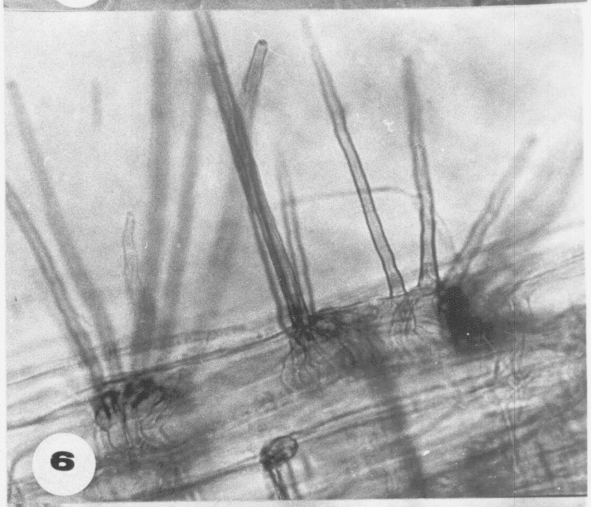


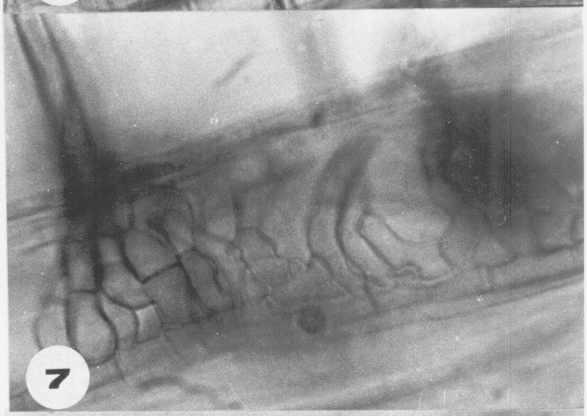



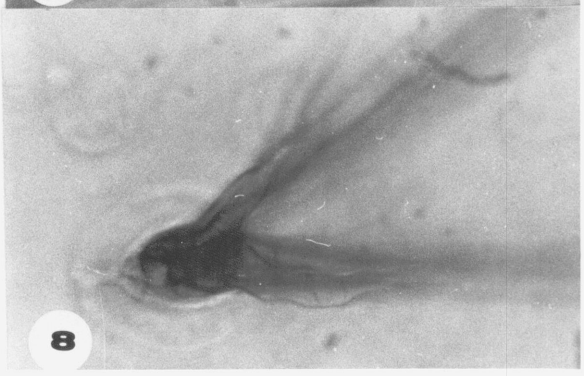




Plate 25

(b) Pseudocercospora cruenta

Conidia of P. cruenta germinated on both leaf surfaces after 3 to 4h incubation after inoculation as was observed for C. canescens. By 48h after inoculation germ tubes had emerged from many cells of the multicellular conidia and had grown extensively with side branching (Plate 26, Fig 1). Some of the intercalary branches formed appressorium-like swellings at their tips either over a stomatal opening (Plate 26, Fig. 3) or on the surface epidemal cells (Plate 26, Figs. 1 and 2).

Some germ tubes were observed to have penetrated stomata and re-emerged from other stomata to form a secondary epiphyllous mycelium from which hyphae penetrated other stomata (Plate 26, Fig. 4).

Germ tube growth and stomatal penetration did not appear to be influenced by the proximity of conidia to stomata. Some germ tubes were observed to pass over stomata without penetrating (Plate 26, Fig. 5). In a few instances, where germ tubes traversed stomata or passed near them, short branches, up to three were produced from the main germ tube proximal to the stomatal opening, and these side

**PLATE 26**

Photomicrographs showing some of the phases in the infection of cowpea cv. Amantin by Pseudocercospora cruenta.

Fig. 1. A germinated conidium (c) with four germ tubes (gt) and short intercalary branches of which the basal one has formed an appressorium-like swelling over the epidermal wall (arrowed) 48h after inoculation.

Fig. 2. A germinated conidium with abnormal swellings along the germ tube and a bulbous swelling over the epidermal wall.

Fig. 3. A bulbous swelling at the proximal end of a germ tube penetrating a stoma.

Fig. 4. Growth of mycelium from a stoma with branched 2ry mycelium forming an appresorium over a stoma.

Fig. 5. A germ tube (gt) with branched hyphae (br) growing over stomata (st).

Fig. 6. Side branching of germ tubes from cells near a stomatal opening several hours after inoculation.

Fig. 7. Short branches from germ tubes forming appressoria over stomata.

Fig. 8. A germinated conidium (c) with 2 germ tubes (gt) from the proximial and end cells of the conidium that have grown for some distance towards a stoma, and formed branches that penetrate through the pore.

Fig. 9. Magnified view (x400) of Fig. 8 in the region where the branched hyphae penetrate the stoma.

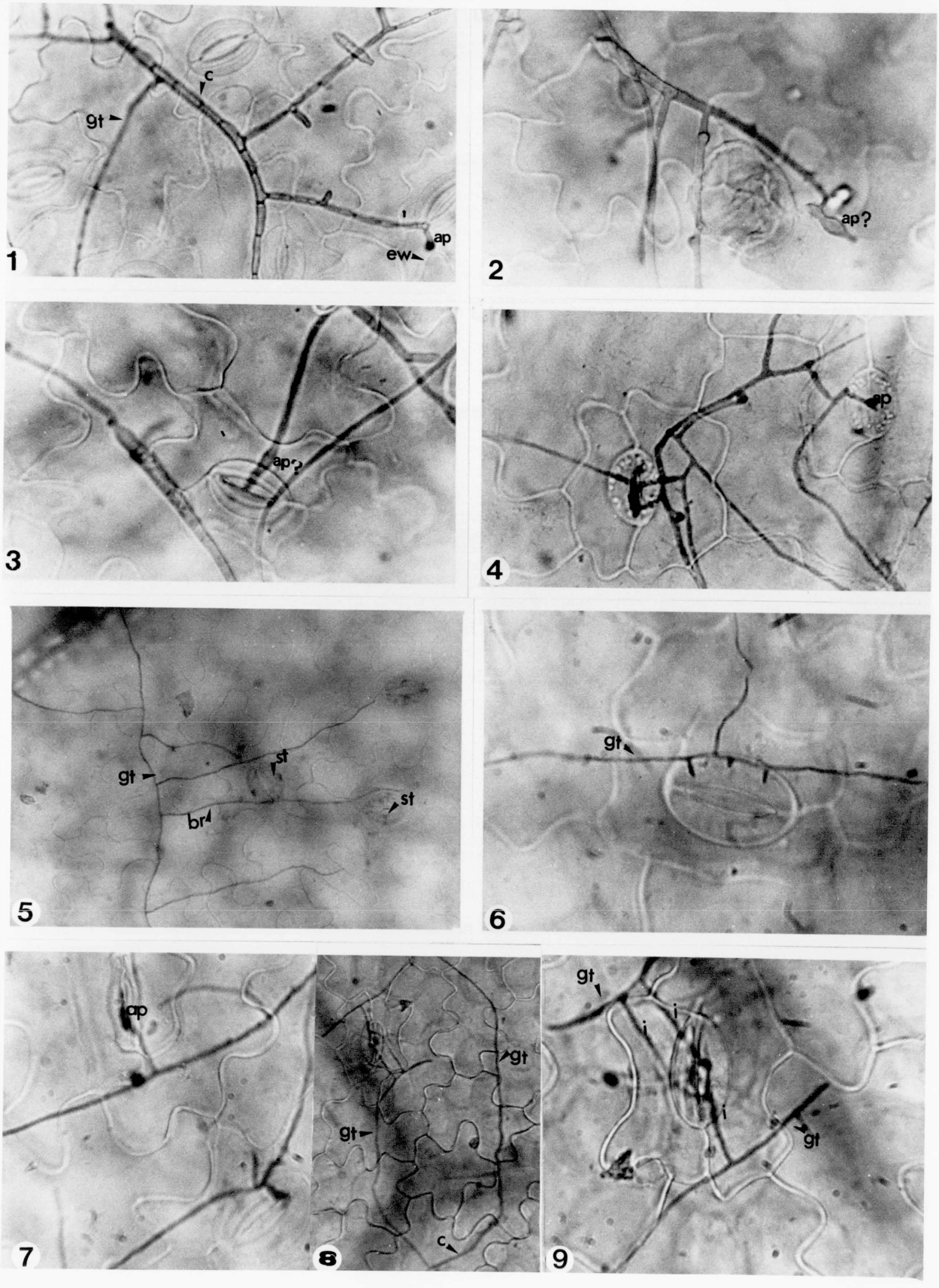



Plate 26

**PLATE 26 (Continued)**

Fig. 10. The development of a bulbous swelling (arrowed) on a branch from germ tube before penetration of stoma.

Fig. 11. The development of side branches and further bulbous swellings on an elongating germ tube (gt) without directional growth towards a stoma.

Fig. 12. Intercellular growth of hyphae in epidermal cells x400.

Fig. 13. Magnified view (x1000) of Fig. 12 to show hyphae attached to and following the contours of host cell walls

Fig. 14. Branched hyphae in substomatal cavity forming stromatic tissue.

Fig. 15. Intercellular growth of mycelium associated with healthy mesophyll cells.

Figs. 16 and 17. Sub-epidermal view showing collapsed (trypan-blue retaining) cells; and haustorium-like swelling on cell walls (arrowed).

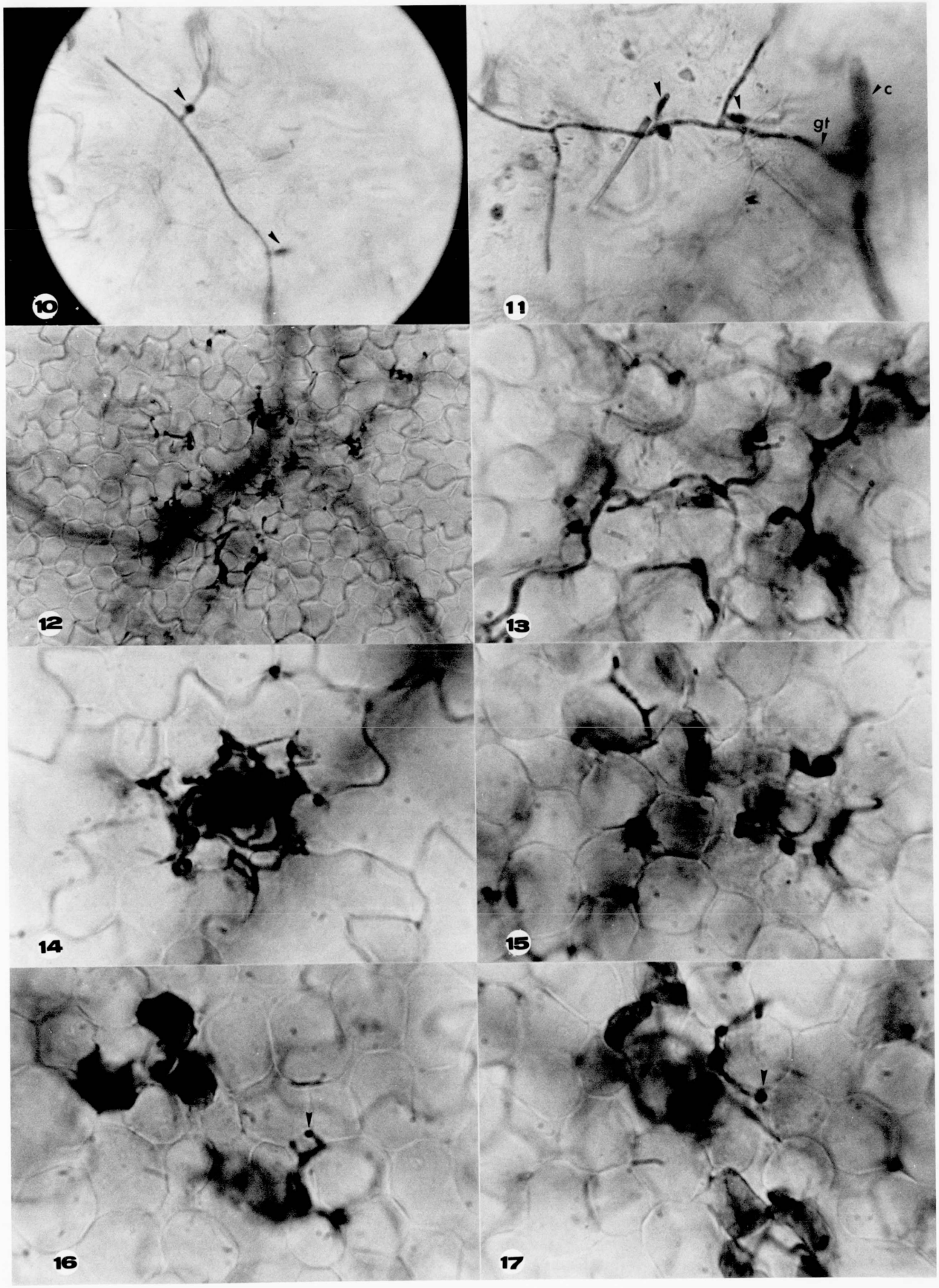



Plate 26

branches grew into the stomata (Plate 26, Figs. 6 and 7). Germ tubes that formed at some distance from stomata, were also observed to grow to considerable lengths before forming branches that then grew towards and penetrated stomata (Plate 26, Figs. 8 and 9). This tropism of germ tubes was observed for conidia that germinated from between 110 to 120 µm from the pore of the stoma. After penetration through the epidermis, secondary mycelia were observed to grow and ramify through intercellular spaces with hyphae adpressed to the host cell walls (Plate 26, Figs. 12 and 13). Bulbous swellings were also observed to develop at the tips of branched mycelium in the substomatal region but the identity of these structures was not confirmed.

For both C. canescens and P. cruenta no evidence was obtained for direct penetration of epidermal cells. The epidermis was penetrated only through stomata and this penetration was preceded often for long periods, by growth of hyphae on the leaf surface.

### 4.3.2 Effects of leaf maturity and plant genotype on the behaviour of P. cruenta

In Section 4.2.5, it was observed that although leaves of all age categories on a susceptible cultivar became

infected, the rates at which lesions developed and the size of lesions differed among leaves at different stages of maturity. It has been suggested that preformed host factors, the concentrations of which are influenced by leaf maturity, affect the germination of conidia, germ tube growth and therefore the rate of penetration of germ tubes and subsequent severity of Cercospora leaf spot of cowpea (Schneider and Sinclair, 1975; Ekpo and Esuruoso, 1977). To verify the effects of leaf age, the behaviour of P. cruenta was examined on three cultivars of cowpea, differing in susceptibility to P. cruenta. Three cultivars; Ife Brown, IT 82D 885 and Muliana; highly suscptible, moderately susceptible and resistant, respectively to P. cruenta (see Table 4.2.8) were used.

(a) Effect of leaf diffusates on the germination of conidia and germ tube growth.

Tagged leaves of three age categories (2, 4, and 8 weeks) were washed in a jet of sterile distilled water and allowed to dry overnight. Drops (0.05ml) of sterile distilled water were placed on the adaxial surfaces of the leaves and allowed to incubate for 24h. 'Leaf diffusates' were collected with sterile disposable pippettes and placed on glass slides in moist chambers. An equal volume of a suspension of conidia ($10^4$ $ml^-$) was added to each drop with four replicates for each treatment. The controls were drops

of sterile distilled water added to an equal volume of conidial suspension. The various treatment combinations were incubated in moisture chambers, 30cm below two rows of white fluorescent tubes (40w) in a controlled temperature room maintained at 25 ± 1°C. Two slides each per treatment were examined 6 and 12h after incubation, with the addition of a drop of lactophenol to stop further growth.

Results: The percentage of germinated conidia, was determined from ten (x40 microscope fields of view for each slide (c. 20-25 conidia per field) and a mean calculated for each of the two periods of incubation for a treatment. The results presented in Table 4.3.1 shows that by 6h after incubation there were only small differences in the numbers of conidia that had germinated in diffusates from the different leaf age/cultivar combinations. After 12h incubation, the mean percentage of germinated conidia in diffusates from the resistant cultivar, Muliana, was comparable to that observed in the distilled water control. Leaf diffusates, therefore did not appear to enhance or inhibit the germination of conidia, neither did the age of the leaf influence the effects of the diffusates.

**Table 4.3.1** Effect of leaf diffusates from cowpea cultivars varying in susceptibility and maturity on the germination of conidia of P. cruenta on glass slides at 25 ± 1°C.

| Cultivar/Leaf age (wks) | | % Germination* of conidia after 6h | 12h |
|---|---|---|---|
| Ife Brown (HS) | 2 | 40.0 d** | 83.0 de |
| | 4 | 41.8 d | 84.3 cde |
| | 8 | 43.0 cd | 81.3 de |
| IT82D885 (MS) | 2 | 33.3 e | 84.4 cd |
| | 4 | 48.5 abc | 79.3 de |
| | 8 | 41.5 d | 79.8 e |
| Muliana (R) | 2 | 42.8 cd | 84.0 cde |
| | 4 | 45.8 bcd | 88.5 abc |
| | 8 | 48.0 ab | 87.3 abc |
| Dist. Water Control | | 55.0 a | 91.5 a |

* Each value is the mean of 20 observations in which percent germination counts were based on 20-30 conidia for each observation. Percent germination data was analysed using arcsine $\sqrt{}$ percentage transformed values:

** In each column, values followed by the same letter(s) are not significantly different (P = 0.05).

(b) Effect of water extracts from leaves at different stages of maturity on conidial germination and germ tube growth

The effects of leaf extracts rather than diffusates on the germination of conidia and germ tube development was examined in this experiment. Water extracts from the leaves at the three stages of maturity were prepared by homogenizing 100g fresh weight of leaves in 1 litre of sterile distilled water and filtering through two layers of cheese cloth. Drops of the filtered extracts (0.05ml) were added to equal volumes of a suspension of conidia ($10^4$ $ml^-$) on glass slides and incubated in moisture chambers as previously described. There were three replicate slides per treatment, and the percentage of germinated conidia was assessed in each of five (x40) fields of view, 12h after incubation. The mean lengths of the longest germ tubes for a treatment were measured for 10 conidia in each of five fields of view.

Results: Table 4.3.2 shows that the rate at which conidia germinated in sterile distlled water was again significantly better than in the leaf extracts. The rate of spore germination in more mature leaves of a cultivar was also found to be better than in the extracts of younger leaves. However, the rates of spore germination between the cultivars at a similar age did not differ significantly but all the differences were small and very unlikely to be

Table 4.3.2 Germination of conidia and germ tube growth of P. cruenta in water extracts from cowpea leaves at three stages of maturity 12h after incubation at 25 ± 1°C.

| Cultivar/Leaf age (wks) | | Conidia % germination* | Germ tube Length µm** |
|---|---|---|---|
| Ife Brown (HS) | 2 | 66 de** | 49 c |
| | 4 | 69 bcd | 52 b |
| | 8 | 76 bc | 56 a |
| IT82D885 (MS) | 2 | 65 de | 43 ef |
| | 4 | 69 cd | 45 de |
| | 8 | 78 b | 46 d |
| Muliana (R) | 2 | 58 e | 34 g |
| | 4 | 68 cd | 41 f |
| | 8 | 70 bcd | 45 de |
| Dist. Water Control | | 84 a | 49 c |

* Mean of three replicates of c. 100-150 conidia in five fields of view. Percentage germination data was analysed using arcsine √ percentage transformed values:

** Each value is the mean of 50 measurements, of the longest germ tubes to the nearest µm for each of ten conidia in five randomly selected fields of view.

*** In each column, values followed by the same letter(s) are not significantly different (P = 0.05).

significant in pathogenesis. The growth of the germ tubes after 12h was found to be slightly longer but with many more branches in extracts of the more mature foliage of the highly susceptible cultivar, Ife Brown, and the mean increase in germ tube length of conidia germinated in distilled water was slightly higher compared to the growth in extracts from the moderately susceptible and resistant cultivars which did not differ. But again, the difference was far too small to have any importance in pathogenesis.

### 4.3.3 Behaviour of conidia of P. cruenta on the leaf surface of cowpea cultivars varying in susceptibility and maturity

The results of the experiments described in Section 4.3.2 suggested that the germination of conidia and germ tube growth of P. cruenta was neither enhanced nor inhibited significantly by leaf diffusates or extracts from cultivars differing in susceptibility. The germination of conidia and germ tube growth was therefore examined on intact leaf surfaces, to determine if there were any differences in the behaviour of conidia on the surfaces of leaves of differing susceptibilities and maturity.

The cowpea cultivars and leaf age categories were as described in the preceeding experiments. Three (trifoliate) leaves of each category for the three cultivars, were

inoculated as a group by spraying the abaxial leaf surfaces with inoculum containing 2 x $10^4$ conidia $ml^{-}$. The plants were kept moist in a humid chamber and transferred to a mist chamber after 48h, where they were exposed to alternating periods of mist and dry periods.

(a) Spore germination and germ tube growth

Whole leaf pieces were examined directly (as described in Section 8.2) for the numbers of germinated conidia 12 and 24h after incubation, and the numbers of germ tubes per conidium, and the mean length of the longest germ tubes determined after 24h. To serve as a control drops of the inoculum were placed on glass slides and incubated in moist chambers in the glasshouse. The percentage of 200 conidia that germinated was assessed on ten (10) leaf pieces sampled randomly from three leaves. The number of germ tubes per conidium was also assessed on 200 conidia, and the lengths of the longest germ tubes was determined from measurements of fifty (50) conidia for each leaf age/cultivar combination; the results are presented in Table 4.3.3. At 12h after incubation, there were small differences in the numbers of conidia that had germinated on the various treatments, and germination was slightly higher in the distilled water control. However, 24h after inoculation most of the conidia (>90%) on all leaf surfaces had germinated and there were no significant differences in the

Table 4.3.3: Observations on the prepenetration growth and development of P. cruenta on leaf surfaces of cowpea cultivars with varying reactions to P. cruenta (at three stages of maturity).

| Cultivar | Leaf age (weeks) | Percent germination[1] of conidia after 12h | Percent germination[1] of conidia after 24h | Number of germ tubes/[2] conidium after 24h | Lengths of longest germ tube[3] µm after 24h |
|---|---|---|---|---|---|
| | | | | mean ± s.d. | mean ± s.d. |
| Ife Brown (HS) | 2 | 75.5[4] c[4] | 90.5[4] a[4] | 2.7 ± 0.20 | 97.1 ± 12.2 |
| | 4 | 80.5 bc | 96.0 a | 2.5 ± 0.44 | 97.9 ± 9.2 |
| | 8 | 77.5 c | 94.0 a | 2.8 ± 0.18 | 100.1 ± 8.8 |
| IT82D (MS) | 2 | 87.5 ab | 97.0 a | 3.0 ± 0.24 | 92.2 ± 10.8 |
| | 4 | 81.5 bc | 93.0 a | 3.3 ± 0.24 | 93.0 ± 6.9 |
| | 8 | 89.5 a | 97.0 a | 3.1 ± 0.18 | 94.4 ± 6.2 |
| Muliana (R) | 2 | 80.5 bc | 96.0 a | 3.1 ± 0.22 | 86.2 ± 9.5 |
| | 4 | 80.0 bc | 95.0 a | 3.2 ± 0.18 | 88.1 ± 9.3 |
| | 8 | 85.0 abc | 96.0 a | 3.3 ± 0.19 | 87.0 ± 6.3 |
| Control (Distilled $H2_O$) | - | 92.0 a | 96.5 a | 3.7 ± 0.25 | 99.8 ± 8.6 |

1. Each percent germination value is the mean of 200 conidia observed on 10 leaf pieces randomly selected from three leaves for each treatment. Percentage germination data were analysed using arcsin $\sqrt{}$ percentage transformed values.
2. The numbers of germ tubes per conidium is the mean (and standard deviation) of 200 conidia, (20 conidia were observed on each of 10 leaf pieces).
3. The lengths of the longest germ tubes (± standard deviation) per treatment was measured on 10 conidia in each of 5 (x 40) fields of view.
4. Values followed by the same letters are not significantly different (P = 0.05).

numbers of conidia that had germinated on the surfaces of leaves of different susceptibilities to P. cruenta leaf spot. The numbers of germ tubes per conidium ranged between 2 and 4, but many more cells of conidia incubated in distilled water, produced germ tubes compared to conidia on leaf surfaces. There was no consistent trend in the differences in the numbers of germ tubes produced per conidium, among the leaves at different stages of maturity. The mean length of the longest germ tube growing from each conidium were also similar among the various treatments.

(b) Stomatal penetration

The numbers of germ tubes that developed from the conidia and the proportions of the total that penetrated stomata either from germ tubes in close proximity to stomata or from germ tubes that had branched from some distance before penetrating, were assessed separately from ten (10) randomly selected leaf samples.

Results: A greater proportion of of stomatal penetration was observed with germ tubes that developed close to a stomatal opening (Table 4.3.4).

The frequency of the total penetration was not affected by the age of the leaf. However, there were significant

Table 4.3.4: Effect of leaf age on penetration by germ tubes into stomata on cowpea leaves differing in susceptibility to P. cruenta.

| Cowpea cultivar | Leaf age (weeks) 2 | | 4 | | 8 | | Mean for cultivar |
|---|---|---|---|---|---|---|---|
| | Percent penetration from germ tubes growth | | | | | | |
| | Short | branched | Short | branched | Short | branched | |
| Ife Brown (HS) | 52.0 | 22.0 | 40.5 | 19.0 | 48.0 | 31.5 | 35.6 a |
| IT82D 885 (MS) | 44.0 | 20.0 | 46.0 | 19.0 | 42.0 | 25.5 | 32.4 a |
| Muliana (R) | 34.8 | 19.0 | 33.5 | 22.5 | 41.0 | 18.5 | 28.1 b |
| Mean of mode of penetration/age | 42.7 | 20.3 | 40.0 | 20.2 | 43.7 | 25.2 | |
| Mean of total penetration for Leaf age | 31.5 ab | | 30.1 b | | 34.4 a | | |

Critical values for comparison (LSD 0.05)
Penetration by leaf age = 3.4
Penetration by cultivar = 3.4
CV X mode x age = 8.2

differences in the rates of stomatal penetration among the cultivars. The frequency of penetration was similar for the highly susceptible cultivar (Ife Brown) and the moderately susceptible cultivar (IT 82D 885) but the proportion was slightly lower on the resistant cultivar (Muliana).

# 5. DISCUSSION

## 5.1 Growth and cultural characteristics

The results of the study has confirmed marked differences in the cultural characteristics of isolates of C. canescens that were isolated from infected leaves from various sources. Mycelial growth, varied both within isolates from cowpea and between isolates from the different legume species. Although the differences in radial growth were found to be statistically significant, it is evident that such differences (usually not more than 4mm) were too small for the parameter to be useful in distinguishing between isolates. The mean increase in growth of the isolates observed on V-8 juice agar after 10 days was however found to be considerably more than that reported by Ekpo and Esuruoso (1978) (31.0mm (range 26.5-33.5) cf. 23.0mm).

The morphology of the colonies formed also varied considerably from the more compact colonies of the isolates from mung bean (VR), bambarra groundnut (VS) to the relatively effuse growth of the cowpea isolates. An interesting observation that emerged from the results is that, although pink pigment production in culture has been reported as being characteristic of the 'true Cercospora' (see Section 2.2.1), the results of the present research did not confirm this view. Secondly, it was observed that the production of the pink/purple pigment is not restricted to media containing dextrose as reported by Mew et al. (1975). Mew et al. (1975) observed that a mung bean

isolate of C. canescens produced a dark purple pigment on potato dextrose agar (PDA) but no similar pigmentation was produced on oatmeal agar or carrot extract plus oatmeal agar. In contrast, in the present study, the mung bean isolate (VR) produced the purple pigment on media containing dextrose (PDA and PDCA) as well as on V-8 juice agar, and potato carrot agar (PCA). However, production appeared to be enhanced in the medium containing both dextrose and carrot juice extract (Potato dextrose carrot agar - PDCA) (see Plate 13). The effects of light on the pigmentation of the isolates were different from those reported by other workers (Fajola, 1978c; Roy, 1982). Fajola (1978c) reported that alternate periods of 12 light and 12h darkness enhanced the production of the purple pigment. Roy (1982) on the other hand, observed that ten (10) species of Cercospora including an isolate of C. canescens from cowpea produced an intense maroon purple pigment on potato dextrose agar (PDA) incubated in continuous darkness. The results obtained in the present study indicated that there was an interaction between the isolates and different regimes of light, and that light is not obligatory for the production of the purple pigment. For example, isolate $ZB_3$ produced relatively more pigment in the dark (Table 4.1.6) and all the other isolates except isolates $KP_1$ and $KP_2$ produced the pigment in the dark. The differences also appeared to relate to the sequences of exposure of the cultures to the different light regimes. Isolate $ZB_2$ produced only traces of the purple pigment when incubated under alternate light/dark conditions (Plate 8).

However, when cultures of the isolate were incubated in the dark initially and exposed daily to periods of 5 to 6h of light, concentric rings of deep purple pigment were produced which corresponded to the periods of light exposure (Plate 9). The results are similar to those reported for C. beticola by Lynch and Geoghegan (1979) in which an isolate 46HC (ATCC 24079), produced only a low yield of the pigment in the dark, but more of it when incubated initially in the dark and exposed daily for five minutes in the light. In contrast an isolate 72R which produced low yields in the dark was induced to produce more of the pigment when incubated initially in the light and subsequently in the dark for various periods of time. They concluded that increasing the initial incubation in light resulted in an increase of the production of the pigment.

As pointed out by Steinkamp et al. (1981) the assessment of pink pigment production and its relation to cercosporin is complicated by the cultural conditions. Certain isolates within a species known to produce the pigment may not produce it under certain conditions because the production of the pigment and indeed of cercosporin in culture, is influenced not only by the medium and light, but also by the temperature, pH and the addition of specific substances (Lynch and Geoghegan, 1979). Apparently cercosporin is red under acidic and neutral conditions and turns green in the base form (Fore et al., 1988).

The results of the comparisons of the sporulation of the isolates of C. canescens revealed that all the isolates sporulated albeit sparsely on all the media tested, except potato dextrose agar (PDA). The results, therefore, agree with those reported by Ekpo and Esuruoso (1975), but differ from the observation made by Mew et al. (1975) who noted that a mung bean isolate of C. canescens sporulated readily but not abundantly on potato dextrose agar (PDA). This suggests that isolates from different sources behaved differently. It however, emerged from the methods used to induce and increase sporulation, that when transfers of macerated mycelium was made from PDA to V-8 juice agar, some isolates produced only conidiophores, and it was only on subsequent transfers of these conidiophores to fresh media that appreciable increases were obtained. To explore the full potential of the sporulative capacity of the isolates, several such transfers involving different combinations of media would have been necessary and require more time than was possible in the present study. Generally the sporulative capacity of the isolates observed with the techniques of multi-point inoculation, was relatively low, when compared to the spore yield reported for other isolates of C. canescens (Schneider et al., 1973; Kwon et al., 1981) and with other species of Cercospora (Chen et al., 1979; Vathakos and Walters, 1979). Schneider et al. (1973) obtained as much as $1.3 \times 10^4$ conidia/cm$^2$ of colony surface on potato dextrose carrot agar (PDCA), the equivalent of about $1.82 \times 10^6$ conidia for 9.0cm Petri plate. In the present study an average of only $2.9 \times 10^5$

conidia was obtained for the whole plate. Similarly the spore yields of a mung bean isolate studied by Kwon et al. (1981) was estimated as 1.2 x $10^5$/cm² of colony surface (the equivalent of 1.75 x $10^6$ conidia for the whole colony surface on mung bean leaf decoction agar. In comparison the mung bean isolate (VR) produced an average of 4.1 x $10^5$ conidia for the whole colony surface. Such differences in spore yields have also been recorded for isolates of other related species. Chen et al. (1979) estimated that an isolate of Cercospora kikuchii from purple stained seed of soyabean in Texas produced 5.7 x $10^4$ conidia/cm² on V-8 juice agar, and Yeh and Sinclair (1980) obtained only 2.5 x $10^4$ conidia/cm² on the same medium. In contrast to the above reports El-Gohil et al. (1982) reported that isolates of C. kikuchii from blighted foliage of soy bean, produced either a smooth colony or one with radial folds. The colonies with radial folds produced 9.6 x $10^4$ conidia per colony surface on V-8 juice agar in 9.0cm Petri plates, the colonies that were smooth produced only 3.5 x $10^4$ conidia/9.0cm plate.

Thus, the observations confirm the inherent variability that exists among isolates of Cercospora species, in respect of their sporulation on agar media. The differences could therefore not be attributed to the methods used to induce the sporulation of the isolates nor the methods used to estimate spore production. It is therefore concluded that the isolates of C. canescens used in the present study could well have been low sporulating

isolates.

Comparisons of the conidia of isolates of C. canescens from the different species of legumes showed a wider range of variation in size. In contrast to the observations made by Ekpo and Esuruoso (1978) the range in the lengths of conidia produced on infected host leaves was wider compared to those produced in culture media (Fig. 4.1.3). Fajola (1978b) proposed that to mitigate the problem of the effects of the environment on the morphology and dimensions of conidiophores and conidia, comparisons should be made on a common and prescribed set of conditions. Using the same ranges of temperature, light and relative humidity, Fajola (1978b) concluded that there were great similarities of conidia and conidiophores of isolates of Cercospora canescens, C. nicotianae and C. ricinella. In the present research, although the mean length of conidia of the cowpea isolate $ZB_3$ and the lima bean isolate (LB) were significantly shorter compared to the other isolates (Table 4.1.5), the ranges of the lengths were similar. The range of the measurements made, were also greater than those reported by other authors (see Appendix Table 4).

## 5.2 Pathogenicity and host-range tests

One of the main purposes of the research was to study the range of parasitism of isolates of C. canescens from different sources with a view to determine forms of physiologic specialization. The results of the preliminary experiments which examined the comparative virulence of the

isolates of Cercospora, confirmed reports in the literature of the substantial difficulties encountered in producing disease under glasshouse conditions. Although lesion development was enhanced with inocula obtained from infected host leaves, compared to that produced in culture, the symptoms that developed were atypical of the usual cherry red to reddish brown lesions associated with the disease (see Plate 2). The relatively long incubation period and the development of 'green island' type of lesions, normally associated with biotrophic fungi, suggested initially that the isolates were behaving as biotrophs for much of their growth in leaves. It later became apparent however, that infection and subsequent lesion development was influenced considerably by the environmental conditions, and in particular the ranges of temperature and the lighting regimes. The temperature range in the glasshouse where the experiments were done fluctuated considerably between 25°C and 15°C. Although the day time temperatures ranged between 22 - 25°C it dropped at night, sometimes to as low as 15°C particularly during the winter months. Later experiments conducted mainly during April to August, (during which time the temperature range was 20 - 35°C during the night and day time respectively) produced distinct necrotic lesions on the inoculated plants (see Plates 19 and 20).

Cross infection by the isolates from different legume species was obtained with the inoculation technique adopted, but the pattern of infection of the different

hosts did not separate the isolates into a pattern of physiologic races. The isolates from cowpea (TZ) lima bean (LB) and groundnut, (AH) had a similar range of parasitism infecting in common cowpea, lima bean, bambarra groundnut, groundnut and soyabean. The remaining isolates from mung bean (VR) and bambarra groundnut (VS) also had a similar host range except that the bambarra groundnut isolate (VS) did not produce any lesions on groundnut as was observed with the mung bean isolate (VR). None of the other isolates including that obtained from infected groundnut leaves isolate (AH) was pathogenic on the groundnut cultivars tested. Thus, the observations confirm other reports in which isolates of C. canescens were found to be non-pathogenic to groundnut, although they were associated with lesions caused by Cercospora arachidicola (see Section 2.2.4).

The isolate from groundnut (AH) on the other hand was found to be virulent on cowpea and weakly pathogenic on lima bean and winged bean, thus indicating some degree of specialization. The range of the isolates from the respective legume species was however, rather narrow for the deductions to be conclusive. Further tests with many more well characterized isolates and a set of differential cultivars would be needed to supplement the results obtained with the range tested in the present study. Nevertheless, the results have indicated that isolates differ considerably in their parasitism. Other isolates of Cercospora from different genera or the same species have

been observed to exhibit differences in host range and pathogenicity. La (1963) and Ruppel (1972) have reported differences in virulence among isolates of C. beticola from different regions of the United States, while Solel and Minz (1971) have confirmed the existence of physiologic specialization of C. beticola among isolates originating in the U.S.A., Japan and Germany tested in Israel. Berger and Hanson (1963b) also observed that isolates of Cercospora from red and white clovers (Trifolium spp.) alfalfa (Medicago sp.) and sweet clover were pathogenic on their hosts or origin and often on other hosts, but none of them was significantly pathogenic on subclover (Trifolium subterraneum). In contrast Pratt (1984) noted that an isolate from subclover in Mississippi produced symptoms only on subterranean and rose clovers (T. hirtum), but not on eight other species of clover and alfalfa tested. Nevertheless in view of the similarities in morphological and other cultural characteristics with those described for Cercospora zebrina, the isolate was conservatively classified as C. zebrina. It is possible that isolates of C. canescens from different hosts that have been so classified might exhibit such differences. The interesting observations that isolates of C. canescens infected sugar beet, okra and celery, and also of the infection of cowpea by the isolate of C. beticola (Plate 23), however supports the contention that isolates of Cercospora with hyaline acicular conidia are all forms of the type species C. apii (Ellis, 1971). Chupp (1953) however, maintained that nearly all species of Cercospora are limited narrowly in

their host range. He pointed out that the results of cross-inoculation tests under high humidity conditions in the laboratory are not acceptable for the Cercosporae.

It is evident from the results obtained with the isolate of Pseudocercospora cruenta, that the methods used in the assessment of the reaction of cowpea cultivars was effective in differentiating between the cultivars. The overall reaction of some of the cultivars however, did not confirm the reactions observed in the countries of origin (Table 3.1). Colossus, a cultivar found to be susceptible to P. cruenta in the U.S.A. was found to be resistant whereas Ala 963.8 classified as resistant in the U.S.A. was moderately susceptible. The differences could be due to isolate variability but since only an isolate of P. cruenta was available for the study the observation was not confirmed.

It was the intention to do as much or more work with P. cruenta than with C. canescens. But this was not possible because of the failure, despite repeated attempts, to obtain more isolates and more infected material from overseas. But it is hoped to continue work with P. cruenta in Ghana.

## 5.3 Infection and host-pathogen relationships

The observations of the host-pathogen interaction confirmed that both C. canescens and P. cruenta penetrated cowpea leaves through stomata but not directly through

undamaged surfaces. Although both leaf diffusates and extracts were not tested for the presence of nutrients, the fact that conidia of P. cruenta germinated readily in distilled water, and the fact that the percentage of germinated conidia was higher than observed in extracts from the more mature foliage of the highly susceptible cultivar (Table 4.3.2) suggests the absence of any factor(s) which significantly inhibited germination of conidia. The germination of the conidia in both diffusates and extracts from the resistant cultivar was also found to be slightly higher than in the highly susceptible cultivar. However, it is clear from the above results that the differences in the observed reactions of cowpea cultivars could not be attributed to differences in the rates of germination of conidia or on the fact that germ tube elongation on intact leaf surfaces varied considerably on both resistant and susceptible cultivars. The results therefore, do not support the claim that conidial germination and germ tube growth is inhibited by leaf diffusates or enhanced by extracts from it (Schneider and Sinclair, 1975; Ekpo and Esuruoso, 1977). Schneider and Sinclair (1975) attributed resistance in cowpea to infection by C. canescens to an inhibition of conidial germination and germ tube growth. Ekpo and Esuruoso (1977) on the other hand suggested that increased susceptibility of mature cowpea leaves to P. cruenta was due to the stimulatory effects on spore germination of some germination principle. Although leaf exudates are known to contain nutrients which may condition the host for

susceptibility by making nutrients available to the pathogen on the leaf surface (Deverall and Wood, 1961; Sharma and Sinha, 1972), the nature of such nutrients was not reported on by Ekpo and Esuruoso (1977).

The methods used in obtaining diffusates and extracts from cowpea leaves in the present study differed from those used in the research cited above; it is however, unlikely that the observed differences could have been due to the methods of extraction alone.

In a study with C. beticola on sugar beet leaves, Solel and Minz (1971) observed no differences in the rates of spore germination on cultivars differing in susceptibility. In contrast Kovacs (1955) (cited by Solel and Minz, 1971) reported that when conidia were washed from inoculated sugar beet leaves the rate of germination differed on various cultivars. Solel and Minz (1971) suggested that the differences in the results could be due to the concentration of mineral substances found in sugar beet leaf washings which either stimulated or inhibited spore germination. Further work is therefore necessary to characterise the nature of the diffusates and to ascertain much more clearly their effects on the behaviour of conidia of both P. cruenta and C. canescens on leaf surfaces of cowpea.

Stomatal attraction of germ tubes was evident from either the directed growth of germ tubes towards stomata

(Plate 26, Figs. 6 and 7) or a definite change in direction (Plate 26, Figs. 8 and 9). However, the directional growth of germ tubes was similar on cultivars with varying susceptibilities. It is therefore more likely that the differential susceptibility of cowpea to infection by P. cruenta is due to factors that limit growth of the pathogen within the leaf. Behaviour of P. cruenta after penetration of cowpea leaves was studied only to a limited extent mainly because of technical difficulties in observing the growth of hyphae in whole leaves. Further studies would seem justified with better histological techniques to determine the differences in the extents of leaf colonization following stomatal penetration of both resistant and susceptible cultivars of cowpea.

The main points from the research described in this thesis are, therefore: the variation in cultural characteristics among isolates of C. canescens from the same host; the observation that not all isolates of C. canescens produce a pink purple pigment in culture on agar media; the striking effect of the type of reproductive unit subcultured on sporulation; the variation among C. canescens isolates in the preferred medium for obtaining abundant sporulation; the slight loss of virulence of conidia of C. canescens isolates obtained from agar culture and the resultant 'green island' type of lesions on cowpea; the very striking absence of symptoms on leaves of cowpea inoculated with C. canescens until leaves became senescent or were detached and incubated in Petri dishes, when

lesions and sporulation develop conspicuously; the cross-infection of one legume species by an isolate obtained from another legume species; the infection of okra, celery and sugar beet by C. canescens; the very striking lesions obtained with infection of cowpea by C. beticola; the lack of evidence of substantial differences in germination and growth of germ tubes in leaf diffusates, leaf extracts and on leaf surfaces of cowpea differing in susceptibility to P. cruenta.

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Appendix Table 1 Comparisons of radial linear growth of cowpea and lima bean isolates of C. canescens colony radii after 10 days at 25 + 1°C (mm). (Text Table 4.1.1).

| | Isolate Code | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Medium | $KP_1$ | $KP_2$ | $KP_3$ | TZ | $ZB_1$ | $ZB_2$ | $ZB_3$ | LB | 292 | 306 |
| PDA | 34.8+ | 37.0 | 27.6 | 31.2 | 31.5 | 31.7 | 28.5 | 30.3 | 29.5 | 34.7 |
| PCA | 30.0 | 29.0 | 27.2 | 28.2 | 27.0 | 28.3 | 27.7 | 27.8 | 30.5 | 32.0 |
| CLDA | 32.3 | 34.7 | 31.8 | 28.3 | 31.8 | 27.5 | 27.0 | 29.3 | 31.8 | 30.2 |
| PDCA | 35.3 | 36.3 | 33.0 | 31.8 | 33.3 | 31.7 | 29.8 | 28.0 | 32.8 | 30.8 |
| V-8A | 33.7 | 33.5 | 32.8 | 28.8 | 31.2 | 27.5 | 26.5 | 30.2 | 33.8 | 33.8 |

Analysis of Variance

| Source of variation | DF | SS | MS | F |
|---|---|---|---|---|
| Isolates | 9 | 1011.8 | 112.42 | 62.46** |
| Media | 4 | 443.6 | 110.90 | 61.61** |
| Isolates x Media | 36 | 710.9 | 19.75 | 10.97** |
| Error | 250 | 450 | 1.8 | |
| Total | 299 | | | |

+ mean of 10 measurements; ** denotes significant differences (P.0.05)

Appendix Table 2 Comparison of the sporulation of C. canescens isolates from cowpea and lima bean on 5 agar media using two types of inocula; mycelial fragments (m) and mycelial fragments plus conidia M+C. (Text Table 4.1.3).

| | Spore counts per plate+ | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Medium | $KP_1$ | | $KP_2$ | | $KP_3$ | | TZ | | $ZB_1$ | | $ZB_2$ | | $ZB_3$ | | LB | |
| | m | m+c | m | m+c | m | m+c | m | m+c | m | m+c | m | m+c | m | m+c | m | m+c |
| PCA | 2.8 | 3.5 | 2.1 | 3.9 | 0.8 | 1.6 | 1.1 | 2.4 | 1.5 | 2.3 | 1.4 | 2.3 | 1.0 | 2.0 | 0.6 | 2.3 |
| CLDA | 2.1 | 4.6 | 3.2 | 5.3 | 0.8 | 2.3 | 2.1 | 5.0 | 1.6 | 3.9 | 0.9 | 2.9 | 1.0 | 3.2 | 1.2 | 3.9 |
| PDCA | 1.7 | 3.6 | 1.6 | 4.1 | 1.7 | 2.4 | 1.4 | 3.4 | 1.3 | 2.7 | 1.5 | 2.1 | 1.0 | 2.0 | 1.9 | 2.7 |
| 1.2% V-8A | 3.3 | 4.7 | 3.6 | 6.0 | 1.0 | 2.6 | 1.6 | 4.8 | 1.6 | 3.5 | 2.2 | 2.3 | 1.4 | 2.0 | 1.0 | 3.5 |
| 2% V-8A | 3.2 | 4.3 | 2.7 | 5.8 | 0.9 | 2.2 | 1.2 | 3.6 | 1.0 | 3.0 | 1.3 | 2.3 | 1.0 | 2.0 | 1.2 | 3.1 |

+ mean of 5 replicate plates to the nearest 0.1 x $10^5$

Appendix Table 3: Summary statistics for the comparisons of the size of conidia of cowpea and lima bean isolates of Cerospora canescens produced on V-8 juice agar (Text Table 4.1.5).

| Isolate Code | Lengths of Conidia | | | | Width of Conidia | | | |
|---|---|---|---|---|---|---|---|---|
| | Range | Mean* | SD | SE | Range | Mean* | SD | SE |
| $KP_1$ | 90-300 | 193 | 63.5 | 9.2 | 2.4-4.5 | 3.2 | 0.6 | 0.08 |
| $KP_2$ | 60-290 | 170 | 47.8 | 6.8 | 2.5-5.0 | 3.2 | 0.4 | 0.06 |
| $KP_3$ | 80-270 | 153 | 49.3 | 7.4 | 2.5-4.0 | 3.2 | 0.4 | 0.05 |
| TZ | 120-280 | 182 | 40.2 | 5.6 | 2.5-4.0 | 3.4 | 0.5 | 0.06 |
| $ZB_1$ | 100-260 | 189 | 40.6 | 5.7 | 2.5-3.5 | 2.9 | 0.3 | 0.04 |
| $ZB_2$ | 100-300 | 204 | 43.7 | 6.2 | 2.5-4.0 | 2.8 | 0.3 | 0.05 |
| $ZB_3$ | 75-230 | 155 | 47.0 | 6.5 | 2.5-5.0 | 3.1 | 0.4 | 0.06 |
| LB | 100-300 | 159 | 44.9 | 6.4 | 2.8-5.0 | 3.4 | 0.5 | 0.07 |

* Mean of 50 observations.

Analysis of variance for lengths of conidia

| Source of Variation | d/f | SS | MS | F |
|---|---|---|---|---|
| Isolates | 7 | 126100 | 18020 | 7.95** |
| Error | 392 | 888700 | 2267 | |
| Total | 399 | 1014800 | 20287 | |

Appendix Table 4: Published dimensions of conidiophores and conidia of isolates of *Cercospora canescens* under varying environmental conditions

| Authors | Source of Isolate | Substrate/Growth Medium | Dimensions µm | | | |
|---|---|---|---|---|---|---|
| | | | Conidiophores | | | |
| | | | Length | | Width | |
| | | | Range | Mean | Range | Mean |
| 1. Chupp (1953) | Not specified | | 20-175 | - | 3-6.5 | - |
| 2. Mulder and Holliday (1975) | Not specified | | 20-175 | - | 3-6.5 | - |
| 3. Ellis, (1976) | Not specified | | 50-200 | - | 4-6 | - |
| 4. Fajola (1978a) (i) | Cowpea leaves | Host | 12-85 | 43.7 | 3.8-5.5 | 4.5 |
| | Cowpea leaves | V-8A | 100-700 | - | 3.75-5.5 | - |
| (ii) | Bambarra groundnut | Host | 20-120 | 68.0 | 3.8-5.0 | 4.3 |
| | Bambarra groundnut | V-A8 | 150-960 | - | 4-5 | - |
| 5. Nowsher *et al* (1978) | Winged bean (Bangladesh) | Infected leaves | 27-91.8 | - | 2.7-4.5 | - |
| 6. Vakili, (1977) | Cowpea (Puerto Rico) | Infected leaves | 75-200 | - | - | - |
| 7. Ilag *et al*, (1977) | Mung bean (Philippines) | Infected leaves | 144-234 | 183 | 5.25-7 | 6.06 |

Appendix Table 4 (Continued)

| Authors | Source of Isolate | Substrate/Growth Medium | Dimensions µm | | | |
|---|---|---|---|---|---|---|
| | | | Conidia | | | |
| | | | Length | | Width | |
| | | | Range | Mean | Range | Mean |
| 1. Chupp (1953) | | | 30-300 | - | 2.5-5.0 | - |
| 2. Mulder and Holliday (1975) | | | 30-300 | - | 2.5-5 | - |
| 3. Ellis, (1976) | | | 50-150 | - | 3-5.5 | - |
| 4. EKpo and Esuruoso, (1978) | Cowpea leaves (cv. New Era) (Nigeria) | Infected host leaves Cowpea stem CSD | - | 109.2 | 1.4-3.5 | - |
| | | CSDA | - | 138.7 | 1.4-3.5 | - |
| | | CLPDA | - | 114.9 | 1.4-3.5 | - |
| | | V-8A | - | 121.1 | 1.6-3.2 | - |
| 5. Fajola (1978a) (i) | Cowpea leaves | Host | 25-248 | 120.9 | 2.8-4.5 | 3.5 |
| | Cowpea leaves | V-8A | 20-180 | - | 2.25-4.0 | - |
| (ii) | Bambarra groundnut | Host | 17-237 | 122.5 | 2.5-4.2 | 3.4+0. |
| | Bambarra groundnut | V-A8 | 25-255 | - | 2.5-4.5 | - |
| 6. Nowsher et al (1978) | Winged bean (Bangladesh) | Infected leaves | 33-210 | - | 2.7-4.0 | - |
| 7. Vakili, (1977) | Cowpea (Puerto Rico) | Infected leaves | - | 200 | - | - |
| 8. Ilag et al, (1977) | Mung bean (Philippines) | Infected leaves | 53-244 | 142 | 3.4-6.9 | 4.9 |

? = not specified; CDSA = Cowpea stem dedoction agar; CLPDA = Cowpea leaf decoction plus 1.2% potato V-8A = V-8 juice agar.

Appendix Table 5: Effects of medium composition and light on pink pigment production by isolates of *C. canescens* on agar media (Text Table 4.1.6).

| | | PDA | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Cowpea | | | | | | | Lima bean | | |
| | | $KP_1$ | $KP_2$ | $KP_3$ | TZ | $ZB_1$ | $ZB_2$ | $ZB_3$ | LB | 292 | 306 |
| Continuous Light | I | 0 | 0 | 3 | 2 | 2 | 0 | 0 | 2 | 2 | 2 |
| | II | 0 | 0 | 3 | 2 | 2 | 0 | 0 | 3 | 2 | 2 |
| | III | 0 | 0 | 2 | 1 | 1 | 0 | 0 | 2 | 2 | 1 |
| Continuous Darkness | I | 0 | 0 | 3 | 1 | 1 | 0 | 0 | 2 | 2 | 0 |
| | II | 0 | 0 | 2 | 1 | 2 | 0 | 0 | 2 | 1 | 0 |
| | III | 1 | 1 | 2 | 1 | 2 | 1 | 2 | 2 | 1 | 0 |
| Alternate 12h light 12h dark | I | 0 | 0 | 3 | 2 | 2 | 0 | 0 | 3 | 2 | 3 |
| | II | 0 | 0 | 3 | 2 | 2 | 0 | 0 | 3 | 2 | 3 |
| | III | 0 | 0 | 3 | 2 | 2 | 0 | 0 | 3 | 2 | 3 |

Appendix Table 5 (Continued):

| | | PCA | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Cowpea | | | | | | | Lima bean | | |
| | | $KP_1$ | $KP_2$ | $KP_3$ | TZ | $ZB_1$ | $ZB_2$ | $ZB_3$ | LB | 292 | 306 |
| | I | 0 | 0 | 3 | 2 | 1 | 0 | 0 | 2 | 2 | 3 |
| Continuous | II | 0 | 0 | 2 | 1 | 1 | 0 | 0 | 2 | 2 | 2 |
| Light | III | 0 | 0 | 2 | 1 | 0 | 0 | 0 | 2 | 2 | 2 |
| | I | 0 | 0 | 2 | 1 | 1 | 0 | 2 | 1 | 0 | 1 |
| Continuous | II | 0 | 0 | 2 | 1 | 0 | 0 | 2 | 0 | 0 | 1 |
| Darkness | III | 0 | 0 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Alternate | I | 0 | 0 | 3 | 2 | 0 | 2 | 0 | 3 | 2 | 3 |
| 12h light | II | 0 | 0 | 3 | 2 | 0 | 1 | 0 | 3 | 2 | 3 |
| 12h dark | III | 0 | 0 | 3 | 2 | 0 | 1 | 0 | 3 | 2 | 2 |

Appendix Table 6: Comparison of the growth of (7) cowpea and (1) lima bean isolates of *C. canescens* in three liquid media. (Text Table 4.1.7)

| Isolates | Dry weight of mycelium (mg) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Potato dextrose broth | | | Cowpea leaf decoction | | | V-8 juice broth | | |
| KP1 | 238 | 251 | 240 | 219 | 220 | 199 | 220 | 211 | 214 |
| KP2 | 220 | 242 | 238 | 216 | 214 | 212 | 200 | 198 | 206 |
| KP3 | 130 | 135 | 145 | 136 | 130 | 135 | 196 | 188 | 175 |
| TZ | 200 | 198 | 186 | 136 | 126 | 122 | 200 | 189 | 186 |
| ZB1 | 190 | 214 | 220 | 196 | 186 | 188 | 230 | 236 | 219 |
| ZB2 | 135 | 140 | 138 | 168 | 159 | 164 | 216 | 220 | 200 |
| ZB3 | 142 | 145 | 136 | 166 | 170 | 168 | 200 | 185 | 193 |
| LB | 216 | 220 | 223 | 212 | 196 | 200 | 196 | 191 | 188 |



Analysis of Variance table for dry weight of mycelium

| Source | DF | SS | MS | F |
|---|---|---|---|---|
| Isolates | 7 | 45230 | 6461.4 | 113.99** |
| Medium | 2 | 7983.4 | 3991.7 | 70.42** |
| Isolates x Medium | 14 | 24986 | 1784.7 | 31.49** |
| Error | 46 | 2607.5 | 56.684 | |
| Total | 71 | 80971 | | |

Standard Error of means

Weight for an isolate in each medium = 4.3
Weight for isolates overall media = 0.9
Weight for all isolates in a medium = 1.5
Weight for each isolate in 3 media = 2.5

**Appendix Table 7** Ranked comparisons of the growth of isolates of C. canescens from cowpea and lima bean, on agar and liquid media.

a. Potato dextrose

| Isolate | Growth on Agar | Growth on Liquid medium | Ranks for Growth on Agar | Ranks for Growth on Liquid medium | di | di² |
|---|---|---|---|---|---|---|
| | (mm) | (mg) | | | | |
| $KP_1$ | 34.8 | 243 | 7 | 8 | -1 | 1 |
| $KP_2$ | 37.0 | 233 | 8 | 7 | 1 | 1 |
| $KP_3$ | 27.7 | 137 | 1 | 1 | 0 | 0 |
| TZ | 31.2 | 195 | 4 | 4 | 0 | 0 |
| $ZB_1$ | 31.5 | 208 | 5 | 5 | 0 | 0 |
| $ZB_2$ | 31.7 | 138 | 6 | 2 | 4 | 16 |
| $ZB_3$ | 28.5 | 141 | 2 | 3 | -1 | 1 |
| LB | 30.3 | 220 | 3 | 6 | -3 | 9 |

$\Sigma di^2 = 28$

$$rs = 1 - \frac{6(28)}{(8)^3-8}$$
$$= 0.67$$

b. Cowpea leaf decoction

| Isolate | Growth on Agar | Growth on Liquid medium | Ranks for Growth on Agar | Ranks for Growth on Liquid medium | di | di² |
|---|---|---|---|---|---|---|
| | (mm) | (mg) | | | | |
| $KP_1$ | 32.3 | 213 | 7 | 7 | 0 | 0 |
| $KP_2$ | 34.7 | 214 | 8 | 8 | 0 | 0 |
| $KP_3$ | 31.8 | 134 | 5.5 | 2 | 3.5 | 12.25 |
| TZ | 28.3 | 128 | 3 | 1 | 2 | 4 |
| $ZB_1$ | 31.8 | 190 | 5.5 | 5 | 0.5 | 0.25 |
| $ZB_2$ | 27.2 | 164 | 2 | 3 | -1 | 1 |
| $ZB_3$ | 27.0 | 168 | 1 | 4 | -3 | 9 |
| LB | 29.3 | 203 | 4 | 6 | -2 | 4 |

$\Sigma di^2 = 30.5$

$$rs = 1 - \frac{6(30.5)}{(8)^3-8}$$
$$= 0.64$$

**Appendix Table 7** (Continued)

c. V-8 juice broth

| Isolate | Growth on Agar | Growth on Liquid medium | Ranks for Growth on Agar | Ranks for Growth on Liquid medium | di | di² |
|---|---|---|---|---|---|---|
| | (mm) | (mg) | | | | |
| $KP_1$ | 33.7 | 224 | 8 | 8 | 0 | 0 |
| $KP_2$ | 33.5 | 216 | 7 | 7 | 0 | 0 |
| $KP_3$ | 32.8 | 152 | 6 | 1 | 5 | 25 |
| TZ | 28.8 | 171 | 3 | 3.5 | -0.5 | 0.25 |
| $ZB_1$ | 31.2 | 209 | 5 | 6 | -1 | 1 |
| $ZB_2$ | 27.5 | 171 | 2 | 3.5 | -1.5 | 2.25 |
| $ZB_3$ | 26.5 | 167 | 1 | 2 | -1 | 1 |
| LB | 30.2 | 205 | 4 | 5 | -1 | 1 |

$\Sigma di^2 = 30.5$

$$rs = 1 - \frac{6(30.5)}{(8)^3-8}$$

$$= 0.64$$

Appendix Table 8: Comparisons of the lengths and width of conidia and condiophores of C. canescens isolates from 5 legume species and an isolate of Cercospora beticola, produced on three substrates (Text figures 4.1.1, 4.1.2 and 4.1.3).

(a) Lengths and width of conidia produced on V-8 agar.

| Isolate Code | Length of conidia | | Width of conidia | | |
|---|---|---|---|---|---|
| | Range | Mean* | Range | Mean* | ± SD |
| VR | 95-350 | 207.5 | 2.8-4.0 | 3.5 | 0.5 |
| VS | 70-350 | 236.5 | 2.5-3.75 | 2.8 | 0.6 |
| AH | 70-250 | 137.7 | 3.75-5.0 | 4.6 | 0.6 |
| LB | 70-260 | 159.5 | 2.5-5.0 | 3.8 | 0.6 |
| $KP_2$ | 145-330 | 211.7 | 3.0-4.5 | 3.5 | 0.4 |
| TZ | 160-410 | 321.7 | 3.75-5.0 | 4.6 | 0.6 |
| CB | 90-260 | 176.3 | 2.5-3.75 | 2.6 | 0.3 |

LSD = 29.5

* = means of 30 conidia

Analysis of Variance

| Source of Variation | DF | SS | MS | F |
|---|---|---|---|---|
| Isolates | 6 | 66130 | 11020 | 32.38** |
| Error | 203 | 69100 | 340.4 | |
| Total | 209 | 135200 | | |

Appendix Table 8 (Continued)

(b) Lengths and width of conidia produced on CLPDA to the nearest 0.1µm.

| Isolate Code | Length of conidia | | Width of conidia | | |
|---|---|---|---|---|---|
| | Range | Mean* | Range | Mean* | ± SD |
| VR | 100-250 | 179.7 | 3.0-5.0 | 3.5 | 0.7 |
| VS | 70-350 | 244.0 | 2.5-3.75 | 3.3 | 0.6 |
| AH | 50-200 | 135.2 | 3.75-5.0 | 4.6 | 0.6 |
| LB | 65-285 | 182.8 | 2.8-5.5 | 3.9 | 0.9 |
| $KP_2$ | 60-240 | 160.0 | 2.5-4.5 | 2.8 | 0.4 |
| TZ | 60-430 | 246.8 | 2.5-4.5 | 3.9 | 0.5 |
| CB | 110-250 | 168.5 | 2.5-3.75 | 2.6 | 0.3 |

LSD = 30.0

* = means of 30 conidia

Analysis of Variance

| Source of Variation | DF | SS | MS | F |
|---|---|---|---|---|
| Isolates | 6 | 31920 | 5320 | 15.1** |
| Error | 203 | 71570 | 352.6 | |
| Total | 209 | 103500 | | |

Appendix Table 8 (Continued)

(c) Lengths and width of conidia on infected host leaves.

| Isolate Code | Length of conidia | | Width of conidia | | |
|---|---|---|---|---|---|
| | Range | Mean* | Range | Mean* | ± SD |
| VR | 50-350 | 250.8 | 3.75-5.0 | 3.9 | 0.5 |
| VS | 180-350 | 283.5 | 2.5-3.75 | 3.0 | 0.6 |
| AH | 135-330 | 227.5 | 3.75-5.0 | 3.4 | 0 |
| LB | 70-330 | 185.7 | 2.5-4.5 | 3.1 | 0.7 |
| $KP_2$ | 165-320 | 219.8 | 2.5-5.0 | 3.8 | 0.6 |
| TZ | 155-380 | 279.8 | 2.5-5.0 | 2.7 | 0 |
| CB | 114-300 | 171.0 | 2.5-3.75 | 2.6 | 0.3 |

LSD = 28.4

* = means of 30 conidia

Analysis of Variance

| Source of Variation | DF | SS | MS | F |
|---|---|---|---|---|
| Isolates | 6 | 33970 | 5661 | 18.03 |
| Error | 203 | 63750 | 314 | |
| Total | 209 | 97720 | | |

Appendix Table 8 (Continued)

(d) Lengths and width of conidiophores of Cercospora isolates formed on infected host leaves incubated in moist chambers.

| Isolate Code | Length of conidiophore | | Width of conidiophore | |
|---|---|---|---|---|
| | Range | Mean + SD | Range | Mean ± SD |
| VR | 200-800 | 579 + 126 | 5.0 | 3.5 |
| VS | 250-260 | 509 + 77 | 5.0-6.25 | 5.2 + 0.4 |
| AH | 740-980 | 848 + 50 | 5.0 | 5.0 |
| LB | 150-440 | 301 + 73 | 5.0-6.25 | 5.2 + 0.4 |
| KP2 | 240-480 | 352 + 58 | 5.0-6.25 | 5.3 + 0.5 |
| TZ | 160-900 | 463 + 210 | 4.8-6.25 | 5.2 + 0.5 |
| CB | 100-310 | 159 + 57 | 5.0-6.25 | 5.3 + 0.5 |

LSD = 17.2

* = means of 30 conidia

Analysis of Variance

| Source of Variation | DF | SS | MS | F |
|---|---|---|---|---|
| Isolates | 6 | 884700 | 147400 | 128.5 |
| Error | 203 | 232900 | 1147 | |
| Total | 209 | 1118000 | | |

Appendix Table 9: Comparison of the incubation periods (days to first lesion) with conidia of C. canescens isolates obtained from culture on V-8A and those from infected host leaves (Text Table 4.2.2).

| Isolate | Incubation period (days) with conidia from V-8A Replicates | | | | | | Host leaves Replicates | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| KP2 | 28 | 26 | 28 | 27 | 24 | 26 | 24 | 22 | 23 | 21 | 24 | 24 |
| TZ | 21 | 24 | 21 | 22 | 21 | 19 | 16 | 18 | 20 | 21 | 14 | 16 |
| ZB1 | 26 | 22 | 26 | 26 | 26 | 24 | 22 | 24 | 22 | 22 | 24 | 24 |
| ZB3 | 24 | 21 | 22 | 23 | 24 | 22 | 20 | 21 | 20 | 20 | 22 | 21 |
| LB | 28 | 24 | 26 | 26 | 24 | 26 | 22 | 20 | 24 | 24 | 22 | 24 |

Analysis of Variance Table

| Source | DF | SS | MS | F |
|---|---|---|---|---|
| Isolate | 4 | 226.43 | 56.608 | 22.86** |
| Medium | 1 | 126.15 | 126.15 | 50.94** |
| Isolate x Medium | 4 | 11.7 | 2.94 | 1.19* |
| Error | 50 | 123.8 | 2.47 | |
| Total | 59 | 488.18 | | |

LSD Comparison of incubation period (number of days to first lesion)

| Mean of Medium | | | Mean of Isolate |
|---|---|---|---|
| V-8A | 24.23 | 24a | KP2 - 24.8 a |
| Host leaves | 21.3 | 21 b | TZ - 19.4 c |
| LSD | | 0.8 | ZB1 - 23.8 a |
| | | | ZB3 - 21.8 b |
| | | | LB - 23.9 a |
| | | | LSD 1.3 |

Appendix Table 10: Comparison of the severity of disease (lesion numbers) obtained with conidia of C. canescens isolates obtained from culture on V-8A agar with conidia from infected host leaves (Text Table 4.2.2b).

| Isolate | Disease severity score with conidia from V-8A Replicate scores | | | | | | Infected Host leaf Replicate scores | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| KP2 | 3 | 2 | 3 | 2 | 2 | 2 | 3 | 3 | 3 | 2 | 2 | 2 | 2.4 ± 0.5 |
| TZ | 4 | 5 | 5 | 4 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 4.8 ± 0.4 |
| ZB1 | 3 | 2 | 3 | 2 | 3 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 2.8 ± 0.5 |
| ZB3 | 3 | 3 | 2 | 2 | 3 | 3 | 3 | 4 | 3 | 4 | 4 | 4 | 3.2 ± 0.7 |
| LB | 4 | 3 | 4 | 3 | 3 | 3 | 4 | 4 | 4 | 3 | 4 | 4 | 3.6 ± 0.5 |
| Mean Rank | | | 26.0 | | | | | | 34.9 | | | | |

Kruskal Wallis statistic 4.304

P value chi-squared approximation = 0.0380

Parametric Analysis of Variance Applied to ranks for the disease scores

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Between | 1 | 11.88 | 11.88 | 4.56* | 0.0369 |
| Within | 58 | 1510 | 260.3 | | |
| Total | 59 | 1629 | | | |

Tabular F value = 4.02

Appendix Table 11: Pathogenicity and cross-infection among isolates of C. canescens from leguminous hosts (Text Table 4.2.3).

| C. canescens Isolate | Disease reaction score* on legume species/cultivars | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | COWPEA | | LIMA BEAN | | MUNG BEAN | | BAMBARRA | | GROUNDNUT | | SOYA BEAN | PIGEON PEA | WINGED BEAN |
| | Amantin | Ife Br | White | Brown | MG50-10A | CES-2F1 | Mbawa | CV18 | TMV2 | Chitemba | | | |
| TZ (Cowpea) | 4.6 (14)** | 4.3 (16) | 4.3 (20) | 4.5 (20) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3.8 (28) | 3.4 (20) |
| LB (Lima bean) | 3.6 (20) | 3.1 (22) | 4.0 (20) | 3.4 (22) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3.4 (28) | 3.6 (28) |
| VR (Mung bean) | 3.1 (22) | 3.7 (20) | 3.0 (21) | 2.8 (24) | 4.6 (14) | 4.0 (18) | 3.7 (28) | 3.9 (26) | 2.7 (28) | 3.0 (28) | 0 | 2.0 (28) | 3.3 (30) |
| VS (Bambarra) | 3.7 (20) | 3.1 (20) | 3.8 (22) | 4.0 (20) | 4.1 (18) | 4.0 (20) | 4.5 (18) | 5.0 (16) | 0 | 0 | 0 | 3.0 (28) | 3.0 (28) |
| AH (Groundnut) | 4.8 (14) | 4.2 (14) | 2.9 (30) | 3.1 (30) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

*Values are means of the scores for lesion type/numbers for 9 trifoliate leaves for each spp./cultivar in which 0 = No lesions observed; 1 = Few 1-20 Chlorotic lesions; 2 = 21 50 chlorotic lesions; 3 = many chlorotic to 'green island' lesions; 4 = Few necrotic lesions; and 5 = Numerous large spreading lesions.

Based on the disease scores, infection types were classified as follows: 0 = Resistant; 1-3 Moderately susceptible (MS) and 4-5 = susceptible (S).

** Values in parentheses are the incubation periods (number of days after which first symptoms appeared).

## 8. NOTES ON OTHER PATHOGENS

### A. Studies on the growth and pathogenicity of an isolate of Corynespora cassiicola associated with lesions caused by Cercospora canescens on field infected leaves of cowpea.

#### 1. Introduction

During the course of isolation and culture of the pathogens associated with leaf spots on cowpea, believed to be lesions caused by Cercospora spp., a crude suspension of infected leaves collected from experimental plots at the Agricultural Research Station, Kpong, Ghana, was used as inoculum to inoculate cowpea seedlings in the glasshouse at Silwood Park, to facilitate better isolations.

Irregular to angular shaped lesions, which developed a straw-coloured centre with reddish-brown margins were observed on cowpea cultivar Adua Ayera, three weeks after inoculation. The lesions partly resembled coalescing lesions caused by C. canescens (Plate 27A). Microscopic examination of such lesions incubated in moist chambers, showed the presence of septate, brownish conidiophores on which were borne catenate, cylindrical, subhyaline to pale olivaceous conidia (Plate 28). The fungus was identified as Corynespora cassiicola based on the descriptions by Ellis and Holliday (1971) and Wei (1950).

C. cassiicola has been described as a widespread plurivorous fungus, which causes leaf spot diseases on a

## PLATE 27

Symptoms of Corynespora cassiicola infected cowpea plants inoculated in the glasshouse at Silwood Park.

A. Irregular to angular shaped lesions with straw-coloured centres produced on cowpea cv. Adua Ayers, inoculated with a crude suspension in water, of field infected leaves believed to be Cercospora lesions.

B. Lesions on cowpea cv. Adua Ayera inoculated with conidia of cowpea isolate (Vu) of C. cassiicola from a pure culture on V-8 juice agar.

C. Minute pin-point lesions on cowpea cv. IT 82D 885 inoculated with a pure culture of cowpea isolate (Vu) of C. cassiicola.

## PLATE 28

Morphology of conidiophores and conidia of C. cassiicola (Isolate Vu) produced on infected leaf petioles of cowpea cv. Adua Ayera incubated in a moist chamber at 25 $\pm$ 2$^{o}$C.

A. Numerous dark brown conidiophores (Cp) arising singly from swollen basal cells, bearing a chain of 2-4 olivaceous brown conidia.

B. Conidiophores showing septa and proliferation X400

C. A single, cylindrical and slightly curves conidium (c) showing a truncate based and dark hilum at the point of attachment to the conidiophore (cp) x 600.

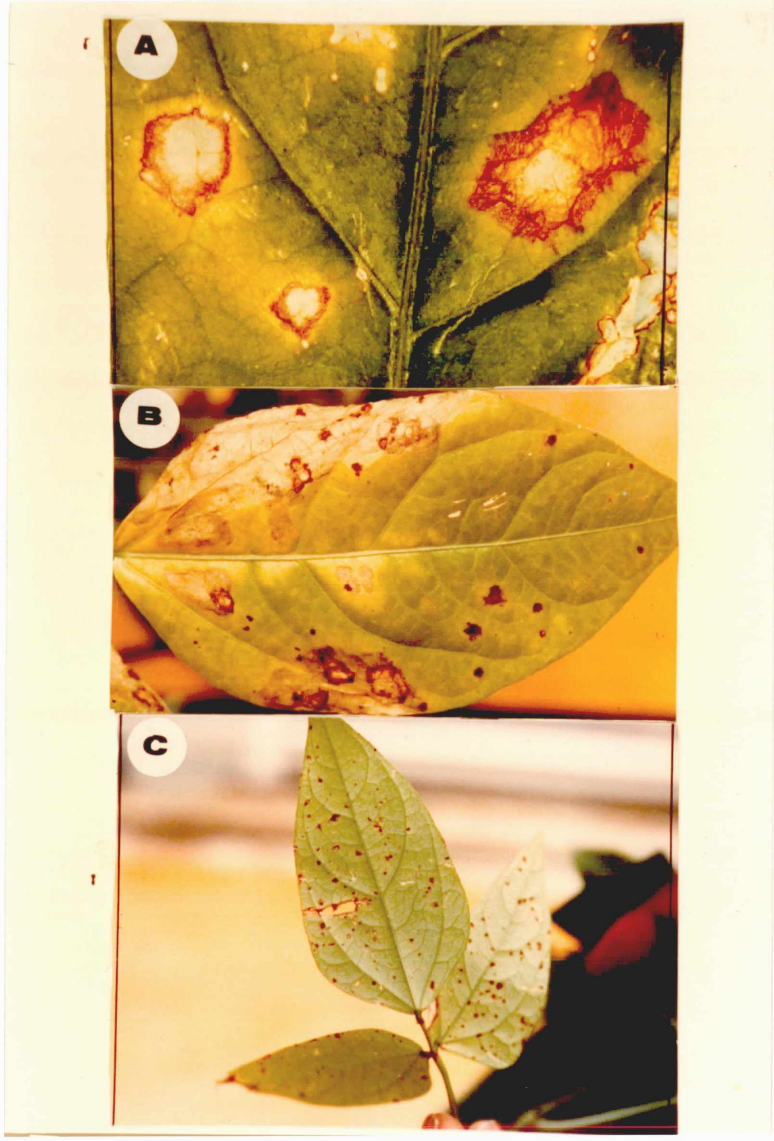


Plate 27

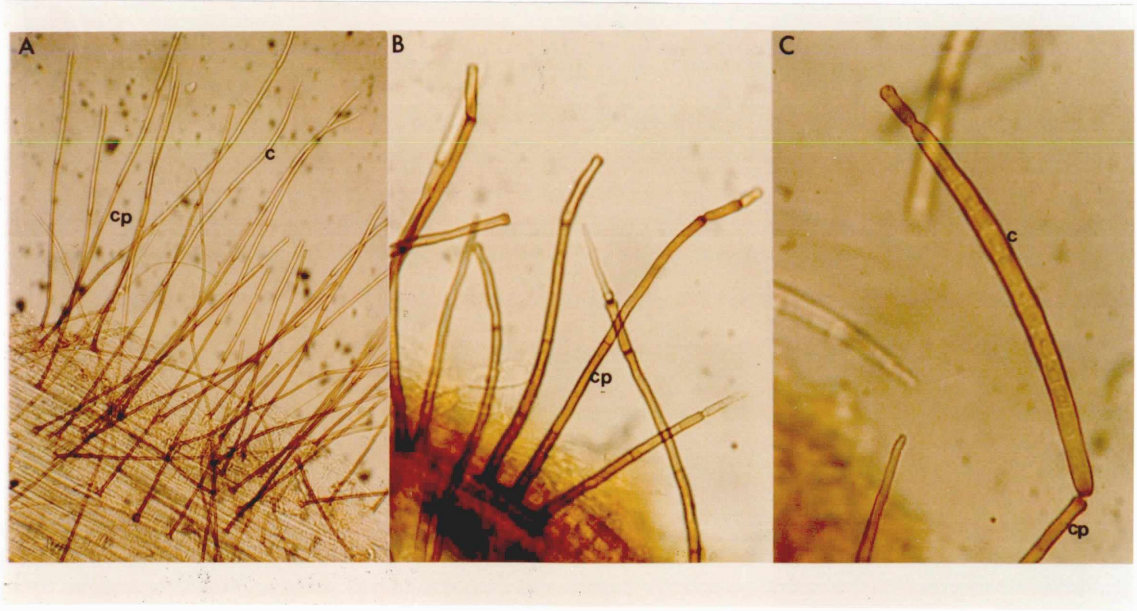


Plate 28

great number of economically important plants including cowpea, soy bean, lima bean, cotton, aubergines, tomato and cucumber (Allen, 1983; Holliday, 1980). The leaf spots caused by C. cassiicola on many of the above hosts, has commonly been called target spot to describe the typical concentric banding of the lesions. On cowpea, several varieties have been observed to develop a high incidence of target spot lesions as the crop is maturing. The early stages of C. cassiicola lesions begin as dark-reddish brown circular spots 1-2mm diameter, which expand with marked concentric banding to become target spots. The dark reddish brown lesions in the early stages of infection of cowpea, can therefore be confused with lesions caused by Cercospora canescens (Vakili, 1977; Williams, 1975).

The lesions observed on glasshouse infected cowpea cultivar Adua Ayera were however atypical of the concentric zonation associated with C. cassiicola lesions on field infected leaves, and resembled coalescing lesions of severe reactions to infection by C. canescens. The long cylindrical conidia also resembled those of Cercospora spp. but were distinguished from the latter by being borne in chains on conidiophores which were erect with successive cylindrical proliferations in contrast to the fasciculate conidiophores of C. canescens that arise from a stroma (Plate 28B).

In view of the apparent confusion with the lesions

observed on glasshouse infected cowpea plants and those of Cercospora sp., the cultural characteristics of the fungus was compared with two isolates of C. canescens and an isolate of P. cruenta, and its relationship with other isolates of C. cassiicola causing diseases on other crops, tomato (Lycopersicon esculentum) rubber (Hevea brasiliensis) and oil palm (Elaeis guinensis) was determined.

## 2. MATERIALS AND METHODS

### Isolates of C. cassiicola

The cowpea isolate was obtained from glasshouse infected cowpea (cv. Adua Ayera) leaves by picking single conidia from sporulating lesions kept in a moist chamber at 25 ± 2°C. The isolates from tomato (IMI 56007), Oilpalm (IMI 61173) and rubber (ex. Sri Lanka) were obtained from Dr. S. Liyanage, who worked with isolates of C. cassiicola from rubber during 1985-1986 in the Plant Pathology section at Silwood Park.

### Cultural Studies

Growth and cultural characteristics of the cowpea isolate of C. cassiicola was compared on potato dextrose agar with two isolates (IMI 185 292 and IMI 185 306) of C. canescens and an isolate, (Pckp) of Pseudocercospora cruenta. Radial growth and other cultural characteristics of the cowpea isolate of C. cassiicola (VU) were compared with those of isolates of the fungus from tomato (Le) rubber (Hb) and Oil palm (Eg) by placing 5mm mycelium-agar

disks cut from 1 week-old cultures of the isolates growing on PDA, in the centre of 9.0cm Petri dishes containing 20cm$^3$ of potato dextrose agar. There were five replicates for each medium and the plates were incubated in the dark at 25$^o$C. Colony diameters of the isolates were measured after 10 days. Sporulation of the isolates was compared on potato dextrose agar (PDA) and V-8 juice agar (V-8A) by determining the number of spores produced per unit area of the colony. Five disks of agar-mycelium (5mm diameter) were cut randomly from each Petri dish and placed in McCartney bottles containing 10cm$^3$ of sterile distilled water; the bottles were shaken on a rotamixer for 3-5 minutes and spore counts were made by taking five sub-samples from each of two plates per medium. The dimensions of 25 conidia of each isolate produced on V-8 juice agar were measured with an ocular micrometer.

Pathogenicity of isolates. The pathogenicity of the isolates was compared on four cultivars of cowpea, two varieties of soyabean and one variety each of tomato, aubergine (Black Beauty) and Okra. Inocula for pathogenicity tests were obtained by washing ten-day-old cultures of the isolates grown on V-8 juice agar with sterile distilled water containing 0.01% Tween 20. The mycelium-spore suspension was filtered through two layers of cheese cloth and the concentration of the conidia adjusted to 5 x 10$^4$ spores/ml. In each inoculation series, four plants, (4 to 6 weeks old) of each species were sprayed with

approximately $50cm^3$ of inoculum per plant and two plants of each species were sprayed with 0.01% Tween 20 in sterile distilled water. The inoculated plants and the controls were incubated in a mist chamber for 48h and subsequently transferred to the glasshouse bench maintained at 25-30°C (June - August 1986).

## 3. RESULTS

Cultural characteristics. The cowpea isolate of Corynespora cassiicola (Vu) formed olivaceous black colonies which almost covered the Petri plates 10 days after incubation on all the three media tested in contrast to olivaceous grey colonies with effuse mycelium formed by the isolates of Cercospora canescens, which also produced a pink-purple pigmentation on potato dextrose carrot agar (Plate 29A). The colony formed by the cowpea isolate of C. cassiicola also differed considerably from the slow growing compact colony formed by the cowpea isolate of Pseudocercospora cruenta (Pc) on potato dextrose agar (Plate 29B). A comparison of the cultural characteristics of the isolates of Corynespora cassiicola from cowpea (Vu), rubber (Hb), tomato (Le) and oil palm (Eg), (Plate 29C) shows that the isolates differed in their rate of growth and other colony characteristics (Table 1). The cowpea isolate (Vu) formed colonies with a light brown centre bordered by an area of white mycelium compared with the other three isolates which formed colonies with very little aerial mycelium but dark sporulating colonies on V-8 juice agar. Radial growth of

**PLATE 29**

A. Comparison of colony characteristics of an isolate of Corynespora cassiicola (Cory) and two isolates of Cercospora canescens (IMI 306 and IMI 292) from lima bean on potato dextrose agar (PDA), cowpea leaf decoction agar (CLDA) and potato dextrose carrot agar (PDCA).

B. Comparison of colony characteristics of an isolate of C. cassiicola (Cory) with two isolates of C. canescens (IMI 292 and IMI 306) and an isolate of Pseudocercospora cruenta (PC) on potato dextrose agar (PDA).

C. Comparison of isolates of C. cassiicola from rubber (Hb), oil palm (Eg), cowpea (Vu) and tomato (Le) on V-8 juice agar at 25$^{o}$C.

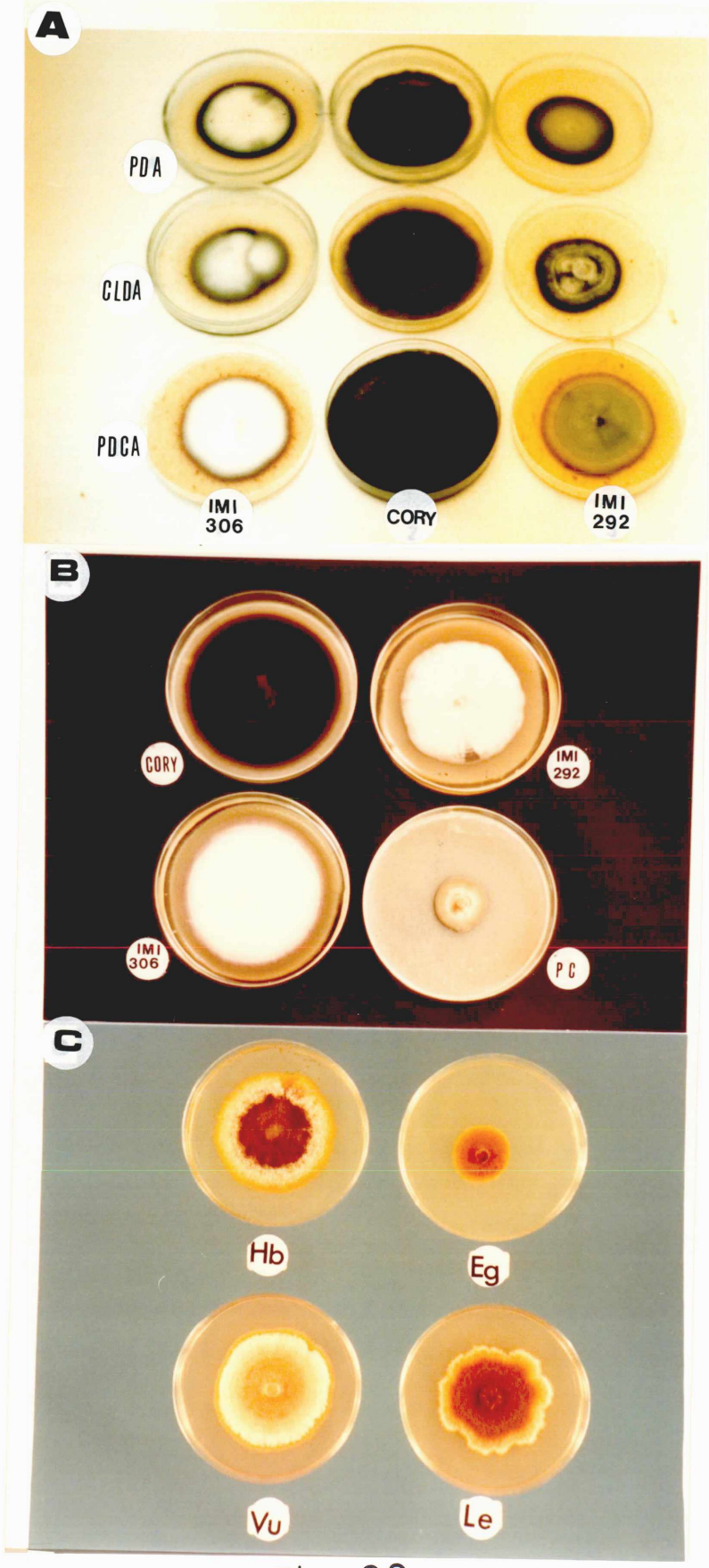
A
PDA
CLDA
PDCA
IMI 306
CORY
IMI 292
B
CORY
IMI 292
IMI 306
P C
C
Hb
Eg
Vu
Le

Plate29

Table 8.1 Comparisons of the growth, sporulation and dimensions of the conidia of isolates of Corynespora cassiicola from four hosts.

| Isolate (Code) | Colony diameter ± sd after 10 days on PDA mm* | Sporulation $x10^4$ spores $ml^{-}$ ** | | | Dimensions of conidia from V-8A*** µm ± SD | |
|---|---|---|---|---|---|---|
| | | PDA | V-8A | Mean of isolate | Length | Width |
| Cowpea (Vu) | 63.1 ± 2.7 | 3.74 | 4.72 | 4.23 c | 51 ± 0.7 (20 - 70) | 7.6 ± 0.5 (6.25 - 8.7) |
| Rubber (Hb) | 43.1 ± 2.9 | 4.84 | 7.48 | 6.16 b | 132 ± 1.3 (40 - 220) | 11.3 ± 1.4 (10 - 13.8) |
| Tomato (Le) | 29.2 ± 2.2 | 4.74 | 6.78 | 5.76 b | 71 ± 1.1 (35 - 122.5) | 6.4 ± 1 (5 - 7.5) |
| Oil Palm (Eg) | 14.9 ± 0.7 | 6.78 | 8.16 | 7.74 a | 54 ± 0.6 (40 - 65) | 7.2 ± 0.5 (6.25 - 7) |

* Mean of 10 measurements in two experiments ± standard deviation.
** Counts based on five sub-samples in each of two plates, means followed by a a common letter(s) are not significantly different ($P < 0.05$) Duncan's multiple range test.
*** Based on measurements of 25 conidia for each isolate Values in parentheses indicate the range of the dimensions of the conidia).

the cowpea (Vu) and rubber (Hb) isolates were similar and slightly faster than the isolate from tomato (Le), which formed colonies with an irregular wavy edge. The oil palm isolate (Eg) grew the least with a more compact colony, the mycelium being mostly immersed. All the four isolates sporulated on both potato dextrose agar (PDA) and V-8 juice agar (V-8A) incubated under alternate light near UV and dark periods, as well as in continuous darkness, but the oil palm isolate (Eg), produced the greatest number of spores (7.74 x $10^4$ spores/ml), and sporulation of the isolates was slightly better on V-8 juice agar (V-8A) than potato dextrose agar (PDA). The cowpea isolate (Vu) produced the least number of conidia on both media (4.23 x $10^4$ spores/ml). The dimensions of the conidia produced on V-8 juice agar by the isolates were very variable, both within an isolate and between the different isolates. The isolate from rubber (Hb) formed significantly longer and wider conidia (mean length of 132 x 11.3um wide) compared to the other three isolates, and the conidia produced by the tomato isolate (Le) were also slightly longer (71µm) than the conidia produced by the cowpea (Vu) and oil palm (Eg) isolates which did not differ significantly (a mean of 51 and 54 µm respectively). The widths of the conidia of the isolates from cowpea tomato and oil palm were also similar in range.

The dimensions of the conidia produced by the cowpea isolate (Vu) on infected leaves and petioles incubated in moist chambers were however considerably longer and more

cylindrical averaging 23$^{6}$ x 6.25 µm (Plate 30, Figures 7 and 8).

Pathogenicity of Isolates. Three to five days after incubation small reddish brown circular spots were apparent on all the four cowpea cultivars inoculated wth the cowpea isolate (Vu). Two weeks after inoculation, the lesions on the cowpea cultivars Adua Ayera and Caloona had developed into large coalescing light brown centred lesions bordered by a reddish brown margin (Plate 27B). The lesions on cowpea cultivar Amantin were few enlarging to about 2-3mm; lesions on cowpea cv. IT 82D 885 were more numerous but remained small (Plate 27C). No lesions were visible on soyabean and tomato cultivars inoculated with the cowpea isolate, but chlorotic lesions which later developed into irregular brown spots were formed on aubergine and okra. The isolate of C. cassiicola from tomato (Le) produced many brown lesions about 2-3mm diameter on tomato leaves and a few pinpoint lesions on cowpea and aubergine leaves. The isolates from rubber (Hb) and oil palm (Eg) were non-pathogenic to any of the plants tested (Table 2).

## 4. DISCUSSON

The results of the study confirmed the observation by other research work that there is a great deal of variation among isolates of Corynespora cassiicola, the morphology and dimensions of conidia being apparently affected by the host (Ellis, 1957; Ellis and Holliday, 1971; Wei, 1950). The

**PLATE 30**

Photomicrographs showing the variation in the morphology of conidia of isolates of Corynespora cassiicola from oil palm (Eg), tomato (Le), rubber (Hb) and cowpea (Vu).

Figures 1, 2, 3, 4, 5 and 6 are conidia from culture on V-8 juice agar. Figs. 7 and 8 are from infected leaves incubated in a moist chamber.

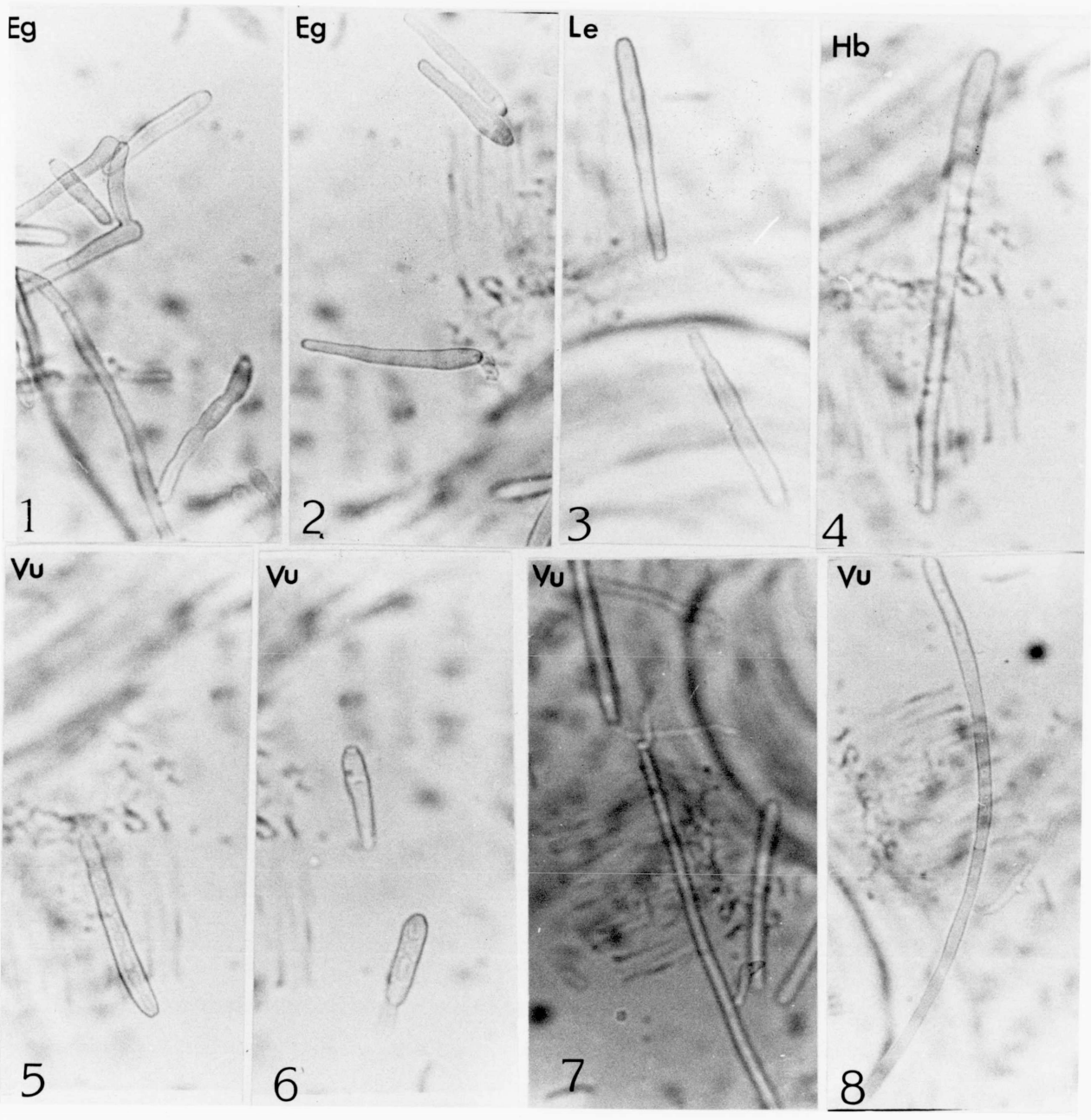


Plate 30

Table 8.2

Pathogenicity of isolates of Corynespora cassiicola from four hosts on five selected plant species.

| Plant species/ cultivar | Virulence* (disease ratings of isolates) | | | |
|---|---|---|---|---|
| | Cowpea (Vu) | Rubber (Hb) | Tomato (Le) | Oil palm (Eg) |
| 1. Cowpea | | | | |
| Adua Ayera | +++ | - | + | - |
| Amantin | ++ | - | + | - |
| Caloona | +++ | - | + | - |
| IT 82D 885 | + | - | + | - |
| 2. Soy bean | | | | |
| Bragg | - | - | - | - |
| S.J.S. | - | - | - | - |
| 3. Tomato | | | | |
| (Wosowoso) | - | - | - | - |
| 4. Aubergine | | | | |
| (Black Beauty) | + | - | - | - |
| 5. Okra | | | | |
| (Local ex Kpong Ghana) | + | - | - | - |

* Virulence and the reaction of plants to the isolates based on a scale:

- \- non pathogenic (no visible symptoms)
- \+ few to many pin-point lesions about 1mm
- ++ moderate lesion size 2-3mm
- +++ Lesions 4-10mm in diameter, with some coalescing to form large necrotic patches.

conidia produced on the same medium by isolates of C. cassiicola from cowpea (Vu) and oil palm (Eg) were particularly variable in shape.

The pathogenicity of isolates of C. cassiicola from some host-species are reported to be infective on botanically unrelated species (Onesirosan et al.1974; Sobers, 1966; Stone and Jones, 1960); however, it has also been noted that host-specific strains may be common (Jones, 1961; Onesirasan et al. 1974; Olive et al. 1945).

Olive et al. (1945) distinguished two races of C. cassiicola based on differential responses of cowpea and soy bean to infection by the fungus. Race 1 caused severe infection of cowpea but only light spotting of soy bean whereas race 2 caused only light spotting of both. Stone and Jones (1960) reported that their isolates from sesame (Sesamum indicum) and soybean did not fit the description of either race. Jones (1961) however, found no differences in isolates from cotton, sesame and cowpea. Spencer and Walters (1962, 1969) also reported that symptoms produced by their cowpea and soy bean isolates corresponded with the previously reported symptoms for races 1 and 2 thus confirming the observations by Olive et al. (1945). Olive et al. (1945) and Spencer and Walters (1969) both recorded that isolates of C. cassiicola from cowpea produced on cowpea small reddish-purple circular spots that enlarged to 5mm with many developing a target effect with concentric

zonation. Olive et al. (1945) also noted that although mature spots of C cassiicola infection on cowpea leaves almost always have a pronounced zonation consisting of a varying number of reddish brown rings against a lighter background, the leaf spots appearing on glasshouse inoculated plants did not have the concentric zonation, associated with lesions on field infected leaves. The results obtained with the cowpea isolate used in the present study is therefore similar to those reported by Olive et al. (1945). However, the virulence of the isolate appears to be different, in that it did not cause any visible lesions on the two cultivars of soyabean tested, and does not therefore fit any of the races suggested. Nevertheless, Onesirosan et al. (1974) also observed that a cowpea isolate of C. cassiicola was not pathogenic to cowpea and soybean but was weakly virulent on aubergine (Solanum melongena) and moderately virulent on cotton. Sobers (1968) on the other hand noted that an isolate from azalea was moderately pathogenic to soybean leaves and two varieties of cowpea (Black Eye and White Cross). Although different cultivars and/or varieties of cowpea and soybean have been used in the research cited above, there appears to be variation in the results of the pathogenicity tests. Further experiments involving many more isolates from cowpea and soybean and of a standard set of cultivars are needed to establish the relationship of the isolate found causing the irregular to angular spots on cowpea inoculated in the glasshouse. The effects of the incubation environment under the glasshouse

conditions however may be a source of the observed differences in symptom expression. It is therefore apparent that the leaf samples collected from the field in Ghana, were infected with both C. canescens and C. cassiicola.

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B. Notes on a Mycosphaerella species associated with lesions caused by Cercospora canescens infection of bambarra groundnut (Voandzeia subterranea).

Microscopic examination of leaf samples of bambarra groundnut obtained from Msekera Regional Research Station, Chipata, Zambia, revealed that numerous black bodies resembling spermagonia were interspersed and associated with conidiophores, and arose from old conidial stromata (Plate 31, Figs. 1, 2 and 3). When such fructifications were squashed in a drop of lactophenol cotton-blue, numerous spermatia-like spores measuring 1.2 - 2µm long were released (Plate 31, Fig. 6). The appearance of these structures was similar to the spermagonia observed on leaf samples of cowpea infected with Pseudocercospora cruenta (Plate 5, Fig. 6 and 7). Further examination of leaf pieces cleared in lactophenol, also showed that other perithecial-like bodies with erumpent short ostioles were present and associated with the spermagonia (Plate 31, Fig. 2 and 7). Samples of the perithecia squashed under a cover slip on a microscope slide released 2-celled ascospores measuring 10.5 - 19.5µm (Plate 31, Fig. 8). The number of ascospores and their shape suggested that they were of a Mycosphaerella species. The spore stages observed, also resembled the descriptions of the Mycosphaerella perfect stages of some Cercospora leaf spots (Latham, 1934; Jenkins, 1939). Although a Mycosphaerella perfect state has been described for Cercospora leaf spots on cowpea, (M. cruenta), groundnuts (M. arachidis and M. berkeleyii) banana (M. musicola and

**PLATE 31**

Photomicrographs of cleared bambarra groundnut leaves showing fructifications of Cercospora and Mycosphaerella sp.

Fig. 1. Fascicles of conidiophores (cp) spermagonium (sp) and perithicium (pe) ? growing on leaf surface of bambarra groundnut.

Fig. 2. Section of Fig. 1 magnified to show the spermagonial (left) and perithecium (right) fructifications.

Fig. 3. Portion of cleared leaf piece of bambarra groundnut with numerous spermagonia and a fascicle of conidiophores (arrowed, but slightly out of focus).

Fig. 4. Conidiophores arising from a stroma.

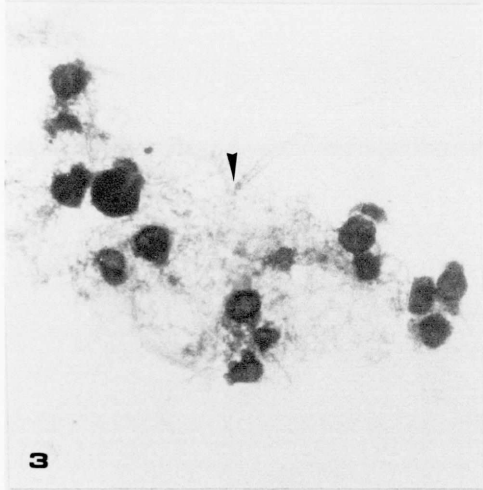
Fig. 5. Development of a spermagonium.

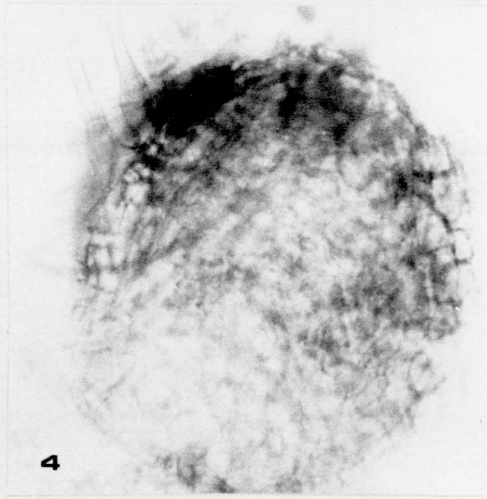
Fig. 6. A spermagonium discharging spermatia after having been squashed under a cover slip.

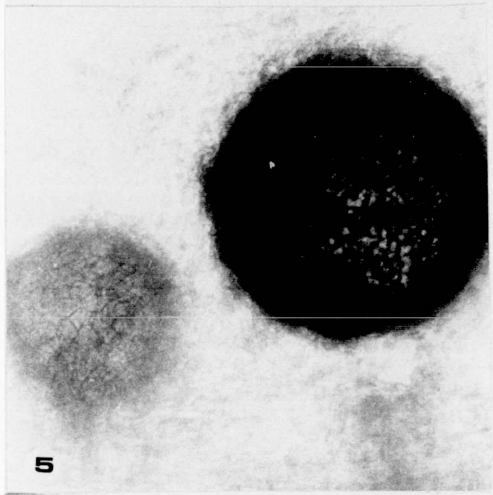
Fig. 7. Development of perithecia.

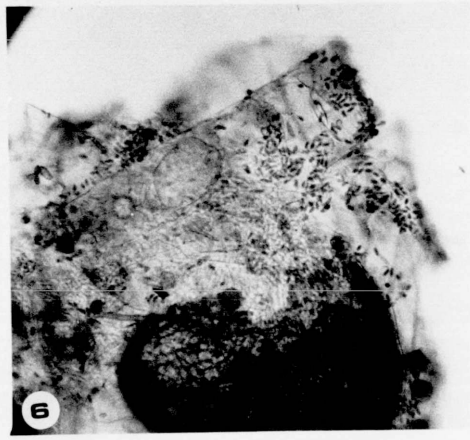
Fig. 8. Ascospores released from a squashed perithecium.







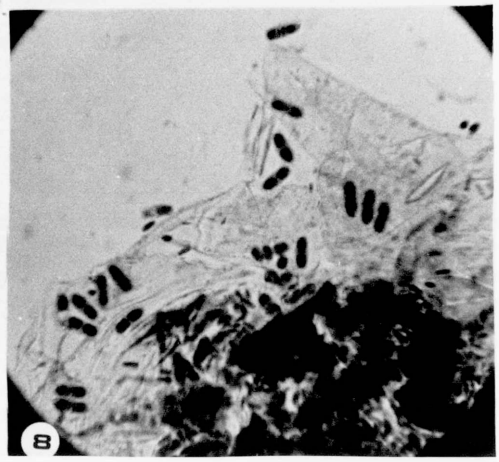



Plate31

cassava M. henningsii), it is now apparent that the species referred to above are not 'true Cercospora' sensu Deighton (1973, 1976).

Most of the species for which a perfect state has been described, have olivaceous to brown conidia and do not have a prominent scar at the hilum in correspondence with the scar on the conidiophores as observed for the true Cercospora (see Section 2.2.1). These species have therefore been transferred to the newly created genera of Pseudocercospora and Paracercospora (Deighton, 1976, 1979).

It therefore appears that no perfect state has been described for the species of Cercospora with hyaline acicular conidia, C. apii and the related species. However, Laterell and Rossi (1977) observed a Mycosphaerella species in overwintered field specimens of maize infected by Cercospora zea-maydis and later reported on the evidence for a genetic relationship between the two stages. Cercospora zea-maydis, the causal fungus of grey leaf spot of maize, has been referred to as representing the section of the genus Cercospora having broad and large hyaline conidia, 70-180 x 5-6µm (Latterel and Rossi, 1983). Latterel and Rossi (1977) noted that spermogonia bearing spermatia, develop in mature lesions from stromatic cells in each stomatal cavity three days of incubation in a moist chambers. The similarity between the fructifications on field infected leaves of bambarra groundnut and those that have been

described for the perfect states of Cercospora leaf spots on cowpea (Latham,1934)., banana (Stover, 1969), groundnuts (Jenkins, 1938, 1939) and maize (Latterel and Rossi, 1977), prompted the need for further examinations of leaf material of bambarra groundnut infected with Cercospora canescens, with a view to identifying the species and further to ascertain the probable connection between the spermogonia and perithecial structures. In view of the fact that a majority of Mycosphaerella species are thought to be saprobic, it was necessary to isolate and germinate single ascospores to compare the cultures from these with those of the Cercospora state. This was not possible, because the leaf samples on which the observations had been made had been treated with hot lactophenol, thus killing the spores. In the absence of further work on the probable connection between the spore types observed, they have been illustrated in Plate 31, and it is being proposed, with caution, that there might be a link between the two states, based on the observations made by Jenkins (1938, 1939) for the perfect state of Cercospora species on groundnuts.

Allen (1983) has pointed out that although a Mycosphaerella perfect state has been described for the cowpea and groundnut leaf spot fungi (Latham, 1934; Jenkins, 1938), they have apparently never been found under African field conditions in Tanzania (Hemingway, 1955), Zambia (Smartt, 1961) and Nigeria (Gibbons, 1966; Fowler, 1970b). Nevertheless, the observation of spermogonia scattered in

and near lesions on cowpea leaves collected from the field at the Agricultural Research Station, Kpong, Ghana (see Section 2, Plate 5) suggests that a perithecial state may exist for Cercospora leaf spot fungi under African conditions but are rare, and that their production might be affected by environmental conditions. Jenkins (1938, 1939) found on groundnuts in Georgia, USA that perithecial stages of Cercospora leaf spot on groundnuts, are initiated only when sufficient rain falls during spermogonial discharges in the autumn, and that temperature plays an important part in their formation. The observations made in the present study, although not confirmed, suggests that there is the need to explore further the perfect states for the Cercospora species causing leaf spots in the tropics and to establish the role that they play in the disease cycle.

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