STUDIES ON PIGEONPEA LEAFSPOT: ETIOLOGY AND THE EFFECT OF PLANT AGE AND LEAF POSITION ON SUSCEPTIBILITY OF PIGEONPEA (*Cajanus cajan*) TO THE LEAFSPOT PATHOGEN (*Mycovellosiella cajani*)

BY

ESTHER WANDIA NJOYA

A thesis submitted in partial fulfilment of requirements for the degree of

MASTER OF SCIENCE

IN

PLANT PATHOLOGY

College of Agriculture and Veterinary Science
Department of Crop Science
University of Nairobi
Kenya

1991

DECLARATION

This thesis is my original work and has not been presented for a degree in any other university

Signed_____ Date 18/4/1991
(NJOYA ESTHER WANDIA)

This thesis has been submitted for examination with our approval as University supervisors.

Signed_____ Date 18/4/1991
(DR. (Mrs.) A.W. MWANG'OMBE)

Signed_____ Date 18/4/91
(DR. P.M. KIMANI)

Signed_____ Date 18/4/91
(DR. MANISHA SHANKAR)

DEDICATION

To my parents for their love and care and in memory of my great grandmother (Mwarari).

TABLE OF CONTENT

LIST OF TABLES

LIST OF PLATES

LIST OF FIGURES

LIST OF ABBREVIATIONS

1. Cv(s) - Cultivar(s)
2. PLDA - Pigeonpea leaf decoction agar
3. CDA - Carrot leaf decoction agar
4. PDA - Potato dextrose agar
5. PCA - Potato carrot agar
6. CA - Carrot agar
7. PMA - Pigeonpea meal agar
8. a.i. - Active ingredient

LIST OF APPENDICES

ACKNOWLEDGEMENTS

This study was financially supported by International Development Research Centre (IDRC) through pigeonpea project and the University of Nairobi. To them I am sincerely grateful.

I would like to thank Dr. P.M. Kimani for his encouragement, helpful suggestions and speed in reading through my draft and also Dr. (Mrs) A.W. Mwang'ombe and Dr. M. Shankar for overall supervision of this work and their encouragement and guidance during laboratory, field work and thesis write up.

I also extended my gratitude to Mr. Kimani Kibuchi for his help in photography, Mr. Gathuma and Mr. Musyimi, pigeonpea project staff for their co-operation during my work.

Finally, I wish to convey my appreciation and gratitude to my sister Mwende and Ms. Jane N. Mbugua for speed and accuracy in typing my work.

ABSTRACT

In recent years incidence of *Mycovellosiella* leafspot damage has increased considerably especially during the wetter seasons in the pigeonpea growing areas. Regular occurrence of the disease has been noted both in the farmers fields and experimental plots in Machakos, Kitui, lower semi-arid areas of Kiambu, Murang'a and Embu districts since 1984. Results on pathogenicity test showed that the disease is incited by *Mycovellosiella cajani* (Henn) Rangel ex Trotter syn. *Cercospora cajani* (Henn = *Vellosiella cajani* (Rangel).

Investigations on *M. cajani* with respect to the effects of culture media, temperature and light regimes on sporulation were conducted on the isolate from Kabete. Factors affecting conidial germination, effect of plant age and leaf position on the infection level of pigeonpea genotypes by *M. cajani* and the response of eight pigeonpea genotypes inoculated with *M. cajani* at seedling stage and at mature plant stage were studied. Mature plants were inoculated both in the glasshouse and in the field.

The results showed that *Mycovellosiella cajani* has a fastidious nutritional requirement for growth than for sporulation. Selective subculturing produced colonies that are pure for sporulating character. Pigeonpea leaf decoction agar medium incubated at room temperature (20 - 24^{o}C) at 24 hour light regime supported growth of many small colonies and gave abundant sporulation 10 days after plate incubation. Sporulation was noted under all environmental factors tested as long as colony growth occurred. *M. cajani* sporulated best in pigeonpea leaf decoction agar. The fungus showed moderate sporulation in carrot leaf decoction agar and PDA. The lowest sporulation was noted in potato carrot agar, carrot agar and pigeonpea meal agar. Highest sporulation occurred 14

days after inoculating Pigeonpea leaf decoction agar plates with conidial concentration of 2 x 10^6 conidia ml^{-1}. There was no colony growth observed in cultures incubated at 5, 10, 30 and 35°C. Colony growth and sporulation occurred at 15°, 20° and 25°C with optimum at 20°C.

M. cajani sporulated both in acidic (pH 4) and alkaline (pH 10) medium. The highest and the lowest sporulation level occurred in culture plates adjusted to pH 5 and pH 10 respectively. Conidial production occurred in all the three light regime tested with the highest in 24 hours light regime 10 days after incubation. Conidia of *M. cajani* germinated at temperatures 15°, 20°, 25° nd 30°C with optimum germination at 25°C in concentration 2 x 10^5 conidia ml^{-1}. Conidial germination was lowest in concentration 2 x 10^4 while there was no significant difference ($P < 0.05$) in germination among concentrations 2 x 10^5, 5x10^4 and 2x10^6 conidia ml^{-1}. Extracts of pigeonpea genotypes either suppressed or promoted conidial germination. Extract from 15 days old plants of cvs ICPL 295, KB 91/1 and 120 day old plants of cv. Katheka significantly suppressed conidial germination when compared to the control. None of the extracts tested promoted conidial germination significantly higher than the control.

M. cajani produced 1 or 2 germ tube that emerged from one conidium and penetrated host tissue passively through the stomata. Koch's postulates were verified to the effect that *M. cajani* was the causal agent of *Mycovellosiella* leafspot of pigeonpea.

The fungus *M. cajani* attacked all the plant parts above the ground except flowers. The fungus caused severe leafspotting and defoliation of pigeonpea genotypes especially in the field. None of the pigeonpea genotypes was immune to *Mycovellosiella* leafspot although disease progress among the genotypes differed. In the glasshouse inoculated plant, disease progress was slow in cv. NPP 670 and high in cv. ICPL 295 while in

the field, disease progress in cv. NPP 670 was much higher than among the other pigeonpea genotypes tested.

Inoculum concentration affected the rate at which disease severity increased on pigeonpea leaves. The rate of increase in disease severity was higher when plants were inoculated with higher level of inoculum concentration (2×10^6 or 2×10^5 conidia ml^{-1}).

Fifty percent disease severity was rarely reached both in the field and the glasshouse. The highest disease severity was obtained on leaves from the lower position of the canopy. In glasshouse inoculated plants, there were no differences in disease severity between the leaves on the upper and the intermediate position of the canopy. While among the genotypes planted in the field disease severity was lower in the leaves on the upper part of the plant canopy as compared to the severity on the intermediate and lower part.

Mycovellosiella leafspot was more severe at Kabete than at Kiboko. There was no significant correlation between seedling susceptibility and adult plant susceptibility.

INTRODUCTION

Pigeonpea (*Cajanus cajan* (L) Millsp. syn. *Cajanus indicus* (spreng)) is the most important grain legume in marginal rainfall areas of Kenya where it is grown on approximately 115,000 ha annually (Anon, 1975). The major growing areas in Kenya are in the Eastern region comprising Machakos, Kitui, and Embu districts and in Central, Rift Valley and Coast Provinces to a lesser extent. It is the third most important legume in Kenya after beans (*Phaseolus vulgaris* (L)] and cowpeas (*Vigna unguiculata* (L) Walp) (Ngugi, 1979). Due to its drought tolerance, pigeonpea gives reasonable grain yield when other crops fail due to drought in the semi-arid areas which constitute about 80 percent of the total land mass in Kenya.

Although pigeonpea is widely grown in the semi-arid areas of Kenya, yields are low averaging 450 to 670 kg ha^{-1}. Research workers have projected that yield could be improved upto 1120 kg ha^{-1}. It has been shown that under research conditions, a yield of between 2637 to 4250 kg ha^{-1} can be realized (Onim, 1983; Kimani, 1987). In Australia, yields as high as 7500 kg ha^{-1} have been recorded under research conditions (Akinola and Whiteman, 1972). The discrepancy between potential and realized yield in farmers' fields is enormous and has been partly attributed to damage by insect pests (Okeyo-Owuor and Khamala, 1980; Kimani, 1985) and lack of suitable varieties, labour shortage, social economic factors (Kimani, 1987).

(i) *Important diseases of pigeonpea*

The main diseases of pigeonpea reported in Kenya include *Fusarium* wilt, *Mycovellosiella* leafspot, *Alternaria* blight and stem canker (Kimani, 1987). Diseases cause considerable reduction in crop yield. *Fusarium* wilt has been reported to affect between 5 to 60 percent of the plants in the

farmers' fields in the semi-arid areas. Losses due to *Alternaria* and sterility mosaic in Kenya apparently are not extensive.

(ii) *Mycovellosiella leafspot*

Mycovellosiella leafspot caused by the fungus *Mycovellosiella cajani* (Henna) Rangel ex Trotter (Syn. *Cercospora cajani* Henn = *Vellosiella cajani* (Rangel) is the most severe leafspot of pigeonpea in areas with high rainfall or during wet seasons in the drier areas (Muller, 1950; Khan and Rachie, 1972). It spreads rapidly during a wet growing season and has been reported as one of the most important diseases of pigeonpea in East Africa. Although *Mycovellosiella* leafspot can be controlled by use of chemicals, the high cost of fungicides prohibit their use especially by the low income subsistence farmers in the semi-arid areas of Kenya. Development of resistant varieties may be the only reliable and cost effective method of controlling this disease.

(iii) *Breeding for disease resistance in pigeonpea*

Breeding for resistance to disease has been a major objective for pigeonpea breeding programme in Kenya. However, much of the effort has been devoted to identification of resistant genotypes to *Fusarium* wilt (Kimani, 1987). In recent years, incidence of leafspot damage has increased considerably especially during wetter seasons in pigeonpea growing areas. Little research work has been carried out to identify resistance to this disease in cultivars released recently, or the new early maturing breeding lines.

Numerous disease resistance-screening techniques have been developed for evaluation of legumes under controlled conditions, including laboratory and greenhouse methods. Such systems of evaluation have the advantage that they tend to be rapid and can usually be employed

throughout the year without reference to season. Implicit in such screening is the accurate control of environment, including the elimination of other pathogens and pests which often complicate field evaluation (Williams, 1974). However, laboratory and greenhouse resistance-screening techniques have not yet been developed for screening pigeonpea plants for resistance against *Mycovellosiella* leafspot.

Foliar symptoms for *Cercospora* leafspot typically develop late in the growth of the crop, often after flowering as reported for leafspots of cowpea (Verma and Patel, 1969; Schneider and Sinclair, 1975; Fery *et al*, 1977; Vakili, 1977) and mungbean (Mew *et al*, 1975). There is evidence that seedling resistance may in certain cowpea cultivars be accompanied by adult plant susceptibility to *Cercospora* leafspot (Schneider and Sinclair, 1975; Ekpo and Esuruoso, 1977). Pigeonpea foliar symptoms due to infection by *Mycovellosiella cajani* have been observed to occur during flowering and pod filling stage.

For effective screening of resistance against *Mycovellosiella* leafspot and to be able to develop laboratory and greenhouse resistance-screening technique that can accurately predict resistance in the field, information about the effect of plant age and leaf position on the susceptibility of pigeonpea plants to *Mycovellosiella* leafspot is necessary. Artificial epiphytotics are also necessary to permit selection for resistant types (Drolsom and Dickson, 1954). Little progress would be made in breeding for resistance to *Mycovellosiella* leafspot since methods for multiplying inoculum in culture have not been developed. One of the greatest difficulties is to obtain adequate quantities of conidia as inoculum for initiating artificial epiphytotics.

Studies on the factors influencing sporulation such as light, temperature and pH have been done on other *Cercospora* species (Diachun and Valleau, 1941; Berger and Hanson, 1963a; Vathakos and Walters, 1979).

Information about nutritional requirements and environmental factors for sporulation of *Mycovellosiella cajani* is lacking. Regular occurence of *Mycovellosiella* leafspot in both farmers' fields and experimental plots in Machakos, Kitui, lower semi arid areas of Kiambu, Murang'a and Embu districts since 1984 (Kimani, 1988) prompted these investigations. This study was undertaken to investigate the effect of environmental factors on sporulation of *Mycovellosiella cajani,* the effect of leaf position on the susceptibility of pigeonpea genotypes to *Mycovellosiella cajani,* the response of different genotypes of pigeonpea to *Mycovellosiella cajani* at different growth stages and to develop a rapid screening technique for assessing resistance to *Mycovellosiella cajani.*

2. LITERATURE REVIEW

2.1 History and geographical distribution of *Cercospora* leafspots

Cercospora leafspots are widely distributed and have been reported in Africa, North and South America, Asia and Europe. From the list compiled by Chupp (1953), the disease occurs on 148 different plant families. In Kenya it has been reported on 60 hosts (Natrass, 1961; Ondiek, 1973).

Singh, (1932), reported a species of *Cercospora* on *Cajanus indicus,* which differed morphologically from *C. cajani* Rangel. In Kenya, *Mycovellosiella* leafspot was first reported by Muller in 1950. It has also been reported to be the most severe leafspot of pigeonpea in areas with high rainfall or during wet growing season in the drier areas (Khan and Rachie, 1972; Rubaihayo and Onim, 1975; Onim and Rubaihayo, 1976; Onim, 1980). Recently, the disease has been reported to occur regularly in the farmers' fields and in experimental plots in Machakos, Kitui, lower semi-arid areas of Kiambu, Murang'a and Embu districts since 1984 (Kimani, 1988). Generally, *Cercospora* leafspot occurs late in the growth stages of the plant. On maize, it normally strikes at silk stage (Hilty *et al*; 1979; Rupe *et al*; 1982), on groundnuts, it strikes eleven weeks after planting (Hemingway, 1954 & 1955) and on the ornamental bells of Ireland (*Molucella laevis*), it normally occurs at the inflorescence stage (Njeru, 1988). Pigeonpea foliar symptoms due to infection by *M. cajani* occur during flowering and pod-filling stage (Kimani, personal communication).

2.2 Economic Importance of *Cercospora* leafspots

Crop damage attributed to *Cercospora* species attack are both direct and indirect. They extend from the field to storage (Hemingway 1954; Garry and Ruppel, 1971; Hilty *et al*, 1979). A decline in photosynthetic area due to both necrosis and defoliation is the initial effect of *Cercospora* leafspot on host plant. On groundnuts, duration of growth is determined by defoliation rate (Elston *et al*, 1976) and complete defoliation results in premature death of the crop (Hemingway, 1954).

In maize, losses ranging from 50 to 100 percent have been reported (Rocane *et al*, 1974; Hilty *et al* 1979; Nevill 1981). In most cases, leaf blight is followed by severe stalk rot and breakage. The breakage does not only result in additional ear damage but also causes inefficiency in the harvesting operation (Hemingway, 1954; Hilty *et al*, 1979).

On sugar beet, 50 percent loss due to rot during storage has been reported (Garry and Ruppel, 1971). Disease incidence in the field closely paralleled the number of harvested roots that rotted in storage.

Little work has been done to establish the losses caused by *Mycovellosiella* leafspot on pigeonpea but a study done in Makerere University, Kampala, Uganda showed that the disease can reduce the grain yield by up to 85 percent (Onim, 1980). These results supported observations made by Khan and Rachie (1972), Rubaihayo and Onim (1975), who indicated that *Mycovellosiella* leafspot was an important disease of pigeonpea.

2.3 Symptomatology

Members of the form-genus *Cercospora* cause leafspot diseases some of which are quite serious. The foliar symptoms typically develop late in the

growth of the crop often after flowering as reported for the leafspots of cowpea (Verma and Patel, 1969; Schneider and Sinclair, 1975; Fery *et al*, 1977; Vakili, 1977); mungbean (Mew *et al*, 1975); groundnut (Hemingway, 1955; Fowler, 1970) and corn (Kingsland, 1963). *Cercospora* species affect leaves, peticles, pods and stems.

symptoms on leaves and petioles

Leafspot varies in distinctness from a faint discolouration to characteristically marked necrotic lesion typical of *Cercospora* leafspots (Chupp, 1953). These turn grey if environmental conditions favour sporulations. Lesion shapes vary from irregular, circular, eliiptical to rectangular as dictated by leaf venation. Rectangular lesions measure 0.5 to 1.0 to 2.5 cm whereas the others have diameter of 2 – 7 mm (Hemingway, 1954; Baxter, 1956; Latch and Hanson, 1962; Kingsland, 1963).

Symptoms induced by *C. canescens* in various hosts are usually circular. Zonate lesions are characteristic of foliar infection by *C. cassicola*. Coalescence produces aggregate lesions of greater dimensions and in corn and cassava severe leaf blight has been reported (Kingsland, 1963, Teri *et al*, 1980). With severe pathogen attack, infected portions get dehydrated, shrink and tear away from the living tissues leaving a short hole effect. Complete defoliation is caused by virulent species.

Cercospora infections on pigeonpeas first appear on the undersurface of leaves as small light brown spots, 1 - 2 mm in diameter (Singh, 1932). These spots are round at first but later become irregular in outline and occasionally several of these coalesce forming irregular areas as large as 15 mm x 15 mm. Spots seldom cross the midrib of the primary veins of the leaflets. The centre of these spots is dark brown and bear the fascule of conidiophores with conidia. On older spots where conidiophores have ceased to form spores, infected areas become thin and translucent. In

advanced stages the whole leaf dries, curls and ultimately falls. The lesions on petioles are less common than on leaves, but more than on stems. These are greyish black and run parallel to the long axis of the petiole (Singh, 1932).

On pigeonpeas, *Mycovellosiella* leafspot is characterized by brown leafspot, sub-orbicular, usually indefinate 1 - 3 mm wide, but sometimes definate sub-orbicular, and as much as 1 cm wide, often the lesion becoming grey in the centre when older (Deighton, 1974).

Symptoms on flowers and pods

Many *Cercospora* species also infect fruits and pods (Chupp, 1953). *Cercospora beticola* Sacc causes lesions on flower bracts, pods, seeds and edible roots. The lesions are similar to those that occur on foliage and petiole. They first appear as small brown flecks with reddish purple borders. When spots attain a diameter of at least 2 mm, they become ashy–gray in the center, but retain the purple border. As spots mature, they often become so thin and brittle in the center that the dead tissue drops out, leaving a ragged hole (Vestal, 1933; Wallin and Loonan,, 1971). *Cercospora capsici* Heald and Wolf cause defoliation and peduncle infections which often results in stunted and irregularly shaped fruit (Muntanola, 1954).

Symptoms on stems

Many *Cercospora* species also infect young stems (Chupp, 1953). Infection has been reported on stems of *Cajanus indicus* which were inoculated with *Cercospora indica* N. sp. (Singh, 1932).

2.4 HOST RANGE

Studies on other *Cercospora* species show that a particular *Cercospora* species may attack only the host plant or several hosts. Verma *et al* (1968) reported that *Cercospora beticola* an incitant of leafspot disease in spinash-beet, also infected *Datura fastuosa, Trifolium alexandrinum, Chenopodium murale, C. album, Amaranthus polygamous, Spinacia tetrandra, S. oleracea, Atriplex hortansis, Portulacea oleracea,* and *Amarathus tricolar.* Sridharan and Rangaswami (1968) while working on two leafspot pathogens of *Abelmoschus esclentus* reported that *Cercospora malayensis* could infect *Hibiscus cannabinus, H. surattensis, H. ficulneus,* while *C. abelmoschi* failed to infect any of the 20 hosts tested, although it readily infected its natural host. *Cercospora indica* when inoculated on *Dolichos lab lab, Glycine hispida,* Maxim, *Phaseolus aconitifolia,* Jacq., *Phaseolus radiatus,* Linn., *Phaseolus mungo* Linn. var *Roxburghii* and *Vigna caljang* Endl., failed to infect. (Singh, 1932).

2.5 Etiology

2.5.1 Classification of the fungus

Mycovellosiella cajani (Henn) Rangel ex Trotter syn. *Cercospora cajani* Henn = *Vellosiella cajani* (Rangel) is the causal agent of *Mycovellosiella* leafspot of pigeonpea (Deighton, 1974). It is grouped together with the form genus *Cercospora* which is placed in the form-family Dematiaceae and in the form-order Moniliales of the form-class Deuteromycetes and subdivision Deuteromycotina (Alexopolous and Minns, 1979).

2.5.2 Pathogen characteristics

Morphological characteristics of the conidia and conidiophore provide the main taxonomic criteria for species delimitation (Hughes, 1953). The distinctive characters of *Mycovellosiella* are thickened conidial scars and the production of an assurgent or repent secondary external mycelium on the hyphae of which the conidiophores are borne terminally and as lateral branches (Deighton, 1974).

Conidiophores of *M. cajani* are terminal or arising as lateral branches on the external mycelial hyphae, pale or rather pale olivaceous, 5 - 35µ long, 4 - 7µ wide. Conidial scars are about 1µ in diameter. Conidia varying from almost colourless to moderately pale olivaceous, catenate (more commonly so in var, *cajani* and var. *indica* rarely so in var *trichophila*), sub cylindric, the shorter ones straight, the longer ones slightly curved, 0-9 septate in var. *cajani* and 10 - 129 X 3 - 6µ, (10 - 35µ long in var. *cajani*). Three varieties *M. cajani* var. *cajani*, *M. cajani* var. *indica*, and *M. cajani* var. *trichophila* have been distiguished (Deighton, 1974).

2.5.3 Cultural Studies of the pathogen

Sporulation of *Cercospora* species is not easy to achieve and some workers have reported on the fastidious requirements for *in vitro* sporulation (Nagel, 1934; Diachun and Valleau, 1941; Goode and Brown, 1970; Smith 1971). When working with *Cercospra* species, it has been common practice to subject a few isolates of a given species to various cultural manipulations. This involves such variables as nutrition, light and temperature (Berger and Hanson, 1963a; Calpouzous and Stallknecht, 1965 and 1967; Miller, 1969; Vathakos and Walters, 1979; El-Gholt *et al*, 1982).

(i) Growth and sporulation on culture media

Cultural studies on a number of *Cercospora* species show that germtubes emerge from any of the conidial cells within 4 hours (Jenkins, 1938; Alderman and Beute, 1986; Cooperman and Jenkins, 1986). In 1969, Sobers noted that on potato dextrose agar (PDA), the genus is characterized by gray colonies with or without a pink pigment. Singh (1932), reported remarkable difference in cultural characters of two isolates of *Cercospora indica*. As early as 1934, Nagel found that although the nature of the nutrient substratum influenced conidial production, the nutrient requirements differed among the species of *Cercospora*.

PDA is common medium for culturing fungi but success in inducing sporulation of *Cercospora* species has been reported only in a few cases (Nagel, 1934; Latch and Hanson, 1962; Sobers, 1969). Carrot leaf decoction agar medium has proved useful in induction of sporulation of *Cercospora* species. Abundant sporulation on carrot medium has been attained with *C. asparagi, C. kikuchii, C. zebrina* and *C. davisii* (Kilpatric and Johnson, 1956; Latch and Hanson, 1962; Berger and Hanson, 1963a; Cooperman and Jenkins, 1986).

Profuse sporulation of various *Cercospora* species on their respective host decoction agar has been reported by a series of workers to occur 7 - 21 days from the time of seeding. This has been observed for *C. nicotianae, C. beticola, C. zebrina, C. arachidicola*, and *C. molucellae* (Diachun and Valleau, 1941; Berger and Hanson, 1963a; Calpouzous and Stallknecht, 1965; Smith, 1971; El-Gholt *et al*, 1982; Njeru, 1988). Despite this, erratic sporulation on some host based media has been reported by Vathakos and Walters (1979) and Cooperman and Jenkins (1986) while working with *C. Kikuchii* and *C. asparagi*, respectively. Vathakos and Walters (1979) observed abundant conidiophore formation and extremely sparse sporulation.

(ii) Light

In 1965, Calpouzous and Stallknecht not only emphasized the importance of selection of an appropriate medium but also the interaction between light and temperature for maximum sporulation. Continuous darkness has been found to be the optimum condition for sporulation of *C. nicotianae, C. davisii,* and *C. kikuchii* (Vathakos and Walters, 1979). Alternate dark and light period favours sporulation of *C. kikuchii, C. zebrina* and *C. asparagi* (Kilpatric and Johnson, 1956; Berger and Hanson, 1963a; Cooperman and Jenkins, 1986). There are yet other *Cercospora* species which require exposure to continuous light for sporulation to occur. This is the case with *C. beticola* as reported by Calpouzous and Stallknecht (1967).

(iii) Temperature

Cercospora species grow and sporulate within a temperature range of 15° - 30°C. The optimum temperature for growth and sporulation reported for *C. zebrina, C. asparagi, C. davasii* is 24°C while for *C. gossypina, C. beticola* and *C. nicotianae* is 21° - 29° C, 15°C, 26°C, respectively (Calpouzous and Stallknecht, 1967; Stavely and Nimmo, 1968; Miller, 1969; Cooperman and Jenkins, 1986).

(iv) pH

The pH of the medium influences sporulation in fungi. Growth of *Cercospora* species has been found to be favoured by acidic medium. Singh (1932) reported that medium adjusted to pH 6.7 supported optimum growth of *C. indica*. The fungus rendered the medium in which it grew acidic. Maximum growth for *C. zebrina* has been reported to be at pH 5.2 (Berger and Hanson, 1963a.).

2.5.4 Factors affecting conidial germination

(i) Moisture

Conidial germination studies show that germination in some *Cercospora* species is inhibited in the presence of free water. Germination of some *Cercospora* species such as *Cercospora omphakodes* was inhibited in free water (Judd and Peterson, 1972). Other *Cercospora* species such as *C. musae* and *C. cruenta* require a film of free water in order to germinate (Meredith, 1970; Ekpo and Esuruoso, 1977).

Conidia of *C. zebrina* were found to germinate well in distilled water (Berger and Hanson, 1963a). Conidia of *C. asparagi* germinated readily in free water both on the leaf surface and on the glass slides (Cooperman and Jenkins, 1986). Ekpo and Esuruoso (1977) found that leaf extract of cowpea enhanced spore germination in *C. cruenta*. Only terminal cells germinated in distilled water whereas high percent germination of both terminal and intercalary cells occurred in leaf extract solutions.

(ii) Temperature

Cercospora species have been shown to germinate at a wide range of temperatures and in the presence or absence of light. Conidial germination of *C . asparagi* occurred between 8 and 36°C with a maximum between 20° and 32°C (Cooperman and Jenkins, 1986). Berger and Hanson (1963a) found that conidia of *C. zebrina* germinated at temperatures ranging from 8 - 36°C with an optimum near 24°C. Ekpo and Esuruoso (1977) reported that the optimum temperature for spore germination of *C. cruenta* was 25°C.

(iii) Time

Germination or disintegration of conidia in culture has been observed in *Cercospora* species (Nagel, 1934). Cooperman and Jenkins (1986) reported that germination of conidia occurred in cultures of *C. asparagi* after 6 days of plate inoculation thus decreasing the number of viable conidia present. Njeru (1988) reported that sporulation of *C. molucellae* increased to a peak and then declined during the time of incubation.

The ability to produce colonies and sporulate in culture was increased with successive transfer of pure sporulating conidia. Selective subculturing, a method of transferring densely sporulating areas, has induced sporulation in other species of *Cercospora* (Nagel, 1934; Calpouzous, 1954; Jones, 1958; Calpouzous and Stallknecht, 1965).

Calpouzous (1954) observed that colonies originally from single isolate, all growing in the same culture plate under identical environmental conditions showed considerable variation in morphology and sporulation. He concluded that the critical factor controlling conidia production in pure cultures of *C. musae* is genetic and is not associated with environmental factors as many had previously thought. He was able to develop cultures that were genetically pure for the ability to produce conidia.

2.6 Resistance-screening technique

Selection for resistance to *Mycovellosiella cajani* in the field has been reported (Onim and Rubaihayo, 1976; Rodriguez and Melendez, 1984). There is evidence that field resistance tends to be confounded by variations in host development, perhaps leading to spurious conclusion in the identification of resistance to *Cercospora* leafspot (Nevill and Evans, 1980).

The expression of resistance to certain pathogens may vary considerably with the growth stage or with the particular plant organ of the host plant. For instance, seedling susceptibility is known to be associated with adult plant resistance in beans to *Rhizoctonia solani* (Bateman and Lumsden, 1965), as well as in certain host-pathogen combination such as bean rust (*Phaseolus vulgaris / Uromyces appendiculatus*) (Atkins, 1973; Ballantyne and McIntosh, 1977). At certain growth stages some parts may be more susceptible than others for instance, young soybean leaves are more susceptible to downy mildew than the old leaves (Sinclair, 1982).

While testing for disease reaction in a selection program a seedling test might prove more efficient but the prime requisite of any such seedling test is that it should accurately predict resistance in the field. Studies on coffee berry disease (CBD) demonstrate clearly that the hypocotyl preselection test, by which 5 - 6 week old seedlings are inoculated with a spore suspension of the pathogen (*Colletotrichum coffeanum*), gives reliable information about mature plant resistance to CBD (Van der Vossen *et al*, 1976). Seedling inoculations have been used also on other crops such as Sudan grass and beans to test varieties for resistance against leaf blight and anthracnose respectively (Drolsom and Dickson, 1954; Hubbeling, 1957). The use of more than one technique may in certain circumstances, assist in differentiation of distinct resistance mechanism as well as help to establish the relationship between the resistance of different parts (Coyne and Schuster, 1974; Allen *et al*, 1981; Patel, 1981).

2.7 The relationship of host age and leaf position to plant susceptibility

The relationship of the age of various host plants to disease susceptibility has been discussed by several workers (Wyllie and Williams, 1965; Mence

and Pegg, 1971; Warren *et al*, 1971; Dickson and Crute, 1974; Nyvall and Haglund, 1976; Hart and Endo, 1981).

Changes in susceptibility have been reported for several host-pathogen systems. Susceptibility of tobacco plants to blue mold changes with age of plants. The younger tobacco plants are more susceptible to blue mold caused by *Peronospora tabacina* than older plants (Clayton, 1945; Hill, 1969; Reuveni *et al*, 1986). Dickinson and Crute (1974) found that the susceptibility of lettuce seedlings to *Bremia lactucae* decreased with age. Mence and Pegg (1971) found that the resistance of pea leaves to *Peronospora viciae* increased with age but declined again in senescence. Headrick and Pataky (1987) demonstrated that sweet corn hybrids became more resistant to *P. sorghi* after anthesis. Younger plants of ornamental bells of Ireland (*Molucella laevis*) are less susceptible to *Cercospora molucellae* than older plants (Njeru, 1988). Similar observations have been made on *Cercospora* leafspot on groundnuts and corn (Hemingway, 1954 and 1955; Hilty *et al*, 1979).

Warren *et al* (1971) reported that the age of plants and position of leaves on the stem influenced susceptibility of potato plants to *Phytophthora infestans*. With older plants, leaf position was important, upper and lower leaves were more susceptible than those of an intermediate position.

Allen and co-workers (1983) reported that lower leaves of sunflower plants are more susceptible to infection by *Alternaria helianthi* than are the upper leaves. Hill (1969) reported that the position of leaves of greenhouse grown tobacco plants influenced susceptibility to blue mold pathogen *Peronospora tabaci*. Inoculated lower leaves produced more lesions of blue mold than did upper leaves.

2.8 Pigeonpea

2.8.1 Origin

Pigeonpea (*Cajanus cajan*) (L.) Millsp.) is a drought tolerant legume widely grown in the tropics. Its origin is controversial and has interested many scientists. Some favour an Indian origin on the basis of diversity and other favour an African origin because of the presence of a single endemic West Africa species, *Cajanus Kerstingii* Harms. In 1915, Harms (cited by Thothathri and Jain, 1980) published the description of *C. kerstingii* Harms from Togo.

Some of the workers (Good 1964; Purseglove 1968; Zeven and Zhukovsky, 1975) are of the opinion that *Cajanus* is probably a native of Africa, from where it spread to India as a secondary centre. Good (1964), while grouping the genera found entirely or predominantly in the tropical regions, excluding those occurring pantropically, placed *Cajanus* under the subgroup Africa, Asia. Sturtevant (1972) mentioned that Schweinfurth during his travels in Africa between 1868 - 1871 reported the presence of a seed of pigeonpea in Egyptian tombs of the 12th Dynasty (2200 - 2400 B.C). It can therefore be tentatively concluded that *Cajanus* is a native of Africa, with India as a secondary center of origin (Thothathri and Jain, 1980).

2.8.2.Taxonomic history

Cajanus as a genus was founded in 1813 by A.P. De Candolle, based on two species, *C. flavus* and *C. bicolor* (cited by Thothathri and Jain 1980). De Candolle attributed both species to India, with a note stating that *C. flavus* also occurs cultivated in America. He cited *Cytisus cajan* (1753) under *Cajanus flavus*, thereby making it clear that the Linnaean plant is the same as his *C. flavus*. This type species of the genus is *Cajanus cajan* (L). Millsp., based on *Cytisus cajan* L.

2.8.3 Host-pathogen interactions

Infection through artificial inoculation of host plants has been accomplished at ages between two weeks and ten months using varying conidial concentrations on leaf surfaces. Conidia of *Cercospora* species germinate from one or more cells within 3 - 6 hours (Jenkins, 1938; Baxter, 1956; Berger and Hanson, 1963b). Passive ingress (through stomata) has been observed on *C. medicaginis, C. zebrina, C. davishii* and *C. moluccellae* with the earliest penetration occurring 24 - 48 hours after inoculation. Except in the cases of *C. beticola*, no appressoria are formed over stomatal opening (Baxter, 1956; Latch and Hanson, 1962; Njeru, 1988). Chupp (1953) and Solel and Minz (1971) stated that *Cercospora beticola* kills the infected parts as it penetrates the host tissue. The lesion size depended upon the host resistance or the ability of the fungus to overcome mechanical obstructions such as veins. Conidia and conidiophores of *Cercospora zebrina* germinated within 3 hours on leaf surfaces of *T. pratense*. Germtubes entered through stomata. After penetration, the fungus branched repeatedly and ramified through the tissue. Invaded cells dried quickly to form necrotic lesions (Berger and Hanson, 1963b).

The incubation periods vary depending on the isolate, inoculum concentration, host plant and prevailing temperature. Berger and Hanson (1963b) reported that the first symptoms appeared on inoculated plants of *T. pratense* 6, 7, 9 and 12 days after inoculation at 28°, 24°, 20°, and 16° C respectively. Symptoms on *Melilotus* sp. inoculated with *Cercospora davisii* took 14 days to appear (Latch and Hanson, 1962), while those on asparagus plants inoculated with *C. asparagi* appeared after 8 days at 15°C. *Cercospora* blight developed in asparagus over a range of temperatures, with the most rapid development and the most severe symptoms at 25°C and the slowest development and the least severe symptoms at 15° and

35° C (Cooperman and Jenkins, 1986). Under favourable conditions, *C. zebrina* sporulated on infected plant tissues. On leaves, stromata form beneath the epidermis in substomatal chambers or between guard cells. As the stromata enlarges, they force guard cells apart and rupture the epidermis. In some cases, the stromata emerge through the stomata and thus are slightly erumpent on leaf surface (Berger and Hanson, 1963b; Beckman and Payne, 1981). In some cases the stromata remain buried beneath the epidermis and emerge through the stomata.

3. MATERIAL AND METHODS

3.1 LABORATORY EXPERIMENTS

3.1.1. Isolation and morphological characteristics of pigeonpea leafspot pathogen

(i) Isolation of *Mycovellosiella cajani*

Infected leaves and petioles were obtained from pigeonpea breeding nursery at Kabete campus of the University of Nairobi at an altitude of 1829 m. The samples were washed in tap water and then surface sterilized in 10 percent solution of sodium hypochlorite for five minutes and then rinsed in five changes of sterile distilled water. The leaves were then incubated for 48 hours in a moist chamber which consisted of a petri-dish containing moistened filter paper. When sporulation occurred, the spores were transferred to petri dishes containing six different types of media in order to identify the media which would support optimum growth and sporulation of the fungus. Petri dishes were inoculated using conidia from sporulating infected host tissues picked using sterile inoculating needles. The six media used included pigeonpea leaf decoction agar (PLDA), carrot agar (CA), carrot leaf decoction agar (CDA), potato carrot agar (PCA), pigeonpea meal agar (PMA) and potato dextrose agar (PDA). The petri dishes were incubated at room temperature (20 - 24°C) for 22 days. The composition of each media is given in (appendix I).

(ii) Single spore isolation

Colonies growing in PLDA culture plates were flooded with sterile distilled water and scrapped off the media using the edge of a sterile glass

slide. The concentration was adjusted to $2x10^5$ conidia ml^{-1}. The conidial suspension was diluted to 200 conidia ml^{-1}. Petri-plates containing 2 percent water agar were inoculated using 0.1 ml of this dilution (about 20 conidia) and the suspension was distributed by swirling the plate. The plates were then incubated at room temperature 20 – 24°C. With the aid of a dissecting microscope, the plates were examined for conidial germination every 3 hours. After 6 hours, when the conidia had began germinating but before germ tube entwined, an agar block bearing a single germinating conidium was cut away and transferred to PLDA medium using a sterile cooled inoculating needle. Four plates were each inoculated using single spores. The plates were incubated at room temperature (20 – 24°C) for 14 days.

(iii) Hyphal growth and spore production

A modification of the slide culture technique described by Riddel (1950) was used to determine the nature of the conidiophores, arrangement of conidia on the conidiophores, and whether or not the conidia were borne in chains, all of which were important features for fungus identification. Two sheets of filter paper, a bent glass rod, microscope slide (on the rod), and cover slip were placed into a petri dish in that order and sterilized.

Meanwhile, sterilized PLDA medium was poured into a sterile 9 cm petri dish to form a layer of about 2 mm deep. When the agar had solidified, 1–cm^2 blocks were cut using a sharp sterile scapel. One agar block was placed on the sterile microscopic slide and seeded at the centre of each edge with conidia of the pathogen using a cooled sterile inoculating needle and then the cover slip was centrally placed on the agar block. It was then incubated at room temperature (20 – 24°C). To maintain high humidity within the petri dish, the filter paper was kept moist by periodic addition of 2 percent aqueous solution of glycerine. After 10 and 14 days of

incubation, the cover slip was lifted carefully and the agar block was discarded. The cover slip was mounted in a drop of lactophenol cotton blue on a clean microscopic slide. The fungal growth on the culture slide was similarly mounted. Examination were done under a light microscope. The nature of conidiophore, arrangement of the conidia and hyphal growth were noted.

3.1.2. Maintenance of fungal cultures

Conidial suspension was prepared from plates that had been inoculated with single spores and incubated at room temperature for 14 days. This suspension was stored in vials at 4° – 6°C. The fungus was subcultured every 3 or 4 weeks and plates incubated for 10 – 14 days. Densely sporulating areas of the colonies were selected and used to prepare fresh conidial suspensions.

3.1.3. Growth characteristic of *Mycovellósiella cajani*

(i) Inoculum multiplication

All inocula for cultural studies were produced by flooding each of the pigeonpea leaf decoction agar (PLDA) petri dish with 1 ml conidial suspension of $2x10^4$ conidia ml^{-1}. The suspension was evenly spread by swirling the petri dishes which were then incubated at room temperature (20 – 24°C) for 14 days.

(ii) Plate inoculation and incubation

All the plates representing different treatments were inoculated using one ml conidial suspension adjusted to $2x10^4$ conidia ml^{-1} after the most suitable concentration had been established. The suspension was evenly

spread on the surface of the medium. The plates were incubated at room temperature (20 – 24°C) unless otherwise indicated.

(iii) Sampling and counting of conidia

Sampling for conidial counts for all the treatments was done on 10,14, 18 and 22 days after plate incubation. At the end of each incubation period, 1 ml of sterile distilled water was added to a culture plate. All the colonies were scrapped off using the edge of a sterile glass slide. The harvested colonies were put in a vial and crushed using a glassrod to release the conidia. The suspension was adjusted to 10 ml and strained through a two layer cheese cloth. The conidial concentration was determined by use of haemocytometer. For each plate sample, four counts were made each consisting of nine values from nine large squares of a haemocytometer. The average value obtained was used to compute the conidial concentration per ml of each sample.

(iv) Experimental design and data collection

Unless otherwise indicated, all the experiments for cultural studies were carried out in a complete randomized design with three replications. LSD was used to compare the treatments.

3.1.3.1. Sporulating capacity of *Mycovellosiella cajani*

(i) Effect of conidial concentration used for plate inoculation

The effect of different inocula concentrations on sporulation of the resultant colonies was determined by inoculating plates of PLDA with 1 ml of suspensions diluted to 20, $2x10^2$, $2x10^3$, $2x10^4$, $2x10^5$ conidia ml^{-1}. Twelve plate cultures were incubated for each treatment. Sampling of three plates was done over four periods and suspensions prepared as

described in section (3.1.3 iii). Conidial concentration was ascertained with the aid of a haemocytometer.

(ii) Effect of media

The main objective of this experiment was to find a medium which would serve as the best medium for sporulation of *M. cajani*. Six different media were tested. These included pigeonpea leaf decoction agar (PLDA), carrot agar (CA), carrot leaf decoction agar (CDA), potato dextrose agar (PDA), potato carrot agar (PCA) and pigeonpea meal agar (PMA) (Appendix 1).

(iii) Effect of temperature

The effect of temperature on sporulation of *M. cajani* was investigated. The PLDA plates were inoculated and incubated at seven different temperatures *viz* 5°, 10°, 15°, 20°, 25°, 30° and 35°C.

(iv) Effect of pH

The effect of pH on spoluration of *M. cajani* was determined in culture by inoculating PLDA plates adjusted to seven pH levels *viz* 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0

(v) Effect of light

The PLDA plates were inoculated with *M. cajani* conidial suspension and incubated under the following light regimes:

(a) 24 hr complete darkness

(b) 12 hr alternate light and 12 hr darkness

(c) 24 hr continous light

Twelve petri dishes were incubated for each light regime and for each of the four periods three plates were sampled. The plates incubated at 24 hrs

complete darkness were wrapped in aluminium foil while the plates incubated under 12 hr light /12 hr darkness regime had the aluminium foil removed after every 12 hrs of darkness. Continous light was provided by use of 40W Philip electric tubes. All the plates were randomly placed on a bench in the culture room.

3.1.4. Factors affecting conidial germination

Unless otherwise stated, conidial suspensions of *M. cajani* were prepared from 14 day old cultures and 0.1 ml of the conidial suspension was transferred to a sterile cavity glass slide contained in a petri dish moist chamber. Percent conidial germination was assessed using not less than 400 conidia per treatment after 3, 4, 8, 12, 24, 48, and 72hr. All the experiments were replicated three times and the experiment repeated once.

(i) Effect of conidial concentration

Conidial suspensions of concentrations 2×10^4, 5×10^4, 2×10^5 and 2×10^6 conidia ml^{-1} were prepared and 0.1 ml of each suspension was placed on a sterile cavity glass slide contained in a petri dish moist chamber. The moist chambers were incubated at room temperature (20 – 24°C) and examined for germination after 3, 4, 8, 12, 24, 48, and 72 hr. The lag period of germination, rate of germination and percent germination were recorded and computed respectively.

(ii) Effect of temperature on conidial germination

To determine the effect of temperature on conidial germination, 0.1 ml of suspension of concentration 2×10^5 conidia ml^{-1} was placed on a cavity slide enclosed in a moist chamber. The moist chambers were incubated at

temperatures 0°, 5°, 10°, 15°, 20°, 25°, 30° and 35°C. Examination of conidial germination was done after 3, 4, 8, 12, 24, 48, and 72 hr of incubation. The lag period of germination, rate of germination and percent germination were recorded and computed respectively.

(iii) Effect of pigeonpea leaf extract on conidial germination

The leaves of 15 and 120 day old pigeonpea genotypes of cvs NPP 670, NPP 673/3, Katheka, ICPL 295, and lines KB 91/1, KO 71/2, KZ 13/2, TK–3, were cut into small pieces and weighed. To prepare the extracts, known volume of sterile distilled water was added to known quantities of leaf sections and the tissue homogenized using a sterile mortar and pestle. To every gram of leaf tissue, 1 ml of sterile distilled water was added. After soaking for 24 hours the homogenate was filtered through a two layer sterilized cheese cloth and filter sterilized using a sintered glass filter.

Conidial suspension of *M. cajani* containing 2×10^6 conidia ml^{-1} was prepared and 0.1 ml of this suspension was added to 9 ml of the leaf extract. Control treatment had 9 ml of sterile distilled water. From the resultant suspension, 0.1 ml was put on a sterile glass cavity slide enclosed in a moist chamber and incubated at room temperature (20 – 24°C). Germination of conidia was examined after 24 hr of incubation. Total percent germination in each replication was calculated.

3.2 GLASSHOUSE EXPERIMENTS

(i) Planting material

Unless otherwise stated eight pigeonpea genotypes were used in the glasshouse experiments. They included an early maturing local cultivar, cv NPP 670, four early maturing breeders lines, KB 91/1, KO 71/2, KZ 13/2, TK–3, two late maturing local cultivars, cvs NPP 673/3 and Katheka, and

one early maturing ICRISAT genotype, ICPL 295. The seeds of the pigeonpea genotypes were obtained from Pigeonpea Improvement Project at the University of Nairobi, Kenya.

(ii) Planting medium

The planting medium used consisted of forest soil, animal (cow dung) manure and stone gravel mixed in ratio of 10:1:1 by volume. Nyabundi (1980) reported good growth of pigeonpea in this soil composition. The soil was put in 5 litres plastic containers.

(iii) Inoculum preparation

Conidial suspension was prepared by flooding 14-day old cultures of the pathogen grown in PLDA medium with sterile distilled water. Conidia were scrapped off using the edge of a sterile glass slide and the concentration adjusted to $2x10^6$ conidia ml^{-1}.

(iv) Plant inoculation and incubation

Plants were inoculated using a modified double inoculation technique of Van der Vossen and co-workers (1976). Conidial suspension of *M. cajani* was applied on both sides of all the leaves present on the plant using a half litre Baygon atomiser (Bayer East Africa Ltd) held at a distance of 10–15 cm away until run off. A double inoculation at 48 hour interval was applied. Control plants were inoculated with sterile distilled water. To maintain high humidity, inoculated plants were covered with transparent plastic bags for 96 hours and thereafter the covers were removed. To increase leaf wetness plants were sprayed with water at least twice a day. The plants were incubated for a total of 35 days in the glasshouse where the average temperatures were 20 – 26°C. Daily observations on symptom development were made until the first appearance of symptoms.

3.2.1. Pathogenicity test

For pathogenicity test, 15 and 120 day old pigeonpea plants of cv NPP 673/3 were used. The test was conducted after isolation in order to prove that the organism isolated was the causal agent of *Mycovellosiella* leafspot. Leaves showing characteristic *Mycovellosiella* leafspot symptoms were detached 35 days after plant inoculation and re–isolation of the pathogen was carried out to fulfill Koch's postulates.

3.2.2. Host – pathogen relationship

3.2.2.1. Pre–penetration and penetration events

Conidial germination on host tissue was monitored in order to establish the time taken to conidial germination after inoculation and also the mode of penetration.

(i) Plant inoculation and incubation

Circles were marked on leaf surfaces to be inoculated using India ink. Leaves of 120 day old pigeonpea plants of cv NPP 673/3 grown in the glasshouse were used. The leaves were inoculated on both sides. Prior to inoculation the potted plants were covered with transparent polythene bags for 30 minutes to increase humidity. The inoculated plants were placed on a laboratory bench and covered with transparent polythene bags for 48 hours to maintain high humidity. The prevailing laboratory temperatures were 20 - 24°C

(ii) Leaf sampling and clearing

Three hours after inoculation, discs of leaf tissue were cut out from the marked areas of the inoculated leaf using a cork borer, size No.5 (1 cm diameter cork borer). The leaf disks were cleared using the method described by Dhingra and Sinclair (1985). The disks of the leaf tissue were put in vials containing Carnoy's solution (1 part glacial acetic acid and 2 parts absolute alcohol) for 24 hours. The specimen were then mounted on cotton blue in lactophenol and observed under the light microscope. Observations were made on spore germination and penetration of host tissue by fungus. This procedure was repeated after 5,12,24,and 48 hours of inoculation.

3.2.2.2. Symptomatology

Diseased plant parts infected by *Mycovellosiella cajani* were collected from inoculated plants in the glasshouse, Kabete and from field plots in Kiboko and Kabete. Description of the symptoms occurring on the leaves, petiole, flowers, pods, and stems were made. Distribution of the symptoms on the whole plants was also observed.

3.2.3. Reaction of pigeonpea genotypes to *Mycovellosiella cajani*

(i) Determination of disease incidence

The artificially inoculated glasshouse pigeonpea plants were assessed for *Mycovellosiella* leafspot incidence by counting the number of infected leaves per plant. Observations for disease symptoms were done on daily basis until the first symptoms were noted. Thereafter, only four records of disease incidence were taken at 4 day interval. The percent disease incidence was calculated for each date of recording.

(ii) Determination of disease severity

Disease severity was estimated by use of a scale based on percent leaf area diseased which was a modification of the scale derived by Horsfall and Barratt (1945). Many leaves that had *Mycovellosiella* leafspot in different proportions were collected from the glasshouse plants and the size of single lesions measured. The average size of one lesion was calculated from those values and used to calculate the actual leaf area diseased. The leaf area was determined for many leaves by use of leaf area meter and from these it was possible to have a good estimate as to how many spots made up a severity of 0.1, 1, 3, 6, 12, 25, and 50 percent. Photographs of infected leaves showing different levels of intensity of the disease were taken. These provided a photographic guide and were used in classifying leaf spotting severity into 6 grades. The disease severity assessment scale included 0–5 grades which were mainly based on leaf area diseased.

3.2.3.1. Effect of inoculum concentration on the infection level on pigeonpea plants by *Mycovellosiella cajani*

A susceptible late maturing cultivar, cv NPP673/3, was used in this experiment. Conidial suspensions were prepared and adjusted to $2x10^4$, $5x10^4$, $2x10^5$, $2x10^6$ conidia ml^{-1}. Each inoculum concentration was applied to two trifoliate leaves of three 120 day old plants of cv NPP 673/3 in two replications. Observations on inoculated leaves were done on daily basis until the symptoms were noted. Thereafter, disease infection level was assessed after every 4 days up to 27 days after inoculation. The final disease records were taken 34 days after plant inoculation when some of the leaves had turned yellow and started defoliating.

3.2.3.2. Effect of plant age on the infection of pigeonpea genotypes by *Mycovellosiella cajani*

Eight pigeonpea genotypes (section 3.2.i) were potted in the glasshouse on different dates to provide plants for simultaneous inoculation when the plants were 1 month, 2 months, 3 months and 4 months old. Four seeds per genotype were planted in each pot and latter thinned to two plants per pot after one month of sowing. Observations for disease incidence were done as described in section (3.2.3.i)

3.2.3.3. Effect of leaf position on the infection of pigeonpea genotypes by *Mycovellosiella cajani* in the glasshouse

One hundred and twenty day old plants of the eight pigeonpea genotypes given under section (3.2.i) were used in this study to assess the effect of leaf position on the plants leaf susceptability to *M. cajani*. The plants were subdivided into three parts according to height and two trifoliate leaves from the upper, intermediate and the lower parts were inoculated using 2 x 10^6 conidia ml^{-1} of *M. cajani*. Thereafter the inoculated leaves were assessed for disease severity which was estimated by use of a 0 – 5 grade scale based on percent leaf area diseased. Defoliating infected leaves were given a score of grade 5. Disease records were taken on different dates ranging from 15 to 34 days after inoculation. Mean disease severity was calculated per leaf position for each genotype. An average score was obtained for the three leaf positions. Data collected 19 days after plant inoculation was used to determine the reaction of the pigeonpea genotypes to *Mycovellosiella cajani*. To assess disease progress, the percentage leaf area infected was recorded and disease progress curves plotted. The experiment was replicated 4 times and each replication had three plants of each genotype.

3.2.3.4 Reaction of pigeonpea seedlings to *Mycovellosiella cajani*

This experiment was carried out in order to develop a method of preselection for resistance to *M. cajani*, by artificial inoculation of young pigeonpea seedlings. This study was also to determine the efficiency of this method by comparing the results of the preselection test with mature plant infection as indicated by artificial inoculation of mature glasshouse and field grown pigeonpea plants.

(i) Plant genotypes and planting

An average of 25 seeds of each of the eight genotypes section (3.2.i) were sown out in moist sterilized sand in plastic boxes measuring 17 cm, 11 cm wide, and 5 cm high with closely fitting transparent lids. The containers were left on the floor of the glasshouse. To provide nutrients for seedlings, Long Ashton Nutrient solution (Hewitt, 1952) was used for watering the plants twice a week. The rest of the time the sand was kept moist using tap water.

(iii) Seedling inoculation and incubation

Inoculum was prepared as described in section 3.2 (iii). The seedling were inoculated when the plants were 15-day old using 2×10^6 conidia ml^{-1} of *M. cajani*. Inoculation of seedling leaves was done using a half litre Baygon atomiser held at a distance of 10–15 cm away until run off. A double inoculation at 48 hours interval was applied. High relative humidity was maintained for the first four days after the first inoculation by covering the boxes with a polythene sheet. To increase leaf wetness seedlings were sprayed with water at least twice a day after the polythene sheet was removed. The seedlings were left on the floor in the glasshouse where the average temperatures were 20 – 26 ^oC.

(iv) Disease assessment

Observation for disease symptoms were done on a daily basis until the symptoms were noted. The seedlings were thereafter scored for disease severity on 17,19,21,23,25 and 27 day after the first inoculation.

The following scale was used to assess the infected leaflets:

Infection scale	Description of infected leaf
0	No disease observed
1	Infection in traces, few spots of >1 mm in size
2	3–6 percent leaf area infected
3	6.1–12 percent leaf area infected, no chlorosis
4	12.1–25 percent leaf area infected, slight chlorosis
5	25.1– > 50 percent leaf area infected and leaves showing chlorosis or severe blight and defoliation

(v) Experimental design

The experiment had three replications.

3.3 FIELD EXPERIMENTS

This study was undertaken to assess the mature plants of different pigeonpea genotypes for *Mycovellosiella* leafspot infections under field conditions both at Kabete and Kiboko.

(i) Plant genotypes

Eight pigeonpea genotypes were planted in November 1989 during the short rain season in the field at Kabete and Kiboko. The genotypes used were similar to the ones used in the glasshouse (section 3.2.(i))

(ii) Planting

Cultivar NPP 670, a local cultivar, was used as the spreader material of the disease due to its high susceptibility to *M. cajani* in the field. The spreader material was planted in rows and spaced 2.25 m apart in each plot. The spreader cultivar also was used as guard rows between replications. Two 3 m rows of each of the eight pigeonpea genotypes evaluated were planted at a spacing of 30 cm within rows and 75 cm between rows. The rows were planted at right angle to and in between the pairs of parallel spreader rows. A space of 1 m at each end of the test rows separated them from the spreader rows. The replicated blocks were bordered and separated by a pair of spreader rows.

(iii) Experimental design

The trial consisted of four replications in a randomized complete block design.

(iv) Disease, Pest and weed control

No fungicides were applied but insect damage was controlled by use of an insecticide, Rogor® L40 which contain 40% W/V (400 ml/l) N-monomethyl-Amide of 0,0 Dimethyldithiophosphoryl-acetic acid (dimethoate).The insecticide was applied at the rate of 25 ml / 20 litres (0.5 g a.i/l). Weeding was done by hand every time the weeds were noted in the experimental plots.

(v) Plant inoculation

Artificial plant inoculation using *M. cajani* at a concentration of 2×10^6 conidia ml^{-1} was done when most of the early maturing genotypes, lines KB 91/1, KO 71/2, KZ 13/2, TK-3, cv ICPL 295 were at flowering and podding stage (120 days after planting). Cultivar NPP 670 had flower buds which later aborted and therefore it was still in a vegetative stage at the time of inoculation. The late maturing cultivars, cvs NPP 673/3 and Katheka were in their vegetative stage.

Leaves were inoculated by spraying on both surfaces using a half litre Baygon atomiser held at a distance of 10 - 15 cm away until run off. A double inoculation at 48 hours interval was applied. There was also alot of natural infection in the field at the time of inoculation.

(vi) Sampling and Disease assessment

Fifteen days after plant inoculation, disease assessment was done and this was repeated on a weekly interval up to 4 weeks after inoculation. An extra sampling was carried out at Kiboko on the 6th week after plant inoculation.

(a) *Determination of disease incidence*

Visual rating for disease intensity was done in the field. The whole plot was examined for either possible variation in disease incidence, such as localised areas with high disease incidence, or for uniform distribution of the disease. The number of plants infected per plot was noted.

(b) *Disease severity as influenced by leaf position*

Infected leaves were gathered and used to determine disease severity. Ten plants per plot were selected for disease assessment. The plants were divided into 3 parts, upper, intermediate and lower parts of the plant canopy. The sampling method consisted of selecting 30 leaflet per plot from each of the selected plants. Samples of 10 of the most severely infected leaflets at each plant position and replication were placed in marked plastic bags and refrigerated until examined. The leaves were graded on 0 - 5 scale based on percent leaf area diseased.

3.4. Relationship between seedling and mature plant susceptibility to *Mycovellosiella cajani*

One of the main objective of this study was to develop a screening test for resistance to *M. cajani* using a seedling technique. The data on *Mycovellosiella* leafspot infection on mature plants in the field, glasshouse and seedling infection were further analysed. The coefficient of correlation between infection levels of mature plants and seedlings were calculated using only the six early maturing genotypes of pigeonpea.

statistical analysis

Genotypic mean infection data from seedlings 23 days after plant inoculation, glasshouse experiment 19 days after plant inoculation, and field experiment 21 days after plant inoculation when the infection level was high and before heavy defoliation took place were correlated.

RESULTS

4.1. Laboratory experiments

4.1.1. Growth characteristics of *Mycovellosiella cajani*

4.1.1.1. Hyphal growth and spore production

Conidia varied considerably in size and ranged from 9 to 36 by 4.5 to 6 µ. The shape was also variable and included subcylindric or slightly obclavate – cylindric, straight, rarely curved, shoe shaped with a distinct scar at one end and 1 - 2 rarely 3 scars at the other end. Some conidia had one septum while others were aseptate (Plate 1a). Occassionally conidia having 3 or 4 septa were observed. Conidia are acrogenous, forming chains or acting as conidiophore. Conidiophores were borne terminally and as lateral branches of secondary mycelial hyphae. They appeared pale brown, smooth, septate or continuous, straight or slightly flexuous, irregularly cylindrical, frequently narrowing at the base. The apical end of the conidia was generally rounded, more or less conical, with a visible scar at the top, sometimes shoe-shaped or with lateral conidial scars. There was some variation on conidiophore. Some conidiophores were short (Plate 1b).

The fungus grew very slowly in all the different media tested. In pigeonpea leaf decoction agar, it was noticed that with monosporic isolations, colonies of 1 - 3 mm were obtained after 14 - 18 days of incubation. After 22 days some colonies measured upto 9 mm. The colonies appeared grey on pigeonpea leaf decoction agar.

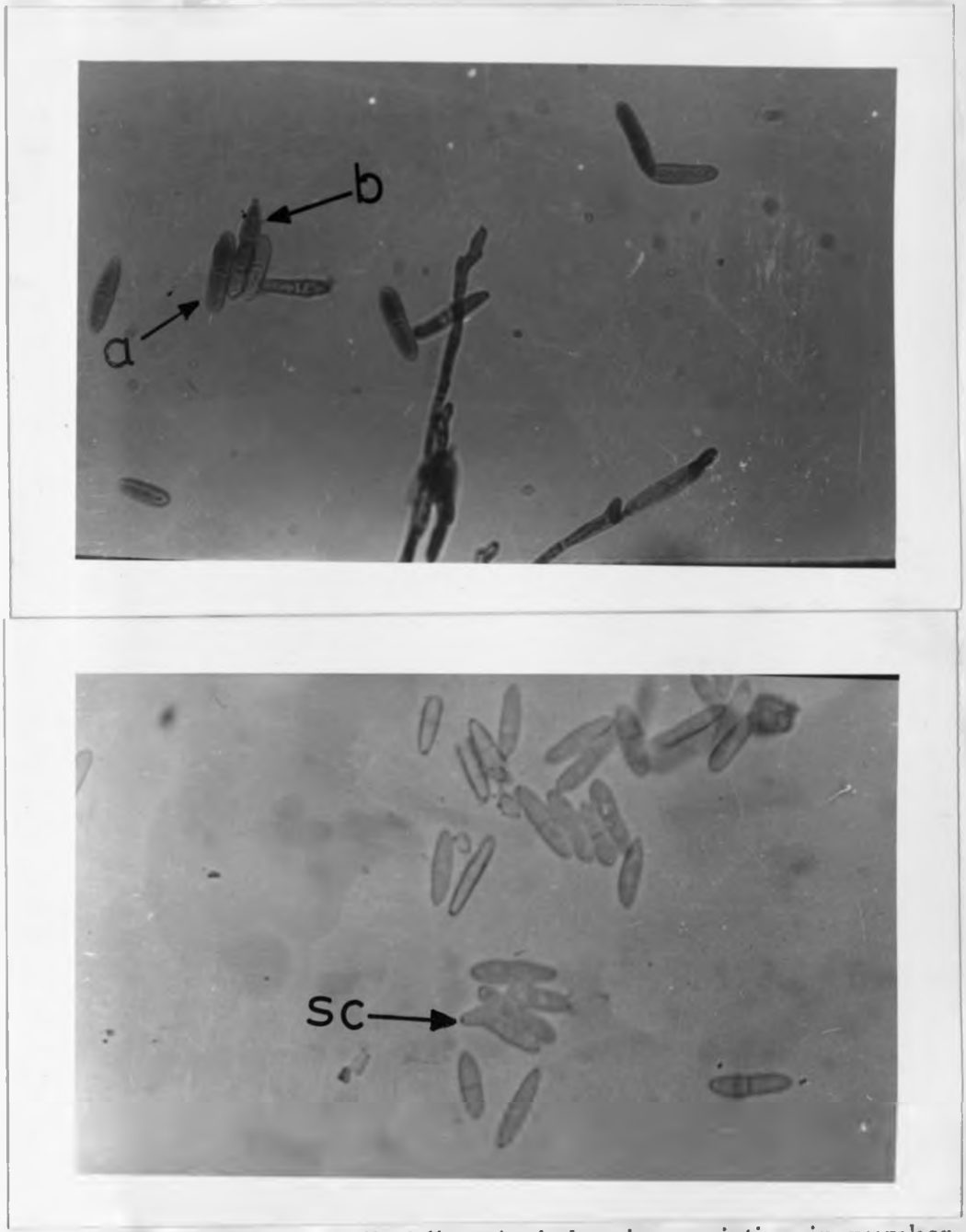

Plate 1a: Conidia of *Mycovellosiella cajani* showing variation in number of septa (a: 1 septa, b: 2 septa), shape, and conidial scar (sc). Magnification x 620 μ.

Plate 1b: Conidiophore (b) of *Mycovellosiella cajani* showing conidia (a) production. Magnification x 620 µ.

4.1.1..2. The effect of media on growth characteristics of *Mycovellosiella cajani*

(i) Mycelial growth characteristics

The size of the colonies in the different types of media used did not vary greatly. The small colonies that developed in different media did not allow for accurate assessment of colony growth rates. Ten days after inoculation the colony sizes measured 1 - 3 mm and there was no appreciable change in size by 22 days of plate incubation (Plate 2a).

Among the six media used in this study, good growth occurred on PLDA (1 - 3 mm), a fair amount of growth developed on CDA and PDA (1 - 3 mm) but fewer larger colonies as compared to PLDA while there was poor growth on PCA, CA and PMA which had colonies less than 1mm in size even after 22 days of incubation. Colonies on leaf decoction agar media (PLDA) were darker in colour than those growing on the other media tested. Colonies on PLDA and CDA media had dark brown to black pigmentation while those on PDA, PCA, CA and PMA had light brown to brown mycelia (Plate 2a).

(ii) Sporulation

On all the agar plates there were many conidia and conidiophore in proportion to the small sizes of colonies that developed. Results on sporulation level of *M. cajani* on the six different media are summarized in Table 1. Maximum sporulation occurred 14 days after plate inoculation on PMA and PLDA and after 18 days on CDA. Thereafter conidial production declined. On CA medium, sporulation was fairly constant from 18 days to 22 days whereas on PDA and PCA sporulation continued to increase from 10 days to 22 days of incubation.

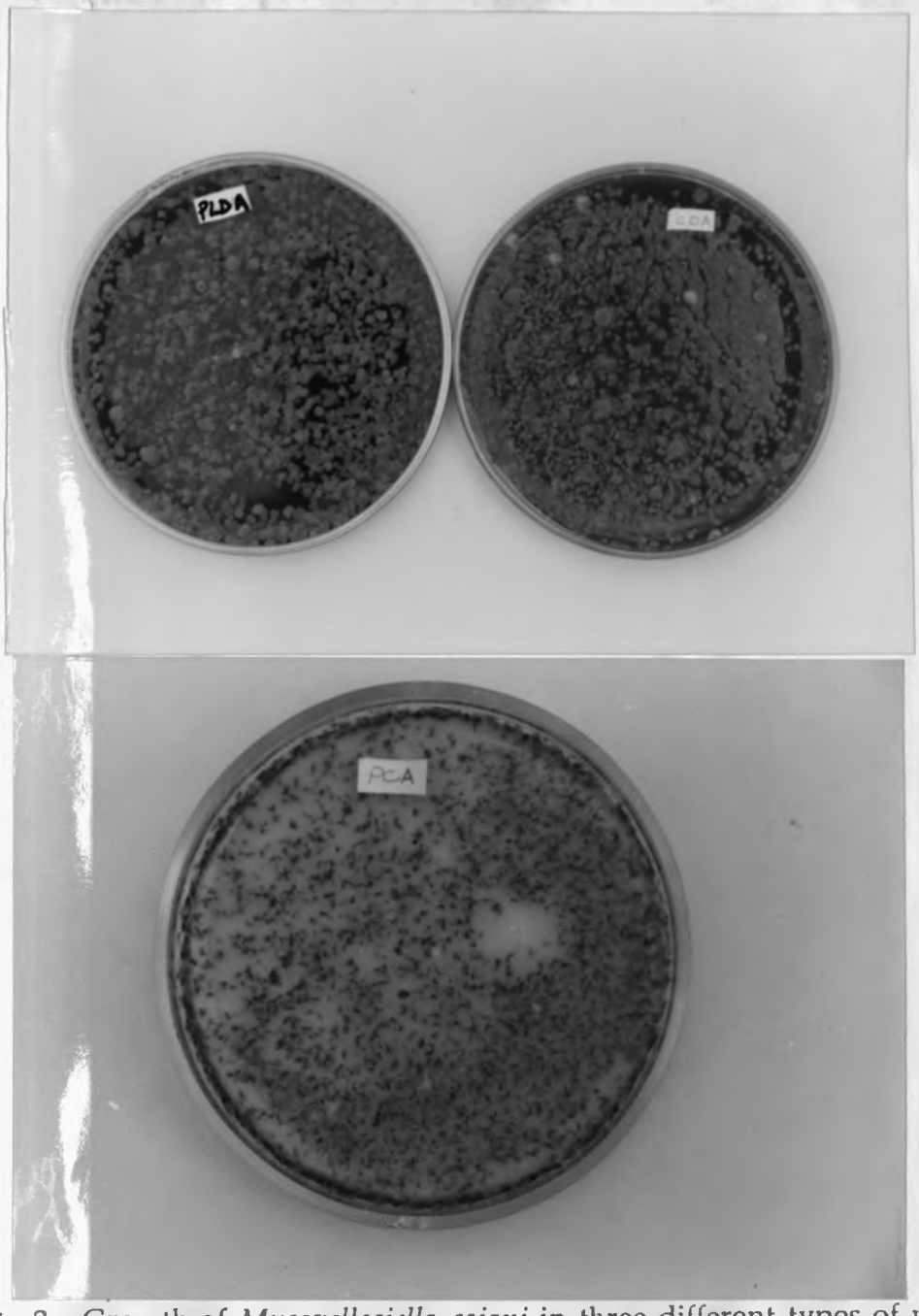

Plate 2a: Growth of *Mycovellosiella cajani* in three different types of media inoculated with conidial concentration of 2×10^4 conidia ml^{-1}.

Table 1. Effect of media and incubation period on sporulating capacity of *Mycovellosiella cajani*

	Days after plate inoculation (Time)				
Media	10	14	18	22	Mean
	Conidia ml-1 x 10'6				
PLDA	8.2	14.2	17.5	11.0	12.7
PDA	4.3	5.7	6.2	8.1	6.1
CDA	2.5	4.0	11.3	4.7	5.6
CA	0.1	1.3	1.6	1.8	1.2
PMA	1.8	2.3	1.3	1.2	1.7
PCA	0.5	1.2	1.7	3.3	1.6
Mean	2.8	4.8	6.6	5.0	4.8

	Media	Time	Interaction (Media x time)
LSD (0.05)	1.0	1.2	2.4
LSD (0.01)	1.3	1.6	3.2

Significant ($P < 0.01$) differences in conidial production were found among the time of recording and also among the formulated media. Some media enhanced conidia production of *M. cajani* while others after some days of incubation depressed it and thus interaction between time of recording and media was highly significant (Appendix 2(i)). Using LSD test for the means over the four incubation periods, it was found that conidial production in PLDA media was significantly ($P < 0.01$) higher than in all the other media tested. On PDA medium conidial production was not significantly ($P < 0.05$) different from that of colonies growing on CDA. However, sporulating capacities of *M. cajani* on these two media were significantly ($P < 0.01$) higher than on CA, PMA and PCA media.

4.1.1.3. Sporulating capacity of *Mycovellosiella cajani*

(i) Effect different conidial concentrations used for plate inoculation

Results on sporulation capacity of *M. cajani* when plates of PLDA medium were inoculated with six different conidial concentrations are summarized in Table 2. Initially, there were increases in the level of sporulation in all the different concentrations after plate inoculation. Thereafter conidial production showed a decline as time progressed especially in higher concentrations. In plates inoculated with conidial concentration of 2×10^4 conidia ml^{-1}, the sporulation level increased up to the 14 days after plate inoculation and then started to decline. Plates inoculated with conidial suspension of 2×10^3, 5×10^4, 2×10^5 conidia ml^{-1} attained maximum sporulation 18 days after plate incubation and thereafter showed a decline, while in the plates inoculated with conidial concentration of 2×10^1 and 2×10^2 conidia ml^{-1} the sporulation level continued to increase up to the 22 days of incubation.

Table 2. Effect of conidial concentration used for plate inoculation and incubation period on sporulation of Mycovellosiella cajani.

Conidial conc.	Incubation period (days)				
Conidia ml-1	10	14	18	22	mean
	(conidia ml-1 x 10'6)				
$2x10^1$	0.0	0.1	0.1	3.0	0.8
$2 x10^2$	0.1	0.5	4.6	6.2	2.8
$2x10^3$	1.5	5.4	6.8	6.2	5.0
$2x10^4$	4.1	9.2	8.5	5.4	6.8
$5x10^4$	3.9	6.8	9.9	5.3	6.5
$2x10^5$	4.2	7.3	7.7	5.8	6.2
mean	2.3	4.9	6.3	5.3	4.7

	conidial conc.	Period	conidial conc. x period
LSD (0.05)	0.8	0.9	1.9
LSD (0.01)	1.0	1.3	2.5

Analysis of variance of data on sporulating capacity of culture plates initiated with different conidial concentration showed that the differences in sporulation among the different inoculum concentrations were significant ($P < 0.01$). There were also significant ($P < 0.01$) differences in sporulating capacity among the different periods of incubation and also the interactions among the periods of incubation and the inoculum concentrations (Appendix 2(ii)). The highest mean sporulation was obtained in plates inoculated with conidial concentration of 2×10^4 conidia ml^{-1} followed by 5×10^4, 2×10^5, 2×10^3, 2×10^2 conidia ml^{-1} and the least mean sporulation was obtained in plates inoculated with 2×10^1 conidia ml^{-1}.

Comparison of means at the end of the four sampling periods showed that sporulating capacity in plates inoculated with conidial concentrations 2×10^4, 5×10^4 and 2×10^5 conidia ml^{-1} were not significantly ($P < 0.05$) different, but their sporulating capacities were significantly higher than in plates inoculated with 2×10^1, 2×10^2, 2×10^3 conidia ml^{-1}. Sporulating capacity in plates inoculated with conidial concentration 2×10^1 conidia ml^{-1} was significantly ($P < 0.01$) lower than that of the plates inoculated with the other concentrations.

(ii) The effect of temperature on sporulation of *Mycovellosiella cajani*

Results on sporulation of *M. cajani* under seven different temperatures are summarized in Table 3. There was no colony growth observed at temperatures 5, 10, 30 and 35°C and thus no records on sporulation were taken. Colony growth occurred at 15°C and sporulation increased with time from 10 to 22 days after plate inoculation. At 20° and 25° C the fungal colonies showed a sporulation peak by the end of 10 days of incubation after which there was a decline. Differences in sporulation among the temperatures and the periods of incubation were significant ($P < 0.01$) and

Table 3. The effect of temperature and incubation period on sporulating capacity of Mycovellosiella cajani.

Temperature (°C)	Incubation period (days)				
	10	14	18	22	mean
	(conidia ml-1 x 10'6)				
5	0.0	0.0	0.0	0.0	0.0
10	0.0	0.0	0.0	0.0	0.0
15	0.9	1.8	3.8	5.5	3.0
20	10.9	8.3	6.4	2.9	7.2
25	8.0	5.2	4.7	2.3	5.0
30	0.0	0.0	0.0	0.0	0.0
35	0.0	0.0	0.0	0.0	0.0
Mean	2.8	2.2	2.1	1.5	2.2

	Temperature	period	Temp. x period
LSD (0.05)	0.4	0.5	1.1
LSD (0.01)	0.5	0.7	1.5

also the interaction between the periods of incubation and the different temperatures were significant ($P < 0.01$) (Appendix 2(iii)).

Comparison of mean sporulation at the end of the four incubation periods showed that the temperatures differed significantly ($P < 0.01$). Among the temperatures where growth took place, the highest sporulation level occurred in plates incubated at 20°C followed by 25°C. The least sporulation level was obtained in plates incubated at 15°C.

(iii) The effect of pH on sporulating capacity of *Mycovellosiella cajani*

Plate 2b shows colonies of *M. cajani* on PLDA plates adjusted to four different pH levels. Sporulation occurred in all the PLDA plates with different pH levels which ranged from acidic (pH 5) to alkaline (pH 10). Results are summarized in Table 4. Sporulation level in culture plates adjusted to pH 5 did not show any variation over the four periods of sampling. In culture plates adjusted to pH 6, 7 and 9 sporulation level increased with time and then started to decline after 18 days of incubation. In culture plates adjusted to pH 4, *M. cajani* showed an increase in conidial production upto 14 days after plate incubation and then started declining while at pH 8 and 10 sporulation level continued to increase from 10 days to 22 days after plate inoculation although at a much slower rate in culture plates at pH 10.

The pH effects on sporulating capacity of *M. cajani* were highly significant ($P < 0.01$). The effects of the incubation periods and the interaction between the pH levels and the incubation periods were also highly significant ($P < 0.01$) (Appendix 3(i)).

Comparison of mean sporulation at the end of the four incubation periods showed that sporulation capacity of *M. cajani* at pH 5 was significantly ($P < 0.01$) higher than at all the rest of the pH levels tested. Sporulating capacity of colonies in plates adjusted to pH 6 was not

Plate 2b: Cultures of *Mycovellosiella cajani* on PLDA plates at 4 pH levels initiated using 2×10^4 conidia ml^{-1}.

able 4. Effect of pH and incubation period on sporulating capacity of *Mycovellosiella cajani*.

pH levels	Incubation period (days) 10	14	18	22	Mean
	(conidia ml-1 x 10'6)				
4	4.7	7.4	6.2	5.3	5.9
5	9.3	9.1	9.5	9.7	9.4
6	5.8	7.7	7.8	7.4	7.2
7	6.6	7.4	7.9	5.4	6.8
8	4.4	4.6	6.8	8.8	6.1
9	1.7	3.1	5.6	3.4	3.5
10	2.1	2.5	2.6	3.4	2.6
Mean	4.9	6.0	6.6	6.3	5.9

	pH	Incub. period	pH x incub. period
LSD (0.05)	0.5	0.7	1.4
LSD (0.01)	0.7	0.9	1.9

significantly ($P < 0.05$) different from culture adjusted to pH 7 but was significantly ($P < 0.01$) higher than that in cultures adjusted to pH 4, 8, 9 and 10. Sporulation capacity in cultures adjusted to pH 7 was found to be significantly ($P < 0.01$) higher than in cultures adjusted to pH 4, pH 9 and pH 10. Sporulating capacity in cultures adjusted to pH 8 was not significantly different ($P < 0.05$) from that at pH 4 but was significantly ($P < 0.01$) higher than at pH 9 and 10. Sporulation capacity in cultures adjusted to pH 9 was highly significantly lower than in cultures adjusted to all the rest of the pH levels tested. The highest and the lowest sporulation occurred in culture plates adjusted to pH 5 and pH 10 respectively.

(iv) Effect of light regimes on sporulation of *Mycovellosiella cajani*

Results are summarized in (Appendix 3(ii). Conidial production occurred in all the three light regimes tested. Differences in sporulation among the light regimes were significant ($P < 0.05$) while the incubation periods and the interaction between the incubation periods and the light regime were highly significant ($P < 0.01$) Appendix 3(iii). The mean sporulation level at the different incubation periods revealed that sporulation level increased to a maximum and then started to decline with time. Ten days after plate inoculation, sporulation was highest in the culture plates that had been incubated in 24 hours light. Fig. 1 shows that ten days after plate inoculation, the plates incubated in 24 hour light regime had already reached maximum sporulation level after which the conidial production started to decline. Sporulation in the culture plates incubated in the 24 hour darkness regime and 12 hour light /12 hour darkness continued to increase up to the 14 days after which there was a decline. The decrease in the level of conidia production was very rapid in

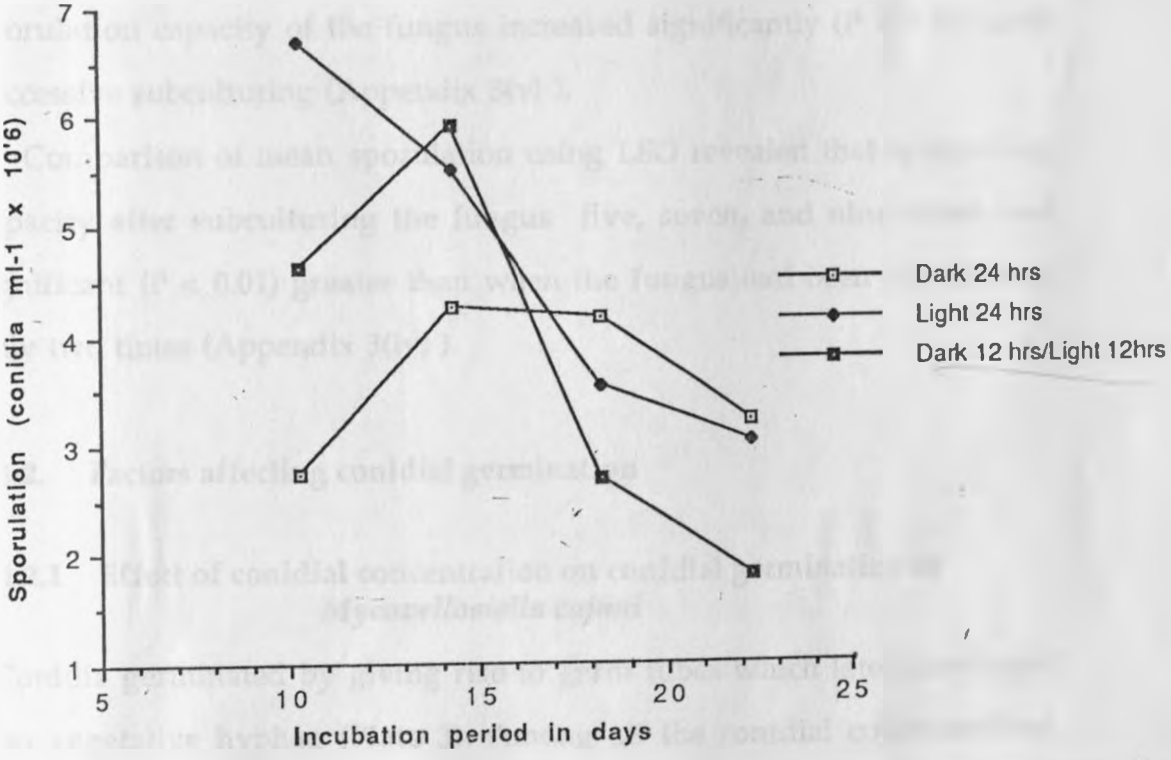

Fig. 1. Effect of three light regimes and incubation period on sporulation of Mycovellosiella cajani.

the plates incubated in 24 hour light regime and 12 hour light /12 hour darkness while the decline noted in culture plates incubated in 24 hours darkness was gradual.

(v) Effect of subculturing on sporulating capacity of *Mycovellosiella cajani*

It was observed that the sporulating capacity of *M. cajani* increased with successive subculturing (Fig 2). Analysis of variance of data obtained after subculturing the fungus 2, 5, 7, and 9 times after isolation showed that sporulation capacity of the fungus increased significantly ($P < 0.01$) with successive subculturing (Appendix 3(v)).

Comparison of mean sporulation using LSD revealed that sporulating capacity after subculturing the fungus five, seven, and nine times was significant ($P < 0.01$) greater than when the fungus had been subcultured only two times (Appendix 3(iv)).

4.1.2. Factors affecting conidial germination

4.1.2.1 Effect of conidial concentration on conidial germination of *Mycovellosiella cajani*

Conidia germinated by giving rise to germ tubes which later developed into vegetative hyphae (Plate 3). Among all the conidial concentrations used, no germination was observed after 3 hours of incubation. Conidial concentration and time of incubation influenced percent germination of *M. cajani* and their interaction was significant ($P < 0.01$) (Appendix 4(i)). The percent of germinated conidia continued to increase with time in all the different conidial concentrations tested.

Great variation in conidial germination was observed 4 hours after incubation. The highest conidial germination was observed in concentration 2×10^5 conidia ml^{-1} (26.4%) followed by concentration 5×10^4 conidia ml^{-1} (21.2%). The lowest percent germination was observed

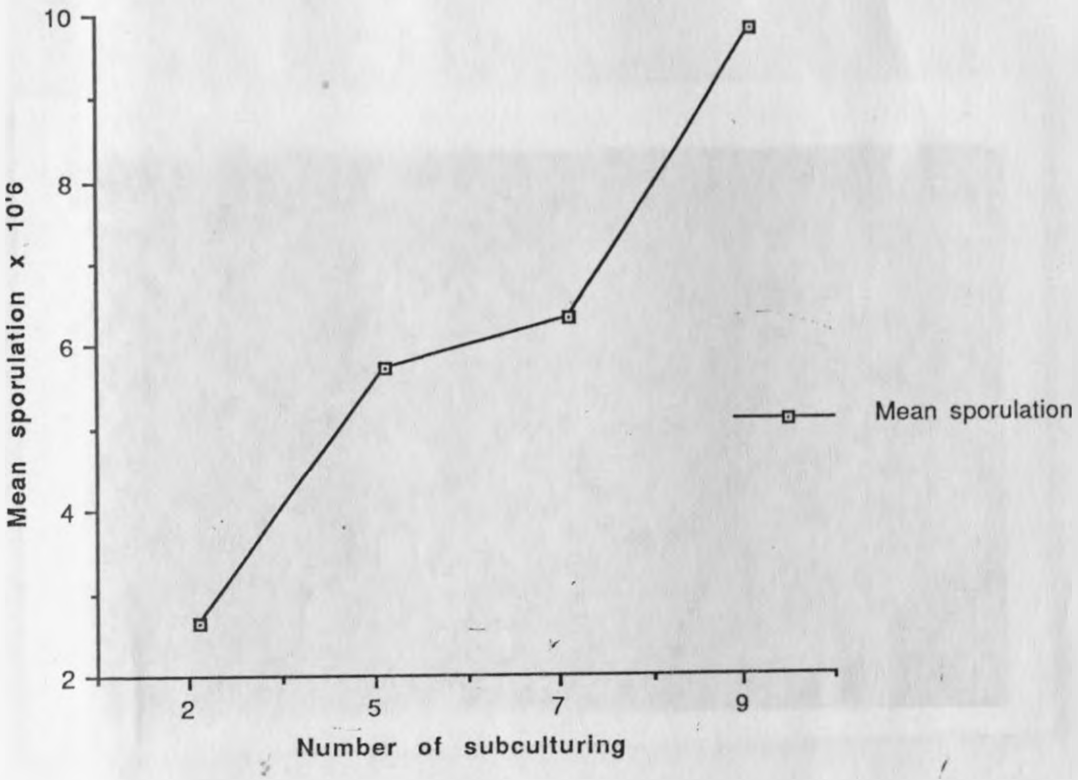

Fig. 2. Effect of subculturing on sporulating capacity of Mycovelloslella cajani

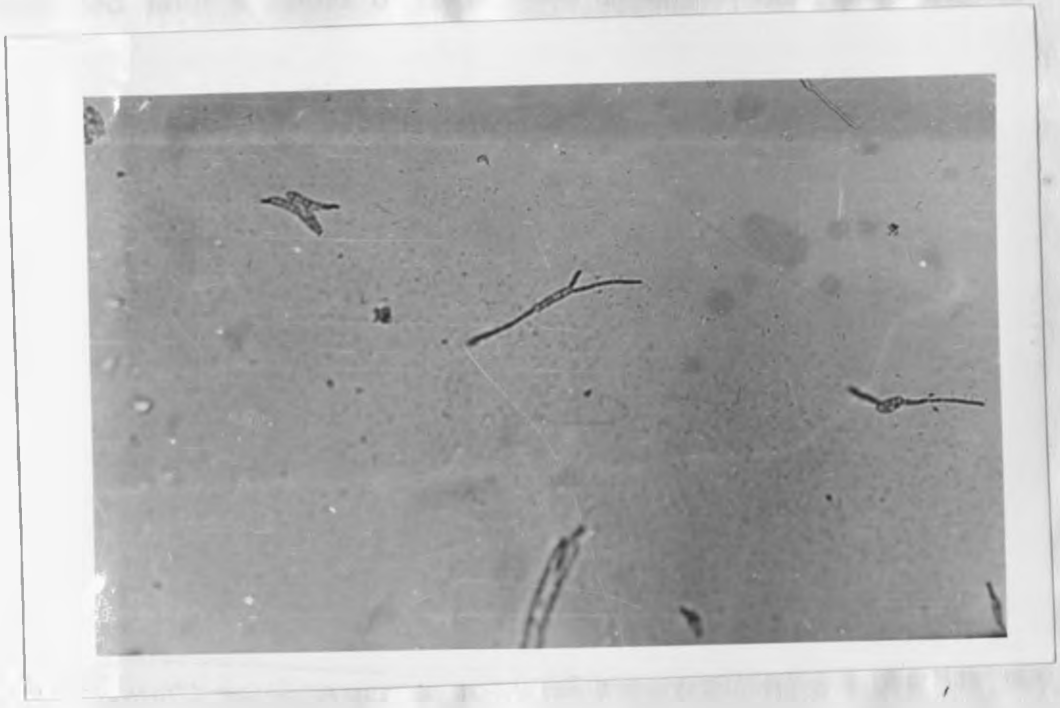

Plate 3: Conidial germination of *Mycovellosiella cajani* at 12 hours of incubation. Magnification x 440 µ.

in concentration 2×10^6 conidia ml^{-1} (4.0%). Percent conidial germinated in concentration 2×10^4 conidia ml^{-1} was (5.3%) which was second from the lowest.

The rate of conidial germination varied with the different concentrations and time of incubation. Percent conidia germinated after 8 hours of incubation was significantly ($P < 0.01$) higher than the percent conidia germinated after 4 hours of incubation among all the four conidial concentrations used (Table 5). The highest rate of germination after 8 hours was observed in concentration 2×10^6 conidia ml^{-1} (27.0%). Between 8 and 12 hours of incubation, the differences in percent conidia germinated were not significant ($P < 0.05$) among concentration 2 x 104, 5×10^4, 2×10^5 conidia ml^{-1} but was significantly ($P < 0.01$) different in conidial concentration 2×10^6 conidia ml^{-1}.

After 24 hours of incubation there were significant ($P < 0.01$) differences in the increase of percent conidia germinated up to 72 hours of incubation in the conidial concentration 2×10^4, 5×10^4, 2×10^5 conidia ml^{-1} but the differences in percent conidia germinated were not significant ($P < 0.05$) in 2×10^6 conidia ml^{-1}. After 48 to 72 hours of incubation, the percent conidia germinated among the concentration 5×10^4, 2×10^5 and 2×10^6 conidia ml^{-1} were not significantly ($P < 0.05$) different but were significantly ($P < 0.05$) higher than in concentration 2×10^4 conidia ml^{-1}.

The highest overall mean percent germination was found in conidial concentration 2×10^5 conidia ml^{-1} while the lowest was in concentraton 2×10^4 conidia ml^{-1}. The overall mean percent germination of different conidial concentration revealed that there were no significant differences ($P < 0.05$) between the concentrations 5×10^4, 2×10^5, and 2×10^6 conidia ml^{-1}. Percent germination in conidial concentration 2×10^4conidia ml^{-1}

Table 5. Effect of conidial concentration and incubation period on conidial germination of Mycovellosiella cajani.

Conidial conc. conidia ml-1	Incubation period (hrs)							
	3	4	8	12	24	48	78	Mean
	(percent germination)							
x 10^4	0.0	5.3	26.3	31.2	48.4	62.8	75.8	35.7
x 10^4	0.0	21.2	38.2	44.0	53.5	74.6	82.8	44.9
x 10^5	0.0	26.4	42.0	45.7	59.6	69.0	77.6	45.8
x 10^6	0.0	4.0	31.0	43.8	64.8	69.0	74.7	41.0
Mean	0.0	14.2	34.4	41.2	56.6	68.8	77.7	41.8

	Conidial conc.	Period	conidial conc. x period
LSD (0.05)	3.0	2.3	6.0
LSD (0.01)	4.0	3.0	8.0

was significantly ($P < 0.01$) lower than in all the other conidial concentrations tested.

4.1.2.2. Effect of temperature on conidial germination

The effect of temperature on spore germination is shown on Fig. 3. Varying temperatures affected lag period, germination rate and total percent germination. Percent conidia germinated data showed that the effects of temperatures, period of incubation and their interactions were highly significant ($P < 0.01$) (Appendix 5(i)).

Out of the eight temperature regimes investigated, conidial germination was not observed in plates incubated at temperatures 0°, 5°, 10° and 35°C. Conidial germination was not observed even after 3 hours of incubation at any temperature regimes tested. Temperatures 15°, 20°, 25° and 30°C supported conidial germination although germination was not observed at 15°C at 4 hours of incubation. Generally the percent conidial germination was low among conidia incubated at 15°C and 30°C with overall mean of 11.7 and 3.7 percent respectively. The highest conidial germination was observed at 25°C (86.5%) and the lowest at 30°C (11.0%) after 72 hr of incubation. The second and the third highest germination were at 20°C (81.7%) and 15°C (29.9 %) respectively.

Among all the temperatures of incubation, the rate of conidial germination increased with time of incubation. Conidia incubated at 15°C showed significantly ($P < 0.01$) higher increase in conidial germination than conidia incubated in the other incubation temperatures upto 48 hours of incubation. Between 48 and 72 hours of incubation the difference in percent conidial germinated also was significant ($P < 0.05$).

The percent conidia germinated at 20°C and 25°C increased with time of incubation but after 24 hours of incubation the increase was not significant ($P < 0.05$). Percent conidial germination at 25°C was significantly ($P < 0.01$) higher than at 20°C. Comparison of overall means of percent conidial

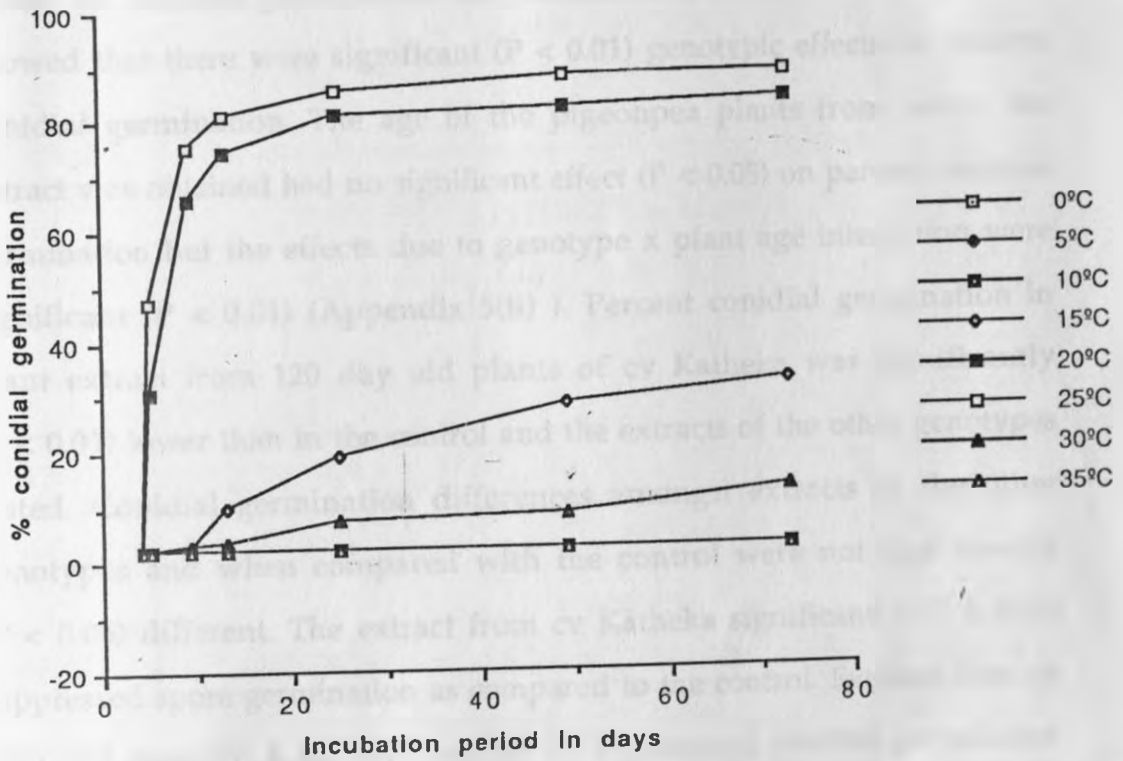

Fig 3. Effect of temperature and incubation period on conidial germination of Mycovellosiella cajani

germination showed that all the temperatures differed significantly ($P < 0.01$) from each other (Appendix 4(ii)). Conidial germination at 20°C and 25°C was generally high with overall mean of 58.0 and 64.9 percent respectively.

4.1.2.3. Effect of pigeonpea leaf extract on conidial germination of *Mycovellosiella cajani*

Results on the effect of pigeonpea leaf extract from 15 and 120 day old plants on conidial germination are summarized in Table 6. The results showed that there were significant ($P < 0.01$) genotypic effects on percent conidial germination. The age of the pigeonpea plants from which the extract was obtained had no significant effect ($P < 0.05$) on percent conidial germination but the effects due to genotype x plant age interaction were significant ($P < 0.01$) (Appendix 5(ii)). Percent conidial germination in plant extract from 120 day old plants of cv Katheka was significantly ($P < 0.01$) lower than in the control and the extracts of the other genotypes tested. Conidial germination differences amongst extracts of the other genotypes and when compared with the control were not significantly ($P < 0.05$) different. The extract from cv Katheka significantly ($P < 0.01$) suppressed spore germination as compared to the control. Extracts from cv ICPL 295, lines TK-3, KZ 13/2 and K0 71/2 promoted conidial germination while extract from cvs NPP 673/3, NPP 670 and line KB 91/1 suppressed conidial germination but not significantly different ($P < 0.05$) from the control.

Percent conidial germination in plant extract from 15 day-old plants of cv ICPL 295 was found to be highly significantly lower than in the control. Extract from lines KB 91/1 and KZ 13/2 significantly ($P < 0.05$) lowered percent conidial germination while extract from lines TK-3, K0 71/2 and cvs NPP 673/3, NPP 670 and Katheka had no significant ($P < 0.05$) effect on

Table 6. Effect of pigeonpea leaf extract from 15 and 120 day old plants on conidial germination of Mycovellosiella cajani.

Genotype	Plant age (days) 15	120	Mean
	(percent conidial germination)		
TK-3	86.2	84.1	81.1
ICPL 295	39.4	83.0	61.2
Katheka	77.2	51.2	64.2
KB 91/1	58.9	78.8	68.9
KZ 13/2	96.3	85.3	90.8
NPP 673/3	95.4	77.6	86.5
KO 71/1	87.9	88.6	88.3
NPP 670	90.6	74.3	82.5
Control (distilled water)	80.1	80.1	80.1
Mean	79.0	78.2	78.6

	Age	Genotype	Age x Genotype
LSD (0.05)	11.4	5.4	16.1
LSD (0.01)	15.2	7.2	21.5

percent conidia germinated as compared to the control. The highest percent conidial germination was obtained in the extract from KZ 13/2 (96.3%), followed by NPP 673/3 (95.4%), NPP 670 (90.7%), K0 71/2 (87.9%), TK-3 (86.2%), control (80.1%), Katheka (77.2%), KB 91/1 (58.9%), and the lowest germination was recorded in the extract from ICPL 295 (39.4%).

Extracts from cv ICPL 295 and line KB 91/1 significantly ($P < 0.05$) suppressed conidial germination than the control. Extract from cv Katheka also suppressed conidial germination but not significantly ($P < 0.05$) lower than the control. Extracts from line KZ 13/2 significantly ($P < 0.05$) promoted conidial germination when compared to the control while extracts from lines KO 71/2, TK-3, cvs NPP 670, NPP 673/3 also promoted conidial germination but not significantly ($P < 0.05$) different from the control.

4.2 GLASSHOUSE EXPERIMENTS

4.2.1 Pathogenicity test

Characteristic *Mycovellosiella* leafspot symptoms were first observed 12 - 14 days after plant inoculation. Thirty five days after plant inoculation the lesions had enlarged to 1 - 3 mm diameter and heavily sporulating. Conidia of *Mycovellosiella cajani* were re-isolated from sporulating lesions.

4.2.2 Host-pathogen relationship

(i) Pre–penetration and penetration events

Conidia on leaf surface germinated within the first six hours with germtubes emerging from the cells. Each conidium, usually had 1 or 2

germtubes (Plate 4a). However on rare occasions more than 2 germtubes per conidium were formed. Although most of the conidia had germinated by the end of 12 hour period, penetration was observed 24 hours after inoculation. Germtubes did not form appressoria and penetration was accomplished through stomata (Plate 4b).

Some germtubes passed near the stomata without penetrating through them but the observations made under light microscope did not provide any evidence for direct penetration.

(ii) Symptomatology

The fungus *Mycovellosiella cajani* attacked all the plant parts above the ground except the flowers. On the leaves, the first evidence of the disease was either the appearance of a bleached irregular spots or dark brown to black spots of less than 1 mm in diameter which were seen only on the upper surface of the leaf. The black spots were numerous or isolated on the leaflets and with or without a halo around them. The leaves turned yellow and dropped without further enlargement of the spots. On yellowing it was noticed that the areas around the spots remained green. (Plate 5a & b)

In some cases the leafspot spread to form a circular lesion of up to 9 mm in diameter or more. The larger spots were dark brown or grey. Some spots had concentric rings while others did not. Sometimes the spots were surrounded by a yellowish halo and coalesced with each other thus becoming irregular in shape. Infections along the veins and veinlets caused raised elongated, brown lesions (Plate 5b).

At the beginning only leafspots were common but later lesions developed on the stems, petioles and pods. On the petioles, stems and pods, the spots were dark and mainly circular or slightly elongated. Later these lesions developed grey centres (Plate 5c).

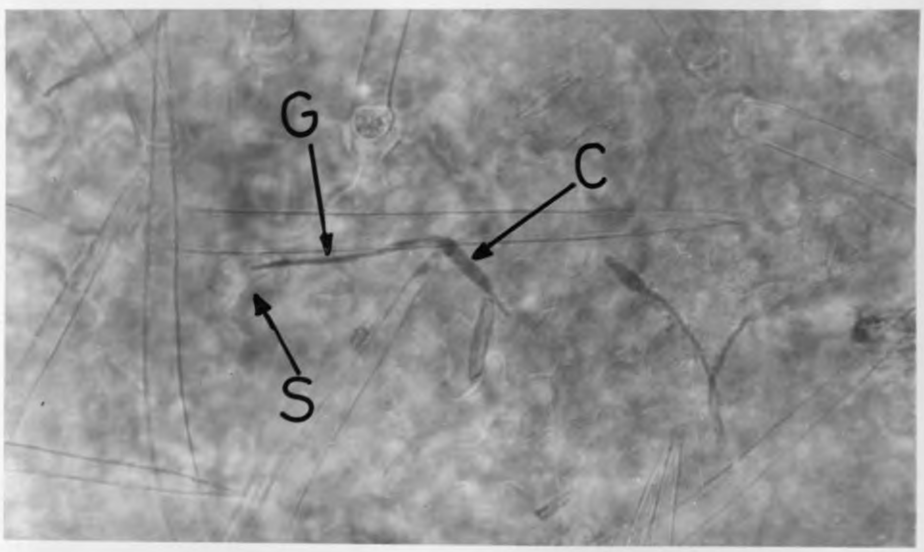

Plate 4a: Germinating conidia (C) of *Mycovellosiella cajani* on cv NPP 673/3 leaf surface with germtube (G) approaching the stoma (S) at 24 hours after leaf inoculation. Magnification x 620 µ.

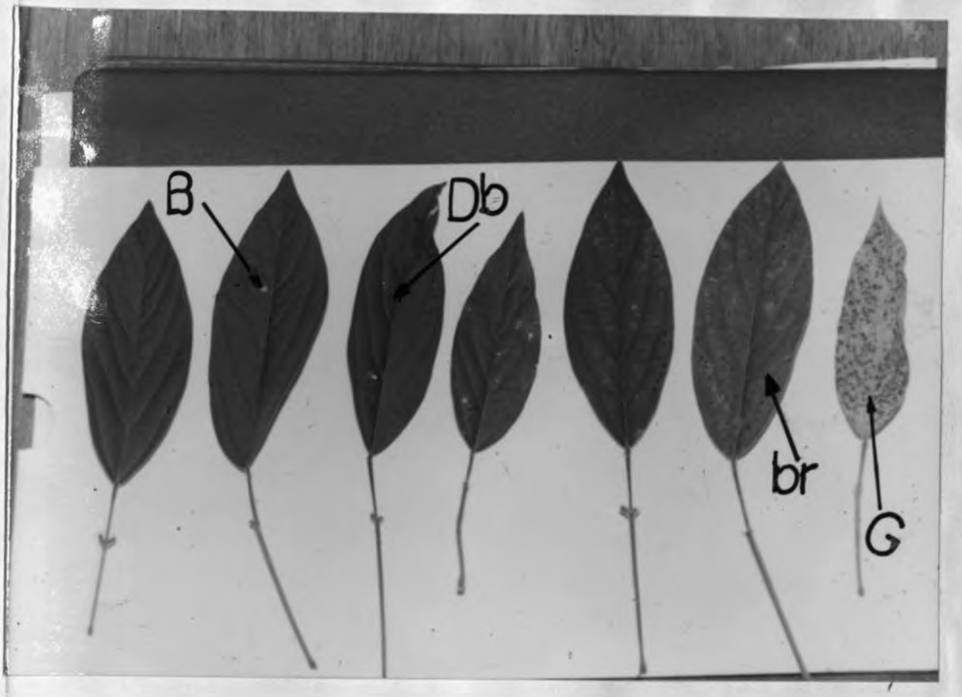

Plate 5a: Symptoms of *Mycovellosiella* leafspot on *Cajanus cajan* leaflets of cv NPP 670. Note the bleached spots (B), the dark brown (Db) and brown (br) spots, and the green islands (G) on the chlorotic leaflet.

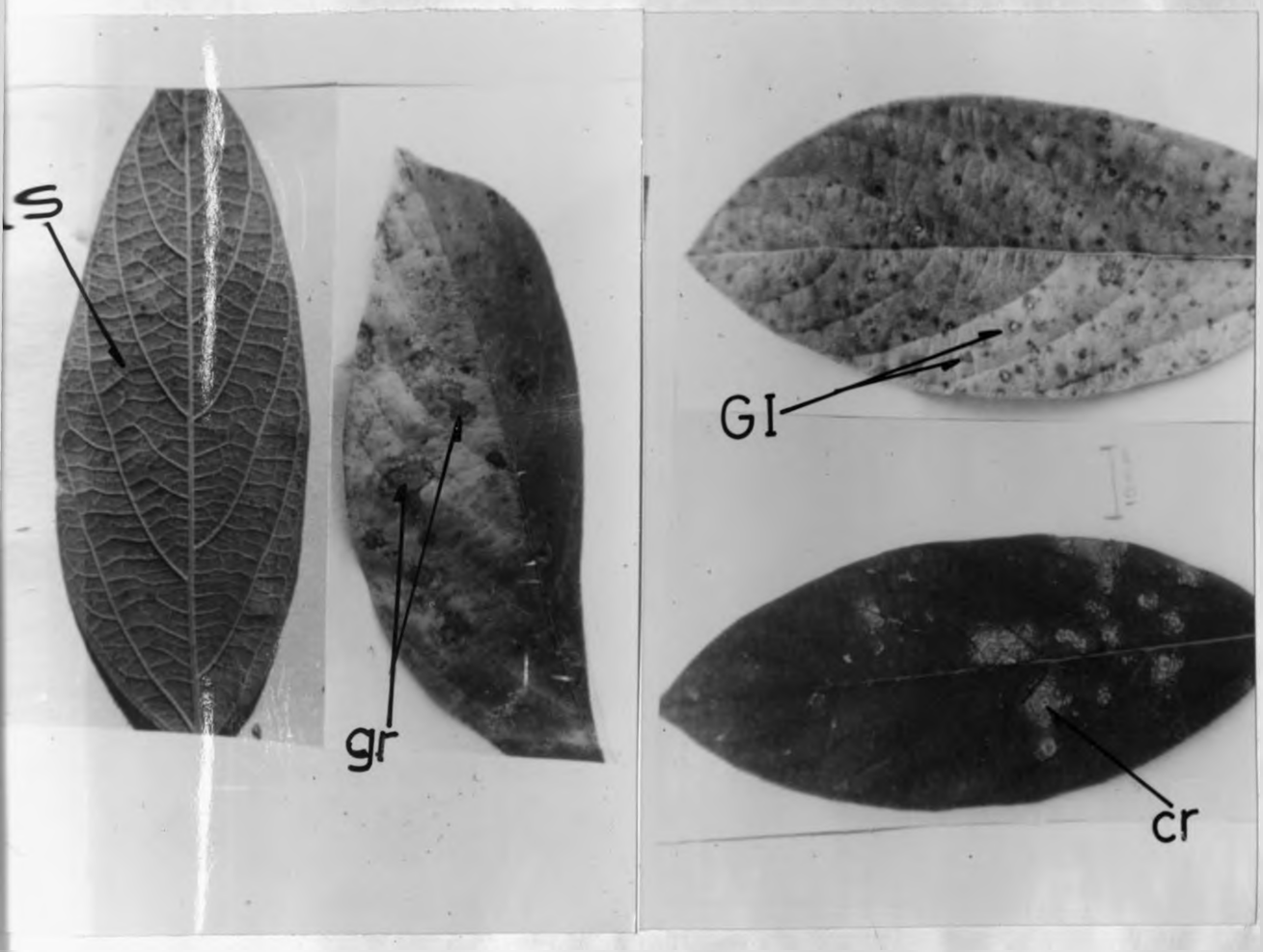

Plate 5b: Leaflets of cv NPP 670 showing symptoms on the lower (l.s.) and upper surface – grey areas on the centre of the spot (gr), green islands around spots on yellowing leaves (GI) and concentric rings (cr).

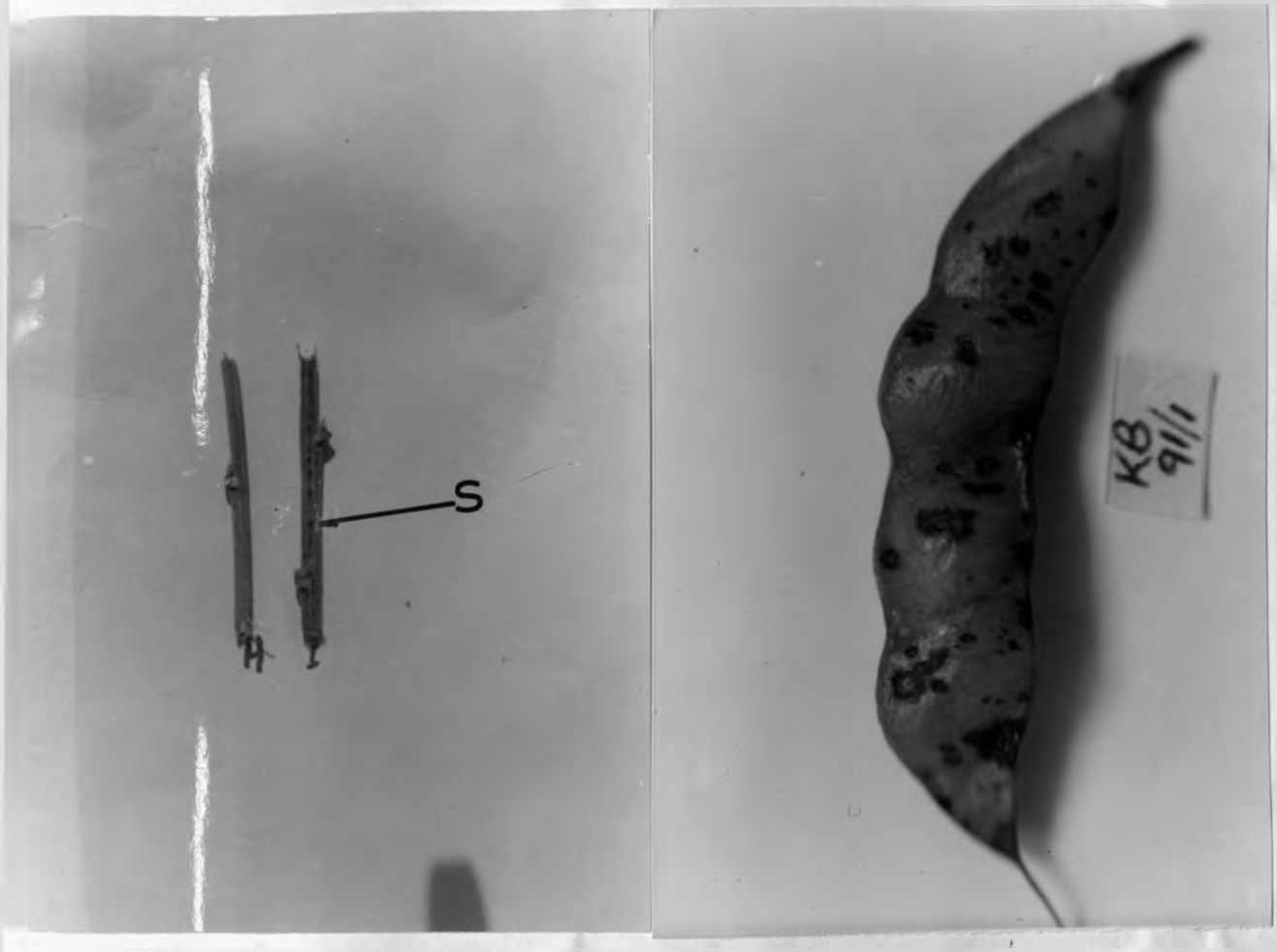

Plate 5c: Symtoms of *Mycovellosiella* leafspots on stem and pods of line KB 91/1. Healthy stem (H) has no dark spots on it while the infected stem (I) has dark spots (S).

In the field lower leaves got infected first and infection progressed upwards to top leaves. Among the short genotypes such as cv NPP 670 at Kabete field, the disease progressed very rapidly and most of the leaves became heavily infected regardless of their position on the canopy from the ground level. In the glasshouse inoculated plants, it was observed that in cv TK-3, the leaves on the upper part of the canopy were just as susceptible as the leaves on both the middle and the lower part of the canopy. The disease caused severe defoliation both in the glasshouse and in the field. In most cases the leaves dropped before turning yellow. Yellowing of leaves was not related to the number of spots on the leaves. Some leaves turned yellow and defoliated although they had few isolated spots (Plate 5d, 5e & 5f). In cases of very severe attack,the leaves of some genotypes developed blight symptoms. The disease caused grey dead spot or extended areas until the leaves were killed. These blighted areas first appeared as faded green patches which later turned to greyish lesion not delimited in size and enlarging rapidly in favourable weather.

The dead areas appeared at the tip or margin of the leaves and spread downwards or inwards. In moist weather, the leaves were shed but when dry weather followed the appearance of lesions the infection advanced slowly and the affected leaves curled. These blight symptoms were mainly observed at Kabete field in some early maturing genotypes, especially lines KB 91/1, TK-3 and cvs ICPL 295, NPP 670 and also on the glasshouse inoculated plants of ages 1 and 2 months.

4.2.3. Disease assessment scale

The leaf spotting severity was classified into six grades *i.e* 0 - 5 where 0 denotes absence of leaf lesion, chlorosis and defoliation and 5 indicates large lesion, severe chlorosis or blighting and severe defoliation (Table 7).

Plate 5d: Cultivar NPP 670 showing leaflets loss (defoliation) due to *Mycovellosiella* leafspot infection in the glasshouse.

Plate 5e: Line TK – 3 showing heavy defoliation (d) and yellowing leaflets (Y) due to *Mycovellosiella* leafspot infection in the glasshouse.

Plate 5f: Cultivar ICPL 295 plants showing defoliation (d) and leaf yellowing (Y) due to *Mycovellosiella* leafspot infection in the field at Kiboko.

Table 7. Disease assessement scale

Grades	Percent diseased leaf area	Description	Plant reaction Classification
0	0	No disease observed	immune
1	0.1 to 3	Infection in traces. 1-10 percent of the total foliage infected	Resistant (R)
2	3.1 to 6	Consisting of very small lessions less than 1mm diameter. 11-25 percent of the total foliage infected	Moderately resistant (MR)
3	6.1 to 12	Including small to medium size lesions 1-2mm in diameter or numerous small spots covering the surface of the leaf . Leaflet has chlorosis 26-50 percent of the total foliage infected with slight defoliation	Slightly susceptible (SS)
4	12.1 to 25	A certain area >12 percent of the leaflet infected or blighted, or consisting of medium to large lesions size 5-9mm mainly on older leaves, or leaves are covered by numerous spots and mild chlorosis. 51-75 percent of the leaves per plant have infections with moderate defoliation	Susceptible (S)
5	25.1 to >50	Indicating large sporulating lesions on leaf surface or leaves severe chlorotic or blighted, lesions covering more than 25 percent of the leaflet surface. 76-100 percent of the total foliage infected and heavy defoliation	Highly susceptible (HS)

Plates 6a - 6g show the scale that was developed for estimating intensity of pigeonpea leafspot. A series of photographs with measured levels of spotting were taken to aid in estimating percent of leaf destruction. The range of severity was mainly between 0.1 percent to less than 50 percent. Fifty percent was rarely reached both in the field and in the glasshouse because leaves dropped or turned yellow before reaching that stage.

4.2.4 Effect of inoculum concentration on the infection of pigeonpea plants by *Mycovellosiella cajani*

The non inoculated plants remained disease free while inoculated ones showed varying degree of leafspot severity. Fifteen days after plant inoculation the infection levels in plants inoculated with inoculum concentrations 2×10^4, 5×10^4, 2×10^5 conidia ml^{-1} were not significantly ($P < 0.05$) different from each other. Infection level in plants inoculated with 2×10^6 conidia ml^{-1} was significantly ($P < 0.05$) and highly significantly ($P < 0.01$) higher than that of plants inoculated with 5×10^4 conidia ml^{-1} and those inoculated with 2×10^4 conidia ml^{-1} respectively. Infection levels among plants inoculated with conidia concentration 2×10^5 and 2×10^6 conidia ml^{-1} were not significantly ($P < 0.05$) different from each other but they were significantly higher than in plants inoculated with 5×10^4 conidia ml^{-1} ($P < 0.05$) and 2×10^4 conidia ml^{-1} ($P < 0.01$) nineteen days after plant inoculation (Appendix 5(ii)).

Infection level increased with time among the conidial concentrations used (Fig 4). Analysis of variance of infection mean grade data showed that the differences due to inoculum concentrations, days after inoculation and their interactions were significant ($P < 0.01$) (Appendix 6(i)).

Thirty four days after plant inoculation, *Mycovellosiella* leafspot infection level on leaves inoculated with concentration 2×10^6 and 2×10^5 conidia ml^{-1} were not significantly ($P < 0.05$) different from each other but

Plate 6a: Grade 0– (0%) No infection

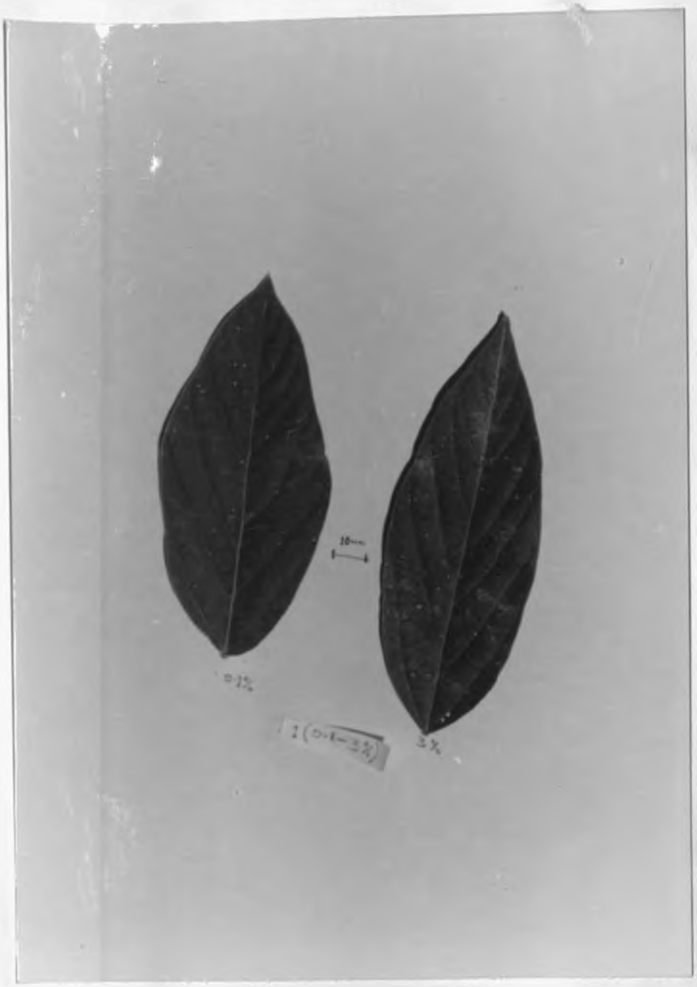

Plate 6b: Grade 1 - Leaf area diseased (0.1 - 3%)

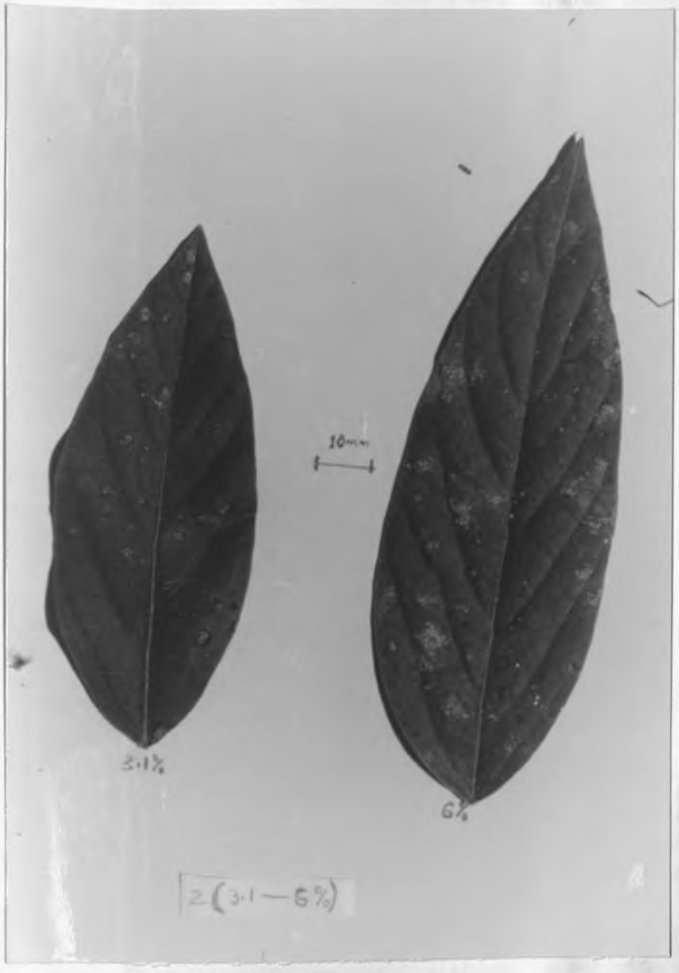

Plate 6c: Grade 2 - Leaf area diseased (3.1-6%)

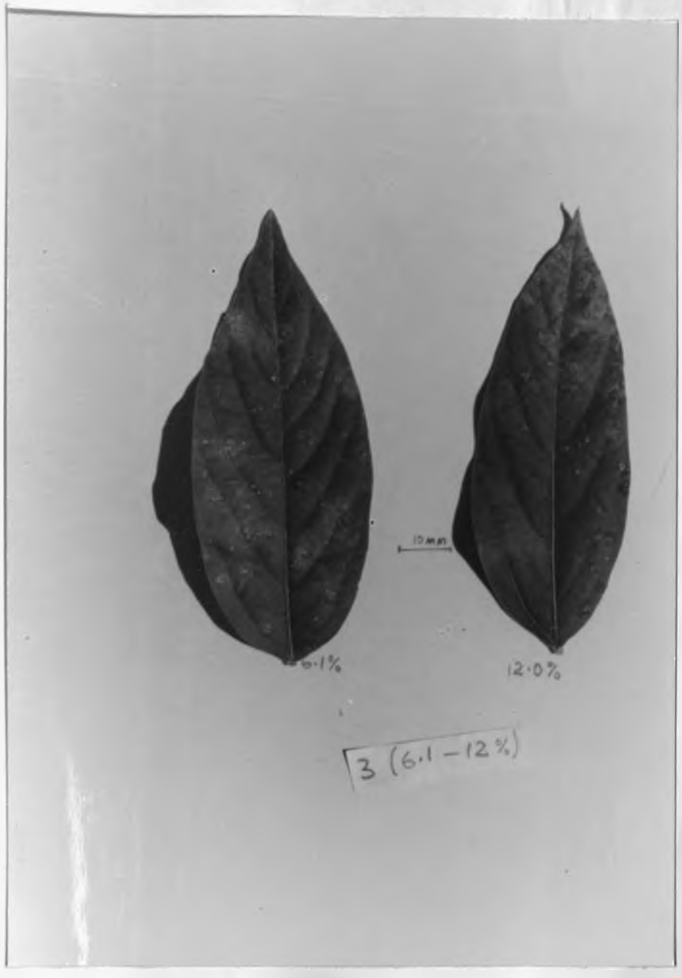

Plate 6d: Grade 3 - Leaf area diseased (6.1-12%)

Plate 6e: Grade 4 - Leaf area diseased (12.1-25%)

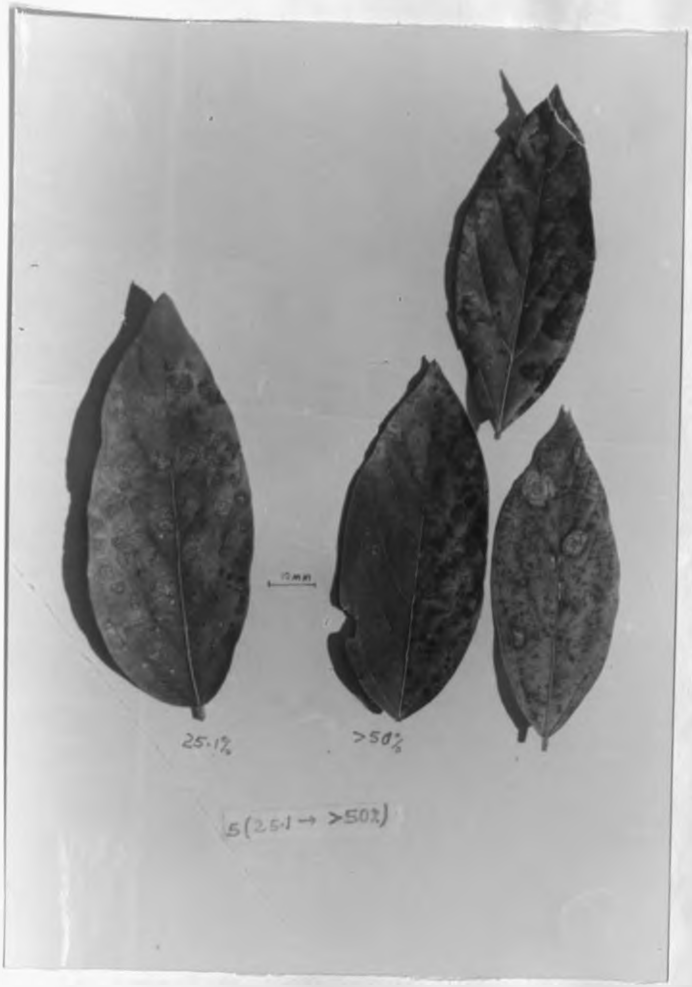

Plate 6f: Grade 5 - Leaf area diseased (25.1 - >50%)

Plate 6g: Infection grades based on percent diseased leaf area used in assessing seedling infection.

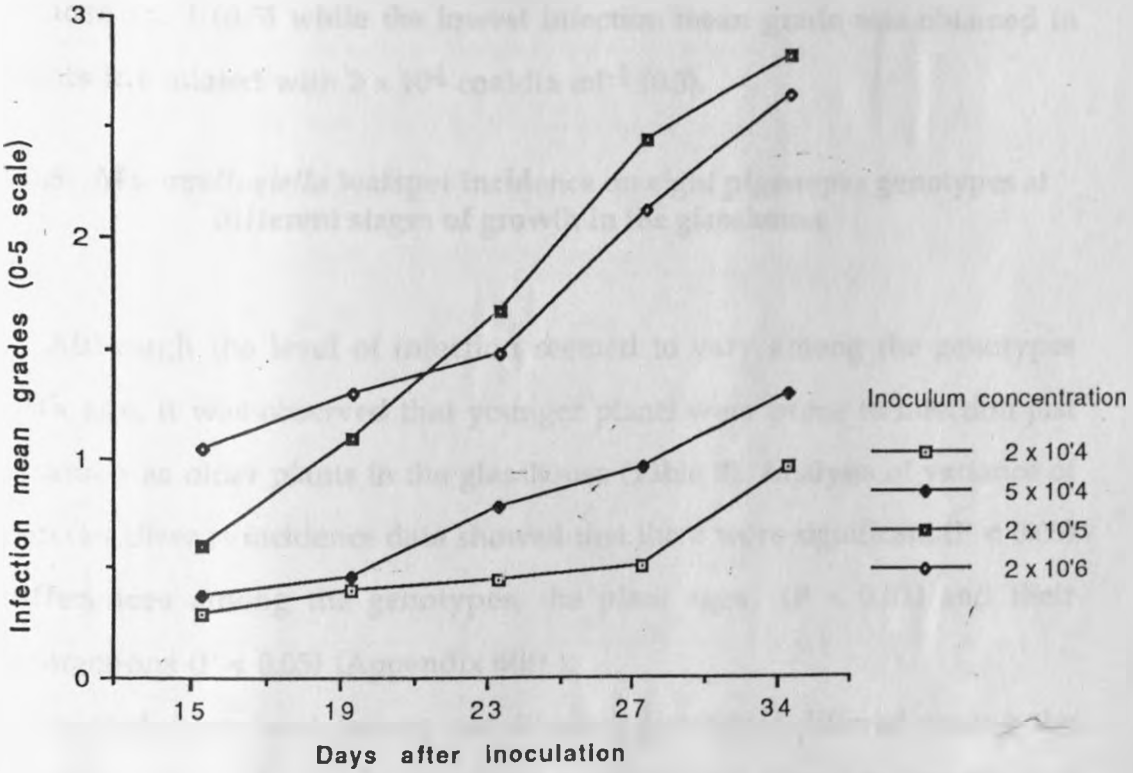

Fig. 4. Effect of inoculum concentration on development of Mycovellosiella leafspot.

were significantly ($P < 0.01$) higher than in those plants plants inoculated with 2×10^4 and 5×10^4 conidial ml^{-1}. Infection levels in plants inoculated with 2×10^4 and 5×10^4 conidia ml^{-1} were not significantly ($P < 0.05$) different from each other.

Comparison of overall means showed that the highest infection mean grade was obtained in plants inoculated with conidial concentrations 2×10^5 conidia ml^{-1} (1.7) and 2×10^6 conidia ml^{-1} (1.7) followed by 5×10^4 conidia ml^{-1} (0.5) while the lowest infection mean grade was obtained in plants inoculated with 2×10^4 conidia ml^{-1} (0.3).

4.2.5. *Mycovellosiella* leafspot incidence on eight pigeonpea genotypes at different stages of growth in the glasshouse

Although the level of infection seemed to vary among the genotypes with age, it was observed that younger plants were prone to infection just as much as older plants in the glasshouse (Table 8). Analysis of variance of percent disease incidence data showed that there were significant ($P < 0.05$) differences among the genotypes, the plant ages ($P < 0.01$) and their interactions ($P < 0.05$) (Appendix 6(ii)).

The infection level among the different genotypes differed among the different plant ages assessed. The lowest disease incidence in line K0 71/2 (53.1%), cv Katheka (52.4%), line TK-3 (50.9%), cv NPP 670 (50.9%) was found in 1 month old plants while line KB 91/1 (51.8%) had the lowest disease incidence in 2 months old plant. Line KZ 13/2 had its lowest disease incidence among 90 days old plant (53.8%), but there were no significant ($P < 0.05$) differences in infection levels between the 90 days and 60 days old plants. The disease incidence in 90 days old plant was significantly ($P < 0.05$) lower than in 30 and 120 days old plants. In some

Table 8. Incidence of Mycovellosiella leafspot on eight pigeonpea genotypes at different stages of growth in the glasshouse.

	Plant age in days				
Genotype	30	60	90	120	Mean
	(percent disease incidence)				
TK-3	50.9	82.6	72.8	85.6	73.0
ICPL 295	66.1	82.5	80.8	80.8	77.5
Katheka	52.6	73.0	78.6	67.9	68.0
KB 91/1	57.4	51.8	79.0	84.4	68.1
KZ 13/2	77.8	65.0	53.8	78.1	68.7
NPP 673/3	77.8	86.1	78.2	83.9	81.5
KO 71/2	53.1	77.1	73.9	89.3	73.4
NPP 670	50.9	71.0	71.8	59.9	63.4
Mean	60.8	73.6	73.6	78.7	71.7

	Genotype	Plant age	Genotype x Plant age
LSD (0.05)	6.9	9.7	19.4
LSD (0.01)	9.1	12.9	25.9

genotypes such as cv 670, low disease incidence was observed in younger plants and older plants which was 51.0 and 59.9 percent respectively while in line K0 71/2 the younger plants showed low disease incidence and the older plants showed high disease incidence which was 53.1 and 89.3 percent respectively. Among cvs ICPL 295 and NPP 673/3, no significant differences ($P < 0.05$) were found in percent disease incidence among the different plant ages assessed.

4.2.6. Seedlings reaction of eight genotypes to infection by *Mycovellosiella cajani*

Results on the reaction of pigeonpea seedlings to *M. cajani* are summarized on Table 9. Analysis of variance showed that the genotypes, days after inoculation and their interactions were significantly ($P < 0.01$) different (Appendix 6(iii)).

The mean genotypes infection grades revealed that infection level increased with time after seedling inoculation. In some genotypes such as cv NPP 670 defoliation started 23 days after inoculation. No significant ($P < 0.05$) disease increase was observed after 23 days of inoculation. For some genotypes such as line KB 91/1 and cv ICPL 295, the infection level remained relatively low as compared to the other genotypes with infection mean score of 2.9 and 3.0 respectively. Significant ($P < 0.05$) increases in the level of infection in these two genotypes were observed up to 27 days after plant inoculation. For assessing susceptibility or resistance of genotypes, data collected 23 days after plant inoculation when the infection level were high among most genotypes and before defoliation started was used. The results showed that line KB 91/1 was moderately resistant, cv ICPL 295 slightly susceptible, lines KZ 13/2 and TK-3 susceptible and cvs NPP 673/3, Katheka, NPP 670 and KO 71/2 were highly susceptible.

Table 9. Seedlings reaction of eight pigeonpea genotypes to infection by *Mycovellosiella cajani*

Genotype	Days after plant inoculation (Period)						
	17	19	21	23	25	27	Mean
	Infection mean grade (0-5 scale)						
TK-3	0.9	1.5	2.9	3.5	4.0	4.4	2.9
ICPL 295	1.5	2.1	2.2	2.4	2.7	3.0	2.3
Katheka	1.1	2.4	4.0	4.5	4.9	5.0	3.7
KB 91/1	0.0	1.6	1.6	1.9	2.6	2.9	1.8
KZ 13/2	2.1	2.9	3.6	4.0	4.5	4.6	3.6
NPP 673/3	1.2	2.5	4.3	4.6	5.0	5.0	3.8
KO 71/2	0.9	2.2	3.5	4.4	4.6	4.8	3.4
NPP 670	0.9	2.7	4.1	4.6	4.8	4.8	3.7
Mean	1.9	2.2	3.3	3.8	4.1	4.3	3.1

	Genotype	Period	Genotype x Period
LSD (0.05)	0.1	0.1	0.3
LSD (0.01)	0.1	0.1	0.3

The overall mean revealed that the infection level on cv NPP 673/3 was significantly higher than that on cvs NPP 670 and Katheka ($P < 0.05$) and also than that on the rest of the genotypes assessed ($P < 0.01$). Infection levels on line KB 91/1 and cv ICPL 295 were significantly ($P < 0.05$) lower than that on the rest of the genotypes assessed while infection levels in line KZ 13/2, cvs NPP 670 and Katheka were not significantly ($P < 0.05$) different from each other. The highest infection mean grade was obtained in cv NPP 673/3 (3.8) followed by Katheka, NPP 670, K0 71/2, KZ 13/2, TK-3, ICPL 295. The least infection mean grade was obtained in line KB 91/1 (1.8).

4.2.7 Progress of *Mycovellosiella* leafspot on eight pigeonpea genotypes in the glasshouse at flowering stage

Analysis of variance of disease severity data showed that the genotypes, days after inoculation and their interactions were significantly ($P < 0.01$) different (Appendix 7(i)). Disease severity increased with time after plant inoculation. Between 19 and 23 days after plant inoculation there was heavy defoliation among the genotypes (Fig. 5). Some of the pigeonpea genotypes such as cv ICPL 295, lines KB 91/1 and TK-3 shed most of their highly infected leaves hence there is a decrease in disease severity after 23 days of plant inoculation. The disease severity continued to increase on other leaves left on the plant. In some of the genotypes such as cv NPP 673/3 and line KZ 13/2, disease severity continued to increase although the plants were defoliating because the leaves that remained on the plants were also highly infected.

Disease development was slowest in cv NPP 670 and highest in cv ICPL 295 (Fig. 5). After 25 days of plant incubation , the highest disease severity score was obtained in cv NPP 673/3. This cultivar retained highly infected leaves longer than the other genotypes tested.

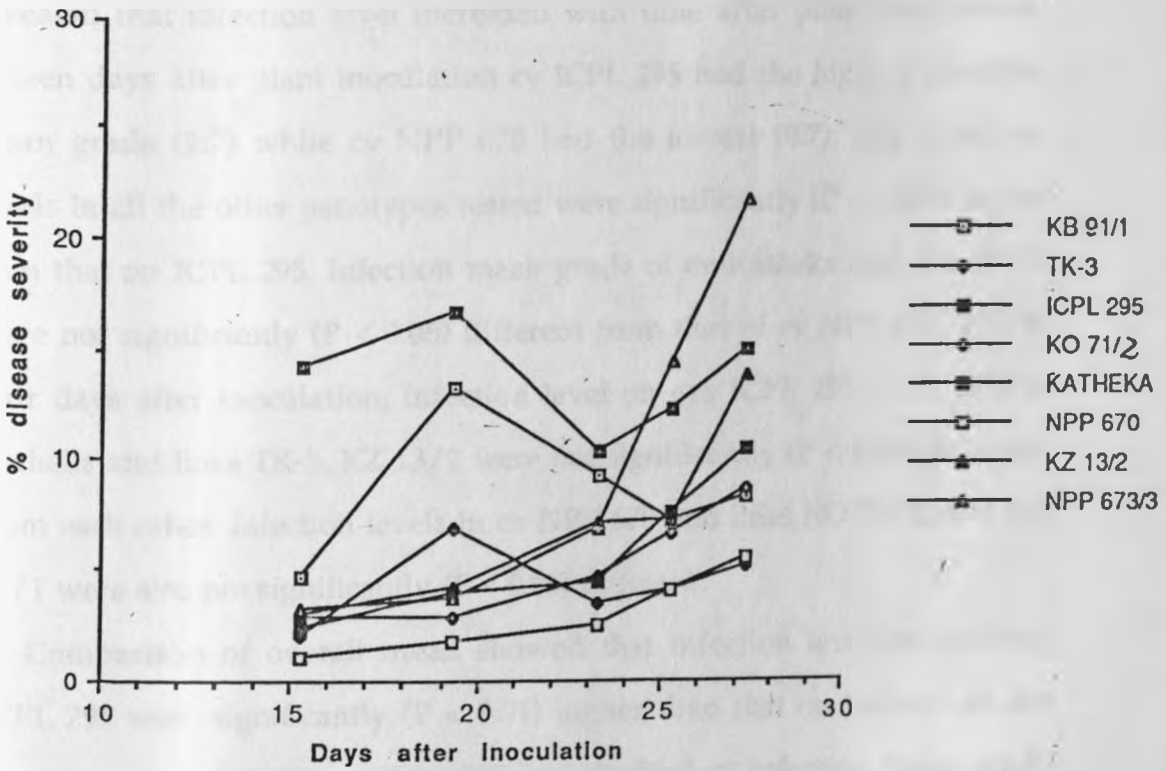

Fig. 5. Progress of Mycovellosiella leafspot on eight pigeonpea genotypes in the glasshouse

4.2.8 Reaction of eight pigeonpea genotypes to *Mycovellosiella cajani* at flowering stage

Results are summarized in Table 10. Analysis of variance of disease infection mean grades data showed that the genotypes, days after plant inoculation and their interactions were significantly ($P < 0.01$) different (Appendix 7(ii)). The data indicated that the infection levels in the different genotypes of pigeonpea varied with date of recording of disease infection level. The mean infection grade at different assessment dates revealed that infection level increased with time after plant inoculation. Fifteen days after plant inoculation cv ICPL 295 had the highest infection mean grade (2.7) while cv NPP 670 had the lowest (0.7). The infection levels in all the other genotypes tested were significantly ($P < 0.05$) lower than that on ICPL 295. Infection mean grade of cv Katheka and line TK-3 were not significantly ($P < 0.05$) different from that of cv NPP 670. Thirty four days after inoculation, infection level on cvs ICPL 295, NPP 673/3, Katheka and lines TK-3, KZ 13/2 were not significantly ($P < 0.05$) different from each other. Infection levels in cv NPP 670 and lines KO 71/2, and KB 91/1 were also not significantly ($P < 0.05$) different.

Comparison of overall mean showed that infection level on cultivar ICPL 295 was significantly ($P < 0.01$) higher than that on the rest of the genotypes tested. Cultivar ICPL 295 had the highest infection mean grade of 4.0, followed by NPP 673/3, KZ 13/2, KB 91/1, Katheka, KO 71/2, TK–3 while NPP 670 had the least mean infection grade of 2.2. Infection mean grade of cv NPP 670 was significantly ($P < 0.01$) lower than that of Katheka, ICPL 295, KB 91/1, KZ 13/2, KO 17/2, NPP 673/3 and TK-3.

The pigeonpea genotypes were graded 19 days after plant inoculation for their reaction to *M. cajani*. Cultivar NPP 670 was classified as moderately

able 10. Reaction of eight pigeonpea genotypes to Mycovellosiella cajani at flowering stage in the glasshouse.

	Days after plant inoculation					
Genotype	15	19	23	27	34	Mean
	Infection mean grade (0-5 scale)					
TK-3	0.8	2.3	2.7	3.2	4.5	2.7
ICPL 295	2.7	3.8	4.1	4.6	4.8	4.0
Katheka	1.0	2.4	3.0	3.7	4.4	2.9
KB 91/1	1.8	2.5	3.1	3.5	3.8	2.9
KZ 13/2	2.0	2.6	3.1	3.7	4.4	3.2
NPP 673/3	2.1	2.9	3.7	4.1	4.5	3.5
KO 71/2	2.1	2.5	2.8	3.2	3.6	2.8
NPP 670	0.7	2.0	2.5	2.9	3.9	2.4
Mean	1.6	2.6	3.1	3.6	4.2	3.0

	Genotype	Days after inoc.	Genotype x days after inoc.
LSD (0.05)	0.0	0.2	0.5
LSD (0.01)	0.2	0.3	0.6

resistant, cvs Katheka, NPP 673/3 and lines KB 91/1, KO 71/2, TK-3, KZ 13/2 as slightly susceptible and cv ICPL 295 as susceptible.

4.2.9. Effect of leaf position on the response of eight pigeonpea genotypes to infection by *Mycovellosiella cajani* in the glasshouse

Results are summarized in Table 11. Analysis of variance of infection mean grades data showed that the differences among the genotypes, leaf position and their interaction were significant ($P < 0.01$) (Appendix 7(iii)). The data indicated that infection level of different genotypes varied with leaf position (Table 11).

Infection level on the upper position of the canopy of cv NPP 673/3 and line KZ 13/2 was significantly ($P < 0.01$) higher than on the intermediate position. For the rest of the genotypes the mean infection score revealed that there were no significant ($P < 0.05$) differences in the level of infection on the upper and intermediate positions. The infection levels on both upper and intermediate positions were significantly ($P < 0.01$) lower than the infection levels on the lower position of the canopy among all the genotypes. Comparison of overall infection mean grades for all the genotypes showed that the highest infection level was obtained on the lower position of the plant canopy while the lowest infection level was observed on the intermediate position.

4.3 FIELD EXPERIMENTS

4.3.1 Progress of *Mycovellosiella* leafspot on eight pigeonpea genotypes at Kiboko

There were significant differences ($P < 0.01$) in percent leaf area infected over the different sampling dates. Genotypic differences and the

Table 11. Effect of leaf position on the infection of pigeonpea genotypes by *Mycovellosiella cajani*

Genotype	Leaf position			
	Lower	intermediate	upper	Mean
	Infection mean grade (0-5 scale)			
TK-3	3.7	2.0	2.2	2.6
ICPL 295	4.7	3.3	3.9	4.0
Katheka	4.0	2.3	2.3	2.9
KB 91/1	4.1	2.3	2.4	2.9
KZ 13/2	4.5	1.9	3.2	3.2
NPP 673/3	4.8	2.1	3.5	3.5
KO 71/2	4.3	1.8	2.4	2.8
NPP 670	3.8	2.0	1.5	2.4
Mean	4.2	2.2	2.7	3.0

	Genotype	Leaf position	Genotype x Leaf post.
LSD (0.05)	0.2	0.4	0.6
LSD (0.01)	0.3	0.5	0.9

interactions between the genotypes x sampling dates also were significant ($P < 0.01$) (Appendix 8 (i)). Leaf area infected increased with time among the different genotypes and then declined. Twenty one days after inoculation, most of the genotypes had their peak leaf infection except lines KO 71/2 and KZ 13/2 which had their peak leaf infection 28 days after plant inoculation. Peak leaf infection was followed by heavy defoliation resulting in low leaf area infection in subsequent dates (Appendix 8 (ii)). For most genotypes except NPP 673/3, the leaf area infected in subsequent dates of sampling continued to decrease with time after the peak leaf infection (Fig 6).

4.3.2 Reaction of eight pigeonpea genotypes to *Mycovellosiella cajani* in the field at Kiboko

The eight pigeonpea genotypes evaluated in the glasshouse were also tested in the field at Kiboko. The results are given in (Table 12). There were highly significant ($P < 0.01$) differences in leafspot infection levels among the genotypes and the samplings dates ($P < 0.01$). The effects of their interaction also was significant ($P < 0.05$) (Appendix 9(i)).

Generally, infection level increased with time, and was then followed by a decline. Infection levels 21 and 28 days after artificial inoculation were not significantly ($P < 0.05$) different from each other but were significantly ($P < 0.01$) higher than at 15 days after plant inoculation. Thirty five days after plant inoculation infection level on the leaves had started declining.

Overall mean score showed that the infection level on cv NPP 670, lines KB 91/1 and TK–3 were not significantly ($P < 0.05$) different from each other. Infection mean grades of lines KB 91/1, TK–3, KO 71/2, KZ 13/2, and cvs NPP 673/3, ICPL 295, Katheka were not significatly different ($P < 0.05$) from each other. The highest infection mean grade was in cv NPP 670 and the lowest in cv Katheka.

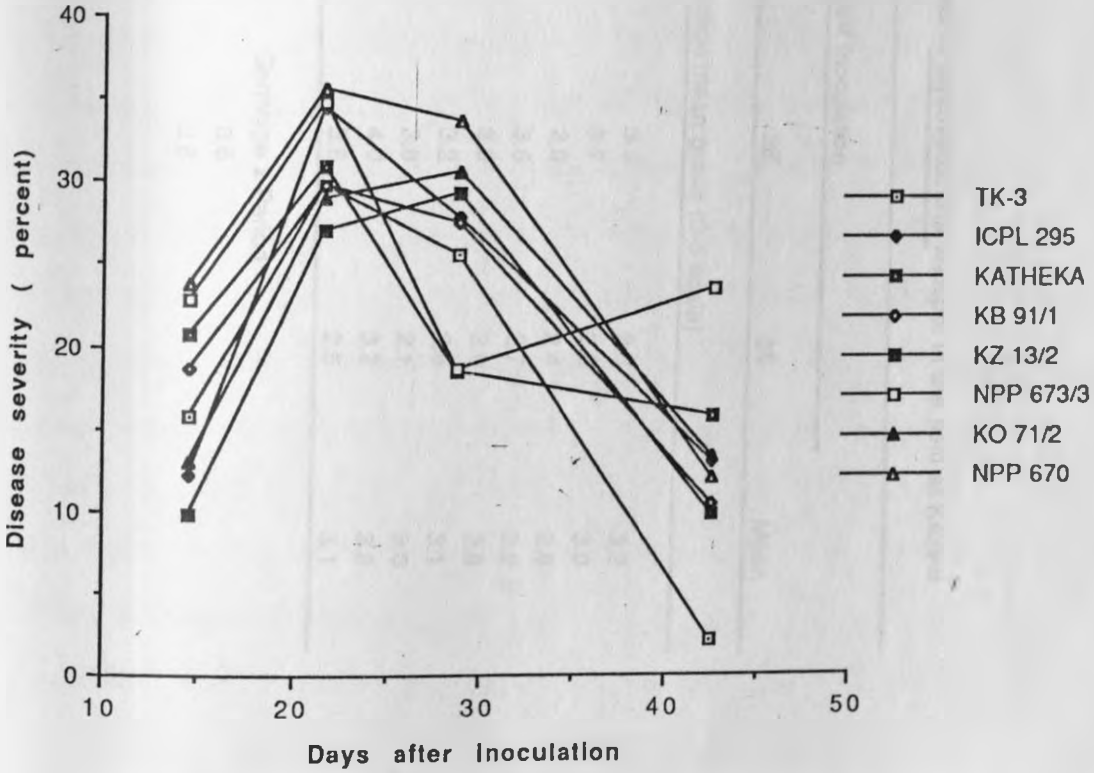

Fig. 6. Progress of Mycovellosiella leafspot on eight pigeonpea genotypes at Kiboko.

Table 12. Reaction of eight pigeonpea genotypes to Mycovellosiella leafspot in the field at Kiboko

	Days after plant inoculation				
Genotype	15	21	28	35	Mean
	Infection mean grade (0-5 scale)				
TK-3	2.5	3.8	3.6	2.8	3.2
ICPL 295	2.2	4.1	3.7	2.2	3.0
Katheka	2.2	3.6	2.9	2.4	2.8
KB 91/1	2.8	3.7	3.6	2.7	3.2
KZ 13/2	1.9	3.6	3.9	2.0	2.8
NPP 673/3	2.9	3.7	3.2	2.6	3.1
KO 71/1	2.4	3.7	3.8	2.1	3.0
NPP 670	3.2	4.0	4.0	3.2	3.6
Mean	2.5	3.8	3.6	2.5	3.1

	Genotype	Period	Genotype x Period
LSD (0.05)	0.2	0.3	0.6
LSD (0.01)	0.3	0.4	0.8

4.3.3 Effect of leaf position on *Mycovellosiella* leafspot infection level on eight pigeonpea genotypes in the field at Kiboko

Results are summarized on Table 13. The analysis of variance of the infection mean grades data showed that there were significant ($P < 0.01$) differences among the genotypes, the leaf positions and their interactions (Appendix 9(ii)).

Comparison of mean infection grades for the genotypes showed that there were no significant ($P < 0.05$) differences in the infection levels on the lower and the intermediate positions. The infection level on the upper position was significantly ($P < 0.01$) lower than that on the intermediate and the lower positions of the plant canopy. Generally the disease progressed upward from the lower to the upper position. The mean infection grades of leaves in the lower and intermediate positions of the plant canopy for all the genotypes was much higher when compared to those of the leaves in the upper position. Some of the genotypes such as cvs Katheka, NPP 670, ICPL 295, and lines TK–3, KZ 13/2, KB 91/1 had lower infection mean grades on the lower position than the intermediate position of the plant canopy.

The shorter early maturing genotypes viz cvs NPP 670, ICPL 295 and line KB 91/1 had higher infection mean grades than those of the taller late maturing cultivars, cvs Katheka and NPP 673/3, on the intermediate and upper leaf position of the plant canopy. Cultivar NPP 673/3 (1.2) had the lowest infection mean grade followed by cv Katheka (1.4), lines KZ 13/2 (1.7), KO 71/2 (1.9), KB 91/1 (2.1), cv ICPL 295 (2.3), and TK–3 (2.4). Cultivar NPP 670 (2.8) had the highest infection mean grade.

Table 13. Effect of leaf position on Mycovellosiella leafspot infection level on eight pigeonpea genotypes in the field at Kiboko.

Genotype	Leaf position			
	Lower	intermediate	Upper	Mean
	Infection mean grade (0-5 scale)			
TK-3	3.1	4.1	2.4	3.2
ICPL 295	3.4	3.5	2.3	3.0
Katheka	3.1	3.8	1.4	2.8
KB 91/1	3.5	4.1	2.1	3.2
KZ 13/2	3.3	3.5	1.7	2.8
NPP 673/3	4.3	3.8	1.2	3.1
KO 71/1	3.6	3.5	1.9	3.0
NPP 670	3.5	4.4	2.8	3.6
Mean	3.5	3.8	2.0	3.1

	Genotype	Leaf post.	Genotype x Leaf post.
LSD (0.05)	0.2	0.3	0.5
LSD (0.01)	0.2	0.4	0.7

4.3.4 Evaluation of eight pigeonpea genotypes for infection by *Mycovellosiella cajani* in the field at Kabete

All the eight genotypes showed high infection level in the field at Kabete (Table 14). From the analysis of variance, there were significant ($P < 0.01$) differences in leafspot infection levels among the genotypes and the sampling dates but their interactions were not significant ($P < 0.05$) (Appendix 9(i)).

Generally, infection level increased steadily with time in all the pigeonpea genotypes tested. Lowest and highest infection mean grade for most of the genotypes was recorded 15 and 35 days after plant inoculation respectively. Fifteen days after plant inoculation cv NPP 670 had the highest infection mean grade while line KZ 13/2 had the lowest. Cultivar NPP 670 had significantly ($P < 0.05$) higher infection mean grade than the rest of the genotypes tested. Infection level on line KZ 13/2 fifteen days after plant inoculation was significantly ($P < 0.05$) lower than at 21 to 35 days after plant inoculation. There were no signficant ($P < 0.05$) differences between 15 and 21 days after plant inoculation in the infection level on cultivar NPP 670 but the infection level 35 days after plant inoculation was significantly ($P < 0.05$) higher than at 15 and 21 days (table 14). Infection levels among the pigeonpea genotypes were not significantly ($P < 0.05$) different at 21, 28 and 35 days after plant inoculation except on line TK-3 where the infection level at 21 days was significantly ($P < 0.05$) lower than at 35 days after plant inoculation.

LSD test indicated that infection mean grade of cv NPP 670 was significantly ($P < 0.01$) higher than that of the rest of the genotypes tested. Infection mean grade of cv ICPL 295 was not significantly ($P < 0.05$) different from that of line KO 71/2 and cv NPP 673/3 but was significantly ($P < 0.05$) higher than that of lines TK-3, KB 91/1, KZ 13/2 and cv Katheka.

Table 14. Evaluation of eight pigeonpea genotypes for infection by Mycovellosiella cajani in the field at Kabete.

Genotype	Days after inoculation (period)				
	15	21	28	35	Mean
	Infection mean grade (0-5 scale)				
TK-3	3.0	3.3	3.5	3.9	3.4
ICPL 295	3.6	3.7	4.0	4.2	3.9
Katheka	3.0	3.4	3.5	3.7	3.4
KB 91/1	2.7	3.6	3.6	3.9	3.4
KZ 13/2	2.9	3.3	3.5	3.8	3.4
NPP 673/3	3.3	3.5	3.7	3.7	3.6
KO 71/2	3.1	3.8	4.1	4.3	3.8
NPP 670	4.1	4.3	4.5	4.7	4.4
Mean	3.2	3.6	3.8	4.0	3.7

	Genotype	Period	Genotype x Period
LSD (0.05)	0.1	0.2	0.4
LSD (0.01)	2.6	0.3	0.5

Overall cv NPP 670 had the highest infection mean grade followed by cv ICPL 295, line KO 71/2, cv NPP 673/3, lines KB 91/1, TK-3, KZ 13/2. The lowest infection mean grade was recorded on cv Katheka.

4.3.5. Leafspot infection level on four pigeonpea genotypes evaluated for their reaction to *Mycovellosiella cajani* in the field at Kabete and Kiboko

Results showed that there were highly significant ($P < 0.01$) location effects on disease development. The pigeonpea genotypes were more infected with *M. cajani* at Kabete than at Kiboko. The most infected genotype was cv NPP 670 with an infection mean grade of 4.4 at Kabete and 3.6 at Kiboko while Katheka (3.4) had the lowest infection mean grade at Kabete and Kiboko. Line TK-3 also had a low infection mean grade at Kabete (3.4) (Table 15).

The interations between the genotypes and the days after inoculation were not significant ($P < 0.05$) (Appendix 10(i)). The infection level increased on all the genotypes during the period of assessment although the disease level was generally higher at Kabete. At both locations highly infected leaves defoliated but those less infected were left on the bránches. Infection mean grades for cvs. NPP 670 and ICPL 295 were not significantly different. However, cv. NPP 670 had significantly ($P < 0.01$) higher infection level than that of cv Katheka and line TK-3.

At Kiboko infection mean grade of cv NPP 670 was not significantly ($P < 0.05$) different from that of ICPL 295 and line TK-3 but was significantly ($P < 0.05$) higher than that of cv Katheka.

Comparison of overall means showed that the infection level among the four pigeonpea genotypes was significantly ($P < 0.05$) higher at Kabete than at Kiboko. Cultivar NPP 670 had the highest infection mean grade on both locations. The lowest infection mean grade was that of cv Katheka.

Table 15. Infection level on four pigeonpea genotypes evaluated at Kabete and Kiboko for reaction to *Mycovellosiella cajani*

	Days after inoculation (period)									
	15	21	28	35		15	21	28	35		
Genotype	Kabete				mean	Kiboko				mean	Genotype Mean
	Infection mean grade (0-5 scale)										
TK-3	3.0	3.3	3.5	3.9	3.4	2.5	3.8	3.5	2.8	3.2	3.3
ICPL 295	3.6	3.7	4.0	4.2	3.9	2.2	4.1	3.7	2.2	3.0	3.5
Katheka	3.0	3.4	3.5	3.7	3.4	2.2	3.6	2.9	2.4	2.8	3.1
NPP 670	4.1	4.3	4.5	4.7	4.4	3.2	4.0	4.0	3.2	3.6	4.0
Mean	3.4	3.7	3.9	4.1	3.8	2.5	3.9	3.5	2.7	3.1	3.5

	Genotype	Location	Period	Genotype x Period x Location
LSD (0.05)	0.2	0.4	0.3	0.6
LSD (0.01)	0.3	0.6	0.4	0.8

4.3.6. The effect of leaf position on the infection of four pigeonpea genotypes by *Mycovellosiella cajani* in the field at Kiboko and Kabete

The effect of leaf position on infection level among the four genotypes at different locations was significant ($P < 0.01$) (Appendix 10(ii)). Comparison of overall means showed that the highest infection mean grade was found in the middle position followed by the lower position. The top position had the lowest infection mean grade. Leafspot infection level on the upper position was significantly ($P < 0.01$) lower than that on the intermediate and lower position of the canopy (Table 16).

Overall, infection level was higher at Kabete than at Kiboko but in both locations infection mean grades was highest on the leaves in the intermediate position.Infection level on the lower position of the canopy was not significanitly ($P < 0.05$) lower than that on the intermediate position. Cultivar NPP 670 had the highest average infection level followed by cv ICPL 295. The lowest infection level was on cv Katheka.

4.3.7 Relationship between seedling and mature plant susceptibility to *Mycovellosiella cajani*

Among the eight pigeonpea genotypes tested, no significant ($P < 0.05$) correlations were observed on the infection levels on mature plants vs seedling infections either in the field or glasshouse. Correlations between infection levels in glasshouse inoculated plants vs field inoculated plants were also not significant ($P < 0.05$) (Appendix 11)

Table 16. The effect of leaf position on Mycovellosiella leafspot infection on four pigeonpea genotypes in the field at Kabete and Kiboko.

		Location(L)		
Genotype(G)	Leaf position(LP)	Kabete	Kiboko	Mean(G)
TK-3	Lower canopy	4.4	3.3	3.9
	Intermediate	4.5	3.9	4.2
	Upper	1.4	2.4	1.9
	Mean(G x L)	3.4	3.2	3.3
ICPL 295	Lower canopy	4.8	3.4	4.1
	Intermediate	4.8	3.5	4.1
	Upper	2.0	2.3	2.1
	Mean(G x L)	3.9	3.1	3.5
Katheka	Lower canopy	4.2	3.1	3.7
	Intermediate	4.3	3.8	4.1
	Upper	1.7	1.4	1.4
	Mean(G x L)	3.4	2.8	3.1
NPP 670	Lower canopy	4.8	3.5	4.2
	Intermediate	4.8	4.4	4.6
	Upper	3.7	2.8	3.2,
	Mean(G x L)	4.4	3.6	4.0
Means (L x LP)	Lower	4.5	3.4	3.9
	Intermediate	4.6	3.9	4.2
	Upper	2.2	2.2	2.2
Means		3.8	3.2	3.5

	Genotype(G)	Location(L)	Leaf position(LP)	(G x L x LP)
LSD (0.05)	0.2	0.3	0.3	0.4
LSD (0.01)	0.3	0.4	0.3	0.6

DISCUSSION

Plant pathology in general is concerned not only with determining the cause of diseases and disorders but also with their treatment and prevention. The methods for diagnosis of plant disease serve two main objectives that can be termed presumptive and confirmatory diagnosis. Presumptive diagnosis of fungal diseases usually used in advisory work, is based on symptomatology and microscopic examination of tissue and recognition of the pathogen on isolation media. Specimens of plants or parts of plants, should be selected to reflect the range of symptoms in the crop. This is imperative because symptomatology is the starting point for diagnosis and because more than one pathogen or disorder may be present.

Most plant pathogenic fungi grow well on a relatively simple media. The more fastidious fungal pathogens either do not grow on artificial media or require host based media. For effective plant breeding programme against plant diseases rapid and efficient screening technique is required to evaluate the germplasm against the important diseases. To create artificial epidermics in the field and greenhouse, sufficient quantity of inoculum is needed and this is usually produced under controlled environment. This study has provided information on the various symptoms associated with *Mycovellosiella* leafspot, a simplified screening technique for assessing the pigeonpea germplasm against *Mycovellosiella* leafspot, the cultural and morphological characteristics of *M. cajani* and factors influencing conidial production and germination. Experiments conducted under this study have also revealed the most ideal medium for production of conidia in culture.

5.1 Morphological characteristics of *Mycovellosiella cajani*

The results indicate that conidiophores of *M. cajani* are terminal or arising as lateral branches of the external hyphae. Most of the conidia observed were sub–cylindric in shape with a distinct scar at one end and one to two scars at the other end. Most of the conidia either had one septa or were aseptate. These observations are in agreement to those reported by Deighton (1974) on *M. cajani*. However there were slight differences in dimensions and shapes of the conidia and this could be attributed to natural variations within the pathogen and environmental conditions.

5.2 Histopathological relationship of *Mycovellosiella cajani* and *Cajanus cajan*

Conidia germinated on the leaf surface by germtubes emerging from one or two cells and penetration was accomplished through stomata. Passive ingress through stomata has been observed on *C. medicaginis, C. zebrina, C. davisii, C. beticola, C. arachidicola, C. molucellae* (Baxter, 1956; Latch and Hanson, 1962; Solel and Minz, 1971; Njeru, 1988). In breeding for resistance, breeders could focus on manipulating stomatal density among other factors which could influence resistance in pigeonpea germplasm to *M. cajani.*

5.3 Symptomatology

Leafspots varying in distinctness from a faint discolouration to characteristically marked necrotic lesions are typical of *Cercospora* leafspot (Chupp, 1953). On pigeonpea, *Cercospora* leafspot is characterized by brown leafspots, usually definate, 1 - 3 mm wide but sometimes indefinate as

much as 1 cm wide often becoming grey in the centre when older (Deighton, 1974). Similar symptoms were observed in this study but with some variations. Necrotic lesions may turn grey if environmental conditions favour sporulation. Lesion shapes varies from irregular, circular, elliptical to rectangular as dictated by leaf venation. Rectangular lesions measure 0.5 to 1.0 or 2.5 cm whereas the other have a diameter of 2 - 7 mm (Hemingway, 1954; Baxter, 1956; Latch and Hanson, 1962; Kingsland, 1963).

Mycovellosiella leafspots were found to vary in shape and size. In some cases necrotic leafspots spread to form a circular or irregular lesion of up to 9 mm or more. Some of the lesions had concentric rings while other did not.

Sometimes the spots were surrounded by a yellowish halo and coaleasced with others thus forming a large necrotic area and becoming irregular in shape. Zonate lesions have been reported in other plant species affected by other *Cercospora* species. Coalescence produces aggregate lesions of greater dimensions and in corn and cassava severe leaf blight has been reported (Kingsland, 1963; Teri *et al.*, 1980).

Singh (1932) reported that in advanced stages of symptoms on pigeonpea plants infected by *Cercospora indica* , the whole leaf dries, curls and ultimately falls. These types of symptoms were observed at Kabete field in some early maturing pigeonpea genotypes such as lines KB 91/1, TK-3, cv ICPL 295, and cv NPP 670 infected by *Mycovellosiella* leafspot and also in the glasshouse inoculated pigeonpea plants at ages 1 and 2 months on some of the leaves of lines KZ 13/2, K0 71/2, TK-3 and cv NPP 670.

5.4 Cultural studies on *Mycovellosiella cajani*

Extensive cultural studies have been carried out worldwide on a number of *Cercospora* species but virtually none has been done on *M. cajani*. The objective of this experiment was to establish the suitable environmental factors for growth and sporulation of *M. cajani* in culture.

Sporulation is not easy to achieve in most of the *Cercospora* species and a series of workers have reported on the *Cercospora* species as being very fastidious in their nutritional requirements for *in vitro* sporulation (Nagel, 1934; Diachun and Valleau, 1941; Goode and Brown, 1970; Smith, 1971). Owing to this, it has been common practice to subject a few isolates of a given species to various manipulations. This brought about investigations on such variables as nutrition, light and temperature (Berger and Hanson, 1963a; Calpouzous and Stallknecht, 1965 & 1967; Miller, 1969; Vathakos and Walters, 1979; El-Gholt *et al.*, 1982; Njeru, 1988).

Nagel (1934) found that the nature of the nutrient substratum has a strong bearing on conidial production. He also reported that a medium suitable to a particular species for optimal sporulation may not prove satisfactory for other species of *Cercospora*.

Among the six media inoculated with spores from pure sporulating colonies of *M. cajani*, numerous colonies occurred on PLDA, a fair number of colonies on CDA, and PDA while there were few or very small colonies on PCA, CA and PMA. The cultures above were more or less similar in morphology. Mycelial growth was very limited in all the environmental factors tested and due to the small colony size of less than 1 - 3 mm accurate measurements on hyphal growth could not be taken. For all cultural studies plates were inoculated by flooding technique and greyish colonies developed which were sampled to determine sporulation capacity on 10, 14, 18 and 22 days after plate inoculation. Conidial

concentration of 2 x 10^4 conidia ml^{-1} was used in these experiments because it gave reasonably high sporulation of 4.12 x 10^6 conidia ml^{-1} after 10 days.

Although no quantitative data is available it was observed that on all the agar plates where colonies appeared, there were many conidia and conidiophores in proportion to the minute mycelial colonies that developed. It seems probable that if any mycelial growth can occur in these pure cultures, it is directed to the formation of sporulating structures and their supporting hyphae. Calpouzous (1954) made similar observations in *Cercospora musae*.

Results indicated that exposure to high temperatures and very low temperatures did not encourage growth of *Mycovellosiella cajani*. Abundant colonies appeared at 20° and 25°C. Fewer colonies appeared in culture plates incubated at 15°C. Sporulation occurred in all the temperatures where colonies appeared. Data on the level of sporulation reflects differences among the temperatures. This could be partly explained by the relative number of colonies that appeared under each temperature regime. Fewer colonies were obtained at 15°C as compared to the abundant number of colonies at 20°C and 25°C. *Cercospora* species grow and sporulate within temperature range of 15°-30°C. The optimum temperature for growth and sporulation reported for *C. zebrina*, *C. asparagi*, *C. davisii* and *C. molucellae* is 24°C while for *C. gossypina*, *C. beticola* and *C. nicotianae* is 21 - 29°C, 15°C, 26°C respectively. This was reported by Calpouzous and Stallknecht (1967), Stavely and Nimmo (1968), Miller (1969), Cooperman and Jenkins (1986) and Njeru (1988).

Growth of *Cercospora* species is favoured by acidic medium. Singh (1932) found that the optimum growth of *C. indica* is pH 6.7. The fungus rendered the medium on which it grew acidic. Maximum growth for *C. zebrina* has been reported to be at pH 5.2 (Berger and Hanson, 1963a) .

Colonies of *Mycovellosiella cajani* appeared and sporulated over a wide range of pH levels. Colony appearance and sporulation were observed in both acidic and alkaline media tested. Optimum sporulation occurred at pH 5.

Alternative dark and light period favours sporulation of *C. kikuchii*, *C. zebrina* and *C. asparagi* (Kilpatric and Johnson, 1956; Berger and Hanson, 1963a; Cooperman and Jenkins, 1986). *C. beticola* and *C. molucellae* require continous light for sporulation to occur (Calpouzous and Stallknecht, 1967; Njeru, 1988). Sporulation of *Mycovellosiella cajani* occurred in all the three light regime tested. Number and size of colonies that grew on plates under the different light regimes were different although sporulation occurred in all the three light regimes. Higher number of colonies appeared in the 24 hour light regime and thus the highest sporulation. U-V light emitted by the inflorescence tubes used for lighting the incubation room could have contributed to the high sporulation in continous light. Because sporulation of *M. cajani* occurred in continous darkness, it seems therefore that no particular wavelength of light is apparently necessary for spore production. Initially, production of conidia was not abundant and neither was it consistent enough to offer a reliable supply of spores for experimental purposes. In 1939, Meredith and Butler (cited by Calpouzous, 1954) made similar observations on *C. musae*.

Mycovellosiella cajani was more fastidious in nutritional requirement for growth than for sporulation. A combination of medium and other external environmental factors were investigated. Among all the environmental factors that were tested, media, pH, temperature and light regimes, sporulation occurred as long as colonies appeared. It was observed that the number of colonies formed in cultures inoculated with conidial concentration 2×10^4 conidia ml^{-1} increased with successive subculturing thus resulting in increased sporulation when highly

sporulating colonies were selected and used to prepare the conidial suspension. The ability to produce colonies and sporulate in culture was increased with successive transfer of pure sporulating conidia. Selective subculturing, a method of transferring densely sporulating areas within a colony, has induced sporulation in other species of *Cercospora* (Nagel,1934; Calpouzous, 1954; Jones, 1958; Calpouzous and Stallknecht, 1965). Calpouzous (1954) observed that colonies originally from single isolate, all growing in the same culture plate under identical environmental conditions showed considerable variation in morphology and sporulation. He concluded that the critical factor controlling conidia production in pure cultures of *C. musae* is genetic and is not associated with environmental factors as many had previously thought. He was able to develop cultures that were genetically pure for the ability to produce conidia. Similarly cultures of *Mycovellosiella cajani* with high sporulating ability were produced.

In most cultures, it was observed that sporulation increased to a peak and then there was a decline. The decline in sporulation could be due to conidia germination or disintegration. Light and high temperatures seemed to promote conidial germination or disintegration. Germination or disintegration of conidia has been observed for other *Cercospora* species (Nagel, 1934; Cooperman and Jenkins, 1986; Njeru, 1988). It is therefore advantageous to use cultures of *M. cajani* of less than 14 days of incubation.

5.5 Environmental factors affecting conidial germination

Some spores may be mature and be able to germinate immediately upon their introduction into a suitable environment. Many spores, however,

undergo a dormancy of varying lengths during which they remain inactive, and germination occurs only when dormancy is broken.

Dormancy may be constitutive and under innate control of the spore or of an exogenous nature and is under control of external factors (Sussman, 1966a, 1966b; Sussman and Halvorson, 1966). Constitutive dormancy may be under control of self inhibitors or of the innate metabolism of the spore. The role of self inhibitors in imposing dormancy is frequently apparent when high concentration of spore fail to germinate, while frequency of germination increases if the spore concentration is decreased.

Certain nutrients and chemicals stimulate conidial germination when supplied at appropriate concentration (Nutman and Roberts, 1962). Brown (1922) showed that high dilutions of certain essential oils stimulated spore germination of *Botrytis cinerea* whereas a concentration of approximately 5 p.p.m of eucalyptus oil had a retarding effect. He found that both ethyl acetate inhibited germination at moderate concentrations and stimulated it at low ones.

It was observed that the rate of germination and total percent germination in *Mycovellosiella cajani* was highest in conidial concentration 2×10^5 and 5×10^4 conidia ml^{-1}. The rate of germination was lowest in conidial concentrations 2×10^4 conidia ml^{-1}. The rate of germination in conidial concentration 2×10^6 conidia ml^{-1} was slow initially but increased rapidly after 12 hours to 24 hours and then became slow upto 72 hours of incubation. Conidial concentration did not affect the lag period of germination. Slow germination rate at low conidial concentration (2×10^4 conidia ml^{-1}) suggest some kind of self inhibition in *Mycovellosiella cajani*. Padwick (1939), reported a case of self inhibition in *Ophiobolus graminis* where ascospores germinated slowly if isolated singly, more rapidly if many spores are present. In *Neurospora crassa*, crowding of conidia reduces the germination rate, but not total

germination (Ryan, 1948). However, this phenomenon may be more dependent on the environment than in self inhibition of spores. Germination of spores may be prevented when the density of the spores is so high that large number of mycelia would compete for the limited food available and undoubtedly face starvation. At low conidial concentration the available nutrient supply might have been too low to allow conidial germination. This may partly explain the reduced initial germination rate at high concentration of *M. cajani* (2×10^6 conidia ml^{-1}) and at low concentration (2×10^4 conidia ml^{-1}). The minimum time required for conidial germination was found to be four hours for all the concentrations tested. Ekpo and Esuruoso (1977) observed no germination in *Cercospora cruenta* after three hours of incubation. Self inhibitors may confer an ecological advantage to those spores which have this mechanism, as they prevent the germination of spores in the fruitification. This is important because an ungerminated spore can be efficiently dispersed, unlike a germinated spore, and is, more resistant to unfavourable conditions (Cochrane, 1958).

Temperature was found to be an important factor in conidial germination of *M. cajani*. High temperatures and very low temperatures did not encourage spore germination. Variations were found in lag period, germination rate, and total percent germination. Conidial germination was not observed after 3 hours of incubation under any of the temperature regime but was observed at 20°, 25° and 30°C after 4 hours and at 15°C after 8 hours of incubation. Conidial germination was not observed in plates incubated at temperatures 0°, 5°, 10°, and 35°C. The minimum, optimum and maximum temperatures for germination were 15°C, 25°C and 30°C, respectively. Best germination occurred between 20° - 25°C. The optimal temperature range for conidial germination in *C. arachidicola* was reported to be 20 - 30°C (Oso, 1972), with little

germination at 30°C. Gobina and Melouk (1981) reported that after 48 hour in a water suspension at 15 - 35°C, germination of *Cercospora arachidicola* was not significantly different. Ekpo and Esuruoso (1977) found that the minimum, optimum and maximum temperatures for germination in *C. cruenta* were 15°C, 25°C and 37°C, respectively.

Some fungal species are known to germinate well in distilled water while for others, certain nutrients such as sugar are required. Duggar (1901) demonstrated species variation with respect to nutrient requirements when spores germinated in water, bean decoction, nutrient-salt solution and cane-sugar solution.

Plant materials besides increasing spore germination through nutritional effects or carbon dioxide evolution, in some cases inhibit germination. Leaf extracts from eight different genotypes of pigeonpea, at the age of 15 and 120 days, either suppressed or enhanced conidial germination as compared to distilled water. Percent conidial germination in leaf extracts from 15 and 120 day old plants were not significantly different but the interactions between the plant age at which the leaf extract was obtained and the genotypes was highly significant ($P < 0.01$). Leaf extract from 120 day old plants of cv Katheka and 15 day old plant of cv ICPL 295 significantly suppressed conidial germination as compared to germination in distilled water. Schneider and Sinclair (1975) reported inhibition of conidial germination and germtube growth of *C. canescens* by cowpea leaf diffusates. Brown (1922) reported better conidial germination of *Botrytis cinerea* when in contact with plant tissue than in distilled water. Leaf extracts from 120 day old plants of cv ICPL 295, lines TK-3,KZ 13/2, and KO 71/2 and from 15 day old plants of cvs NPP 673/3, NPP 670 and lines KO 71/2, TK-3 enhanced conidial germination though not significantly higher than in distilled water. The stimulatory effects on spore germination by extracts of some pigeonpea genotypes may be due to

the presence of some substances which could be an acid, sugar or metabolite of undescribed constitution. Differences in genotypes suggest that some germination principle may be at higher concentrations in some genotypes than in others.

In disease resistance screening tests, it would be essential to consider this host pathogen interaction.

5.6 Reaction of pigeonpea genotypes to *Mycovellosiella cajani*

5.6.1 Effect of inoculum concentration on disease severity

Although symptoms occurred at almost the same time with all the inoculum levels, fewer leaves among the ones inoculated with low inoculum level had symptoms. Consequently the infection level was also low. Disease development in plants inoculated with 2×10^4 and 5×10^4 conidia ml^{-1} was slow as compared to disease development in plants inoculated with conidial concentration of 2×10^5 and 2×10^6 conidia ml^{-1}. Plants inoculated with these two latter concentrations showed no significant differences in their infection means but they were significantly ($P < 0.01$) different from concentrations 2×10^4 and 5×10^4 conidia ml^{-1}. Plants inoculated with conidial concentrations 2×10^5 and 2×10^6 conidia ml^{-1} had started yellowing and defoliating 34 days after inoculation. Conidial germination experiment in this same study had clearly shown that high percent conidial germination could only be attained at concentrations 2×10^5 and 2×10^6 conidial ml^{-1}.

With higher inoculum concentration levels, the probability of more conidia accomplishing the infection process was higher. Also leaf tissue colonization by the pathogen after infection might have proceeded more rapidly due to the higher number of spores which accomplished infection. When screening genotypes for disease resistance, high inoculum

concentrations should be used (2×10^5 or 2×10^6 conidia ml^{-1}). Otherwise, use of low inoculum concentrations may result in low infection rates and this may lead to genotypes that are susceptible being classified as resistant.

5.6.2 Effect of host plant age on disease incidence

With respect to host age, it appears that disease incidence in 30-day old plants was less than in 60, 90 and 120 day old plants in most of the glasshouse inoculated genotypes. In some lines such as lines KB 91/1 and KZ 13/2, the least infection level was found in 60 and 90 day old plants respectively. In other genotypes such as cvs ICPL 295 and NPP 673/3, there were no significant differences in disease incidence among the different host plant ages.

Under field conditions, it was observed that *Mycovellosiella cajani* caused epiphytotics when the plants were not less than 120-150 day of age. Disease was not observed at Kiboko until some of the early maturing genotypes had started flowering. Very low incidences of leafspot were observed at Kabete when the plants were one month old. This could be because the environment was cooler and more humid. High leafspot incidences and severities were not observed until some of the plants had started flowering and early podding. Even after flowering had started, infection level remained relatively low both at Kabete and Kiboko field until most early maturing genotypes, lines KB 91/1, K0 71/2, KZ 13/2, TK-3 were at podding stage. The late maturing genotypes were also heavily infected especially on lower and intermediate leaf canopy although they were still in their vegetative stage at Kiboko. At Kabete the late maturing genotypes, cv Katheka and NPP 673/3 had started flowering at 130 – 140 days after planting.

The apparent increase in cultivar susceptibility in the field can be partially explained by the presence of more favourable enviornmental conditions. Prolonged periods of high moisture and moderate temperatures have been reported to favour leafspot of peanuts (Jensen and Lytton, 1965). Presence of more favourable microclimate as plants mature is due to the shading effect of the canopy as well as the denseness of the canopy. All these favour plant infection and disease development. The presence of an increased supply of host exudates could also be contributed to increased susceptibility. Other reasons could be that the annual disease cycle of *M. cajani* commences with low levels of inoculum and disease build up occurs during host vegetative growth period as a result of conidia formed on attacked leaves. This high level of inoculum combined with favourable weather conditions cause epiphytotics.

The influence of physiologic age on epiphytotic cannot be ruled out especially due to the results obtained on inoculated plants in the glasshouse in some of the genotypes, for example seedling of cv ICPL 295 and KB 91/1 showed low susceptibility while mature plants of the same cultivar showed high susceptibility both in the glasshouse and in the field. The relationship between age of plants and increased susceptibility within a cultivar has been discussed by many workers (Wyllie and Williams, 1965; Mence and Pegg, 1971; Waren *et al.*, 1971; Dickson and Crute, 1974; Nyvall and Haglund, 1976; Hart and Endo, 1981). Changes in plant susceptibility to pathogens have been reported in several hosts. Dickson and Crute (1974) found that the susceptibility of lettuce seedlings to *Bremia lactucae* decreased with age. Mence and Pegg (1971) found that the resistance of pea leaves to *Peronospora viciae* increased with age but declined again during senescence. Younger plants of ornamental bells of Ireland (*Molucellae laevis*) were found to be less susceptible to *Cercospora* leafspot than older plants (Njeru, 1988). Similar observations have been

made on *Cercospora* leafspot of groundnuts and corn (Hemingway, 1954 and 1955; Hilty *et al.*, 1979). Plant susceptibility to *M. cajani* varied among pigeonpea genotypes. The resistance to *Mycovellosiella* leafspot among genotypes at different ages appears to reduce rates of infection but not defoliation. Timing of defoliation may be very important in determining the extent of resulting crop losses. Reduced infection rates may delay onset of defoliation beyond some critical point in host development and yield accumulation. Pigeonpea genotypes with reduced infection rate at flowering and podding stage may still give economic yield despite infection by *M. cajani.*

5.6.3 Pigeonpea genotypes reaction to *Mycovellosiella cajani* and progress of *Mycovellosiella* leafspot

Mycovellosiella leafspot was found to be an important disease of pigeonpea. It was the only leaf disease that was found attacking pigeonpea plants at Kiboko and Kabete experimental plots during the short rains 1989/90. The disease was also prevalent in pigeonpea breeding nurseries at Thika, Machakos, Kiboko and Makueni. The disease caused severe defoliation in pigeonpea plants in the experimental plots both at Kabete and Kiboko and also in the breeding nurseries that were surveyed for *Mycovellosiella* leafspot. The breeding nurseries had been naturally infected. Although the experimental plots were artificially inoculated there was also a lot of natural infection that took place prior to plant inoculation. The growing season was very wet in all the locations surveyed and the disease spread very rapidly attacking all the plants in the field. This agrees with the report made by Rubaihayo and Onim (1975) and Onim (1980).

All the eight genotypes of pigeonpea inoculated in the glasshouse were susceptible to *M. cajani.* By 12 - 14 days after inoculation, *Mycovellosiella*

leafspots were noted in some inoculated leaflets. An incubation period of 12 days has been reported in groundnuts – *C. arachidicola* host-parasite system (Nevill, 1981; Alderman and Beute, 1986). Although infection began approximately at the same time after inoculation on all the genotypes in the glasshouse, significant differences in disease severity on the leaflets among the lines were observed 19 to 23 days after plant inoculation. The genotypes exhibited different rates of disease increase but still attained maximum disease severity when the leaf either turned yellow or defoliated. This agrees with observations made by Alderman and Beute (1986) on early leafspot of peanut caused by *C. arachidicola*. Although complete resistance was not found among the various pigeonpea genotypes, increased incubation period and increased time to defoliation seem to suggest that there may be partial resistance within the genotypes to *M. cajani*.

Khan and Rachie (1972) reported that there was a wide variation of pigeonpea lines in their resistance to *M. cajani*. Such situation was also observed by Onim and Rubaihayo (1976) while screening pigeonpea cultivars to *M. cajani*. They suggested that resistance to *M. cajani* may be polygenic. The number of genes conferring this resistance may vary among cultivars being more in resistant ones.

Differences in partial resistance to early leafspot have been reported in peanut. Some genotypes developed fewer lesions per leaf (Sowell *et al.*, 1976; Hassan and Beute, 1977). Number of lesions resulting from inoculations in the glasshouse screening have been an unreliable measure of partial resistance in peanuts to peanut leafspots (Walls, 1984; Walls *et al.*, 1985; Ricker *et al.*, 1985; Anderson *et al.* 1986). Reduced sporulation per lesion (Foster *et al.*, 1980) and percent of lesions sporulating (Ricker *et al.*, 1985) as well as increased latent period (Nevill, 1981) and increased time to defoliation (Ricker *et al.*, 1985) have been reported in other host-pathogen

systems. There is need to identify the resistance components of partial resistance (lesion size, spore production, infection period, infection frequency and latent period) which most highly correlate with the disease progress in the field to be able to efficiently select pigeonpea genotypes for resistance to *Mycovellosiella* leafspot.

In this study, ranking of genotypes in the glasshouse was different from the field ranking. In the glasshouse, cv NPP 670 was found to be moderately resistant while the same cultivar was found to be susceptible in the field. In the glasshouse cv ICPL 295 had the highest mean infection (4.0) and cv NPP 670 had the lowest (2.4). In the field at Kiboko cv NPP 670 had the highest mean infection (3.6) and cv Katheka had the lowest (2.8). In the field at Kabete cv NPP 670 had the highest mean infection (4.4) while cv Katheka had the lowest (3.4). Environmental variation has been suggested as a cause of shifts in ranking of genotypes (Nevill, 1981; Walls *et al.*, 1985; Anderson *et al.*, 1986).

The disease was more intense among the genotypes at Kabete than at Kiboko. Defoliation which occurs naturally and as a result of leafspot diseases (Leath, 1981) affects the observable disease severity. Defoliation of highly diseased leaves and growth of new healthy leaves limit the maximum level of disease attainable. Infection level at Kiboko increased to a peak by the third week after inoculation and then started to decline. This is because the most severely infected leaves were defoliated and also the weather conditions at the time of defoliation was unfavourable for disease development. Infection level at Kabete increased with time from 15 to 35 days after plant inoculation. Although the plants were heavily defoliating, the infection level recorded did not decrease with time for most genotypes since most of the remaining leaves were still heavily infected.

5.6.4. Effect of leaf position on susceptibility of eight pigeonpea genotypes to *Mycovellosiella cajani*

Although symptoms occurred at the same period, 12 - 14 days after plant inoculation, on leaves at different leaf positions in the glasshouse, differences among the genotypes were observed. In some genotypes such as cv NPP 673/3 and line KZ 13/2, the average infection level on the upper leaf position was significantly ($P < 0.01$) higher than that on the middle position while in most of the other genotypes, the differences between upper and intermediate position were not significant ($P < 0.05$).

Infection level was consistently more on the lower third of the canopy than on the upper and intermediate position. The influence of the plant canopy on the plant microclimate seems to be important. The shading effect of the canopy apparently has a direct influence on prolonging humid conditions within the plant population.

In the field both at Kabete and Kiboko, infection level was consistently higher on lower half of the canopy than on the upper half. Leaves on the lower position showed infection earlier and infection progressed upward from bottom to top. The differences in leaf positions were more pronounced 21 days after plant inoculation when the infection level was high. Initially, defoliation of highly diseased leaves and growth of new healthy leaves limited the maximum level of disease attainable at the lower part of the stem but as disease progressed the new leaves were also highly infected and defoliated. Environmental conditions in March and April 1990 appeared to be the most favourable for growth of pigeonpea and rapid development and spread of *Mycovellosiella* leafspot both at Kabete and Kiboko. In the field, disease severity on the lower and intermediate position were found not to be significantly different but the lower and intermediate positions were highly significantly different from the upper position.

Changes in susceptibility with leaf position have been reported in other host-pathogen systems. Allen and co-workers (1983) reported that lower leaves of sunflower plants were more susceptible to infection by *Alternaria helianthi* than were upper leaves. Similar observations were made on older potato plants inoculated by *Phytophthora infestans* by Warren and co-workers (1971). Upper and lower leaves were more susceptible than those of intermediate position.

When evaluating pigeonpea for resistance to *M. cajani* one should carefully assess the various components of resistance within the plant.

5.6.5 Relationship between seedling and mature plant infection

Among the six pigeonpea genotypes tested no significant ($P < 0.05$) correlations ($r = 0.07$, $r = 0.28$, $r = -0.36$) were observed between infection levels of mature plants either in the field at Kiboko, Kabete or in the glasshouse respectively and seedling infection levels.

Sometimes it can be risky to extrapolate observations of cultivar reaction in the seedling state to the adult plant stage in the field. Reaction of seedlings and adult oat cultivars to *Puccinia graminis f. sp. avenae* was found to differ considerably (Sztejnberg and Wahl, 1977).

Evaluation of components of partial resistance should be done at the correct plant stage or leaf development (Umaerus, 1970). In 1977, Dehne (cited by Parlevliet, 1979) observed that the wheat cultivars he studied were all highly susceptible to yellow rust in the seedling stage, but variation in resistance in the field correlated much better with the components of resistance measured at adult plant stage. Similar observations were made in barley-leaf rust host-parasite system. Resistance in the field correlated better with components of resistance measured at flag leaf stage than at seedling stage (Parlevliet and Ommeren, 1975).

CONCLUSIONS

The results obtained in this project are a useful guide for further research on *Mycovellosiella* leafspot disease of pigeonpea.

For abundant sporulation, *M. cajani* should be cultured on pigeonpea leaf decoction agar incubated at room temperature (20 - 24°C) under 24 hour light regime. Conidia should be harvested 10 – 14 days after plate inoculation to ensure a high number of viable conidia. The optimum pH for sporulation is pH 5.

The optimum temperature for conidia germination is 25°C. Conidia concentration affect the rate of conidia germination. Lag phase was longer in low (2×10^4 conidia ml^{-1}) concentrations of *M. cajani*. Also very few colonies appeared in plates inoculated with conidia concentration 20 and 200 conidia ml^{-1} thus resulting in poor sporulating capacity. At high conidial dilution levels the nutrients available were not enough to stimulate conidial germination or probably conidia of *M. cajani* may have self-inhibitors that maintain dormancy until optimum conidial concentration is obtained.

Tall early maturing genotypes of pigeonpea may escape damage by *Mycovellosiella* leafspot in the field since there is delayed infection for the upper leaf canopy.

Conidia of *M. cajani* germinate by germ tubes emerging from one or two or more scars on the cells and penetrate host tissues, passively through the stomata. *M. cajani* is not merely associated with *Cajanus cajan* but is the causal organism of *Mycovellosiella* leafspot.

Delay in the increase in the infection levels among some pigeonpea genotypes such as lines KZ 13/2, KO 71/2, cv ICPL 295 shows that some early maturing genotypes may give good yield inspite of heavy defoliation.

There were no correlations between susceptibility of seedlings and mature plants pigeonpea genotypes to *Mycovellosiella cajani* .

FURTHER RESEARCH

1. There is need to identify the best storage condition of *M. cajani* in order to avoid germination and disintegration of conidia.

2. Survival of the pathogen from year to year or source of primary inoculum.

3. Epidemiological studies in order to establish the correlation between disease and environmental factors under field conditions.

4. Studies on components of partial resistance (lesion size, spore production, infections period, infection frequency and latent period) and the age and leaf position at which they correlate best with resistance in the field.

5. *Mycovellosiella* leafspot screening to identify resistant pigeonpea genotypes and studies on the effect of *Mycovellosiella cajani* on total yield and yield components of different genotypes of pigeonpea.

REFERENCES

Akinola J.O. and P.C. Whiteman, 1972. A numerical classification of *Cajanus cajan* (L.) Millsp. accessions based on morphological and agronomic attributes. Australian Journal of Agriculture Res. **23**:995 -1005.

Alderman, S.C. and M.K. Beute, 1986. Influence of temperature and moisture on germination and germtube elongation of *Cercospora arachidicola*, Phytopath **76**: 715-719.

Alexopolous, C.J. and C.W. Minns, 1979. Introductory mycology. 3rd edition, pp. 566-567. Wiley international edition, New York.

Allen, D.J., Nebane, C.L.N and J.A. Raji (1981). Screening for resistance to bacterial blight in cowpea, Trop. pest. Manag. **27**:218-224.

Allen, S.J., J.F. Brown and J.K. Kochman, 1983. The effect of leaf age, host growth stage, leaf injury and pollen on infection of sunflower by *Alternaria helianthi*. Phytopath. **73**: 896-898.

Anderson, W.F.; J.C. Wayne; Green; C.C. and M.K. Beute, 1986. Combining ability and heritability of resistance to early and late leafspot of peanut. Peanut Sci. **13**:10-14.

Anon. , 1975. Statistical Abstract, Central Bureau of Statististics, Ministry of Economic Planning and Community Affairs, Kenya.

Atkins, J.C. 1973. The importance, epidemiology and control of bean rust (*Uromyces appendiculatus*) on white haricot beans (*Phaseolus vulgaris*) in Uganda, M.Sc. pp 1-80 Thesis , Makerere Univ.Uganda.

Ballantyne, B. and R.A. McIntosh, (1977). The genetic basis of rust resistance in beans, 3rd Int. Congr. SABRAO, Canberra, Plant breeding papers I. 4(a) :13-16.

Bateman, D.F. and R.D. Lumsden (1965). Relation of calcium content and nature of the pectic substances in bean hypocotyls of different ages to susceptibility to an isolate of *Rhizoctonia solani*, Phytopath. 55: 734-738.

Baxter, J.W. 1956. *Cercospora* black stem of alfalfa. Phytopath. 46: 398-400

Beckman, P.M. and G.A. Payne. 1981. Penetration, infection and development of *Cercospora Zeae maydis* in corn leaf. Phytopath. 71:202.

Berger, R.D. and E.W. Hanson, 1963a. Pathogenecity host parasite relationship and morphology of some forage legume *Cercosporae* and factors related to disease development. Phytopath. 53: 500-508.

Berger, R.D. and E. W. Hanson, 1963b. Relationship of environmental factors to growth and sporulation of *Cercospora zebrina*. Phytopath. 53: 286-294.

Brown, W. 1922. Studies on the physiology of parasitism ix. The effect on the germination of fungal spores of volatile substances arising from plant tissues. Ann. Bot. London 36:285-300.

Calpouzous, L. 1954. Controlled sporulation of *Cercospora musae* Zimm. in pure culture. Nature 173:1084-1085.

Calpouzous, L. and G.H. Stallknecht, 1965: Sporulation of *Cercospora beticola* affeted by an interaction between light and temperature. Phytopath. 55: 1370-1371.

Calpouzous, L. and G.H. Stallknecht, 1967. Effects of light on sporulation of *Cercospora beticola*. Phytopath. 57: 679-681.

Chen, M.D., S.D. Lyda and R.S. Halliwell, 1979. Environmental factors influencing growth and sporulation of *Cercospora kikuchii*. Mycologia 71: 1150-1157.

Chupp, C. 1953. A monograph of the genus *Cercospora*.Ithaca, New York. 13: 269.

Clayton, E.E. 1945. Resistance of tobacco to blue mold (*Peronospora tabacina*). J. Agric. Res. 70: 79-87.

Cochrane, V.W. 1958. Physiology of fungi. pp 524. John Wiley & Sons, Inc., New York.

Cooperman, C.J. and S.F. Jenkins, 1986. Conditions influencing growth and sporulation of *Cercospora asparagi* and *Cercospora* blight development in asparagus. Phytopath. 76: 617-619.

Coyne, D.P. and M.L. Schuster, 1974. Differential reaction of pods and foliage of beans (Phaseolus vulgaris) to *Xanthomonas phaseoli* D.L. Pl. dis. Reptr. 58:278-282.

Deighton, F.C. 1974. Studies on *Cercospora* and allied genera v. *Mycovellosiella* Rangel and new species of *Ramulariopsis*. Mycological papers No. 137: 3 - 6.

Dhingra, O.D. and J.B. Sinclair, 1985. Basic plant pathology methods. pp 276. CRC Press, Inc. Boca Raton, Florida.

Diachun, S. and W.D. Valleau, 1941. Conidial production in culture by *Cercospora nicotianae*. Phytopath 31: 97-98.

Dickson, C.H. and J.R. Crute, 1974. The influence of seedling age and development on the infection of lettuce by *Bremia lactucae*. Ann. Appl. Biol. 76: 49-61.

Drolsom, P.N. and J.G. Dickson, 1954. Seedling and mature plant inoculation and Sudan grass with *Helminthosporium turcicum*. Phytopath 44: 188-192.

Duggar, B.M., 1901. Physiological studies with special reference to germination of certain fungus spores. Bot. Gaz. **31**:38-66.

Ekpo, E.J.A. and O.F. Esuruoso, 1977. Factors affecting spore germination in cowpea isolates of *Cercospora cruenta* Sacc. Phytopath Z, **89**: 249-255.

El-Gholt; S.A. Alfieri; W.H. Ridings and C.L. Schoulties, 1982. Growth and sporulation *in vitro* of *Cercospora apii, Cercospora arachidicola, Cercospora kikuchii* and other species of *Cercospora*. Can. J. Bot. 60:862-869.

Elston, J.; D. Macdonald; and C. Harkness. 1976. The effect of *Cercospora* leaf disease on the growth of groundnuts in Nigeria. Samaru Research Bulletin. **261**: 39-49.

Fery, R.L.; P.D. Dukes and F.P. Cuthbert. 1977. Yield loss of Southern pea (*Vigna unguiculata* (L) Walp) J. Am. Soc. hort. sci. 105:671-674.

Foster, E.J.; M.K. Beute and J.C. Wynne, 1980. Spore production and latent period as mechanism of resistance to *C. arachidicola* in four peanut genotypes. Peanut Sci. **7**: 88-90.

Fowler, A.M. 1970. The epidemiology of *Cercospora* leafspot disease of groundnuts. Somaru Agric. Newsl. **12**: 66-69.

Garry A.S. and E.G. Ruppel. 1971. *Cercospora* leafspot as a predisposing factor in storage rot of sugar beet roots. Phytopath. **61**:1485-1487.

Gobina, S.M. and H.A. Melouk, 1981. Effect of temperature on sporulation and conidia germination *Cercospora arachidicola* (Abstr.). Phytopath **71**: 876.

Good, R. 1964. The geography of the flowering plants (3rd ed.).pp 97. Longsmans, Green, London.

Goode, M.J. and C.R. Brown, 1970. Detection and characterization of *Cercospora citrullina* isolates that sporulate readily in culture. Phytopath. 60: 1503-1508.

Hart, L.P. and R.M. Endo, 1981. The effect of length of exposure to inoculum, plant age, root development root wounding on *Fusarium* yellows of celery. Phytopath 71: 77-79.

Hassan, H.N. and M.K. Beute, 1977. Evaluation of resistance to *Cercospora* leafspot in peanut germplasm potentially useful in a breeding program. Peanut Sci. 4: 78-83.

Headrick, J.M. and J.K. Pataky, 1987. Expression of resistance on common rust in sweet corn hybrid at various host growth stages. Phypath 77: 454-458.

Hemingway, J.S. 1954. *Cercospora* leafspot of groundnuts in Tanganyika E. Afr. Agric. J. 19: 263-269.

Hemingway, J.S., 1955. The prevalence of two species of *Cercospora* on groundnuts. Trans. Brit. Myc. Soc. 38: 243-246.

Hewitt, E.S. 1952. Sand and water culture methods used in the study of plant nutrition. pp 6 - 7 Commonwealth Bur. Hort. Tech. Com.22, East Malling.

Hill, A.V. 1969. Factors affecting viability of spore inoculum in *Peronospora tabacina* Adam and lesion production in tobacco plants. Aust. J. Biol. Sci. 22: 399-411.

Hilty, J.W.; C.H. Hadden and J.T. Garden, 1979. Response of maize hybrids and inbred lines to grey leaf spot disease and the effects on yield in Tennessee. Plant Dis. Repr. 63: 515 - 518.

Horsfall, J.G. and R.W. Barratt, 1945. An improved grading system for measuring plant disease. Phytopath 35: 655.

Hubbeling N. 1957. New aspects of breeding for disease resistance in beans *Phaseolus vulgaris* (L). Euphytica 6: 111 - 141.

Hughes, S.J. 1953. Conidiophores, conidia and classification. Can. J. Bot. 31: 577-659.

Jenkins, W.A., 1938. Two fungi causing leafspot of peanuts. J. Agric. Research 56: 317-332.

Jensen E.R. and W.B. Lytton. 1965: Effects of temperature, relative humidity, precipitation on peanut leafspot. Pl. Dis. Reptr. 49: 975 – 978.

Jones, J.P. 1958. Isolation of sporulating strain of *Cercospora kikuchii* by selective subculturing. Phytopath 48: 287-288.

Judd, R.W. and J.L. Peterson, 1972. Temperature and humidity requirements for the germination of *Cercospora omphakodes* spores. Mycologia 64: 1253-1257.

Khan, T.N. and Rachie, K.0., 1972. Preliminary evaluation and utilization of pigeonpea germplasm in Uganda. E. Afr. Agr. For. J. 38: 78-82.

Kilpatric, R.A. and H.W. Johnson, 1956. Sporulation of *Cercospora* species on carrot leaf decoction agar. Phytopath 46: 180-181.

Kimani, P.M. 1985. The second progress report for pigeonpea project (Kenya) Phase III. International Development Research Centre (IDRC), Ottawa, Canada.

Kimani, P.M. 1987. Pigeonpea Breeding in Kenya. Methods and Objectives. Paper presented at pigeonpea scientist meeting 2-5 June, 1987, Nairobi, Kenya.

Kimani, P.M. 1988. The second report of pigeonpea project (Kenya) phase IV. International Development Research Centre (IDRC), Ottawa, Canada.

Kingsland, C.G. 1963. *Cercospora* leaf blight of corn. A case history of a local epiphytotic in South Carolina. Pl. Dis. Reptr 47: 724 - 725.

Latch, C.G. and E.W. Hanson, 1962. Comparison of three stem diseases of *Melilotus* and their causal agents. Phytopath 52: 300-350.

Leath, K.T. 1981. Pest management systems for alfafa diseases pages 293-301. In: Handbook of pest management in Agriculture. Vol. III. D. pimental, CRC Press Boca Raton, Florida.

Mence, M.J. and G.F. Pegg, 1971. The biology of *Peronospora viciae* of pea. Factors affecting the susceptibility of plants to local infection and systemic colonization. Ann. Appl. Biol. 67: 297-308.

Meredith, D.S., 1970. Banana leaf spot disease (Sigatoka) caused by *Mycosphaerella musicol*a Leach. Phytopathol. paper II. Common W. Mycol. Inst. Surrey. England.

Mew, P.C.; T.C. Wang and T.N. Mew, 1975. Inoculum production and evaluation of mung bean varieties for resistance to *Cercospora canescens*. Pl. Dis. Repr. 59: 397-401.

Miller, J.W. 1969. Cultural conditions affecting sporulation of *Cercospora gossypina*. Phytopath. 59: 511-515.

Muller, A.S. 1950. A preliminary survey of plant diseases in Guatemala. Pl. Dis. Reptr. 34. 203-207.

Muntanola, M. 1954. A study of a newly indentified pepper disease in the Americas.(*Cercospora unamunoi*). Phytopath 44: 233–239.

Nagel, C.M., 1934. Conidial production in species of *Cercospora* in pure culture. Phytopath 24: 1101-1110.

Natrass, R.M. 1961. Host list of Kenya fungi and bacteria. Mycological papers No. 81: 2 - 34.

Nevill, D.J. 1981. Components of resistance to *Cercospora arachidicola* and *Cercosporidium personatum* in groundnuts. Ann. appl. Biol. 99: 77-86.

Nevill, D.J. and A.M. Evans, 1980. The effect of host development on the field assessment of disease resistance to *Cercospora* leafspots in groundnuts (*Arachis hypogaea* (L). J. Agric. Sci. 94: 229-237.

Ngugi, D.N. 1979. Study of the effect of plant population and phosphate fertilizer on growth and yield of pigeonpeas in Kenya. Paper presented at Symp. 1979 on grain legume improvement in East Africa at Nairobi, Kenya.

Njeru, R.W. 1988. Etiology and chemical control of *Cercospora* leafspot of the ornamental Bells of Ireland (*Molucella laevis*). M.Sc. thesis,pp. 1-89. University of Nairobi, Nairobi; Kenya.

Nutman, F.J. and Roberts, F.M. 1962. Stimulation of two pathogenic fungi by high dilutions of fungicides. Trans. Brit. Mycol. Soc. 45(4):449-456.

Nyabundi, J.O. 1980. A study on drought resistance in pigeonpeas (*Cajanus cajan* L. Millsp.) Msc. Thesis, pp. 1 - 144. Nairobi Univ. Kenya.

Nyvall, R.F. and Haglund, W.A., 1976. The effect of plant age on severity of pea wilt caused by *Fusarium oxysporum* f.sp.*pisi* race 5. Phytopath 66: 1093-1096.

Ondiek J.J. (1973). Host list of Kenya fungi and bacteria. E.Afr. Agric. For. J. 38: 1 - 25

Okeyo-Owuor, J.B. and C.P.M. Khamala, 1980. Insect podborer infestations and their influence on developing pods and final seeds yields by pigeonpea *Cajanus cajan* in Kenya. K.J. Sci. and Tech. 1: 79-86.

Onim, J.F.M., 1980. Foliar fungicides for the control of *Mycovellosiella cajani* causing the leafspot of pigeonpea. Indian J. Agric. Sci. 50(8): 615-619.

Onim, J.F.M. , 1983. On farm testing of improved pigeonpea (*Cajanus cajan*) . Cultivars in Kenya. In Kirkby, R.A. ed. Crop improvement in Eastern and Southern Africa Research Objectives. IDRC, Ottawa, Canada.

Onim, J.F.M. and P.R. Rubaihayo, 1976. Screening pigeonpea for resistance to *Mycovellosiella cajani*. Sabrao Journal 8(2):121-125.

Oso, B.A. 1972. Conidial germination in *Cercospora arachidicola*. Hori. Trans. Brit. myco. soc. **59**:169-172.

Padwick, G,W. 1939. Note on the limitation of infection of wheat by ascospores of *Ophiobolus graminis* Sacc. A possible explanation. Ann. appl. Biology. **26**:823 - 825.

Parlevliet, J.E. 1979. Components of resistace that reduce the rate of epidermic development. Ann. Rev. Phytopath. **17**:203-222.

Parlevliet, J.E. and A. van Ommeren, 1975. Partial resistance of barley to leaf rust, *Puccinia hordei*. II. Relationship between field trials, micro-plot test and latent period. Euphytica **24**: 293-303.

Patel, P.N. 1981. Pathogen viability and host resistance in bacterial pustule disease of cowpea in Africa. Tropical Agric. (Trin.). **58**:275 - 280.

Purseglove, J.W. 1968. Tropical crops. 2. Dicotyledons. pp 236 - 241. Longmans,Green. London.

Reuveni, M.S., S. Tuzum, J.S.. Cole; M.R. Siegel and J. Kuc'. 1986. The effect of plant age and leaf position on the susceptibility of Tobacco to Blue mold caused by *Peronospora tabacina*. Phytopath. **76**: 455-458.

Ricker, M.D., M.K. Beute and C.L. Campbell, 1985. Components of resistance in peanut to C. *arachidicola*. Pl. Dis. 69: 1059-1064.

Riddel, R.W. 1950. Permanent stained mycological preparations obtained by slide culture. Mycologia 42: 265-270.

Rocane, C.W., R.L. Harrison and C.F. Genter. 1974: Observations of gray leafspot of maize in Virginia. Pl. dis. Reptr. 58: 456-459.

Rodriguez, R. and P.L. Melendez, 1984. Field screening of pigeonpea (*Cajanus cajan*) for resistance to foliar diseases in Puerto Rico. Journal of Agric. of the Univ. of Puerto Rico 68(3):275 - 279.

Rubaihayo, P.R. and J.F.M. Onim, 1975. A study of some characters of pigeonpea. Sabrao Journal. 7(2): 183 - 187.

Rupe, J.C., M.R. Siegel and J.R. Hartman 1982: Influence of environment and plant maturity on grey leafspot of corn caused by *Cercospora zea maydis*. Phytopath. 72:1587 - 1591.

Ryan, F.J. 1948. The germination of conidia from biochemical mutants of *Neurospora*. Am. J. Botany 35: 497 – 503.

Schneider, R.W. and J.B. Sinclair, 1975. Inhibition of conidial germination and germtube growth of *Cercospora canescens* by cowpea leaf diffusates. Phytopath. 65:63 - 65.

Sinclair, J.B. (1982). Compendium of soybean disease, 2nd ed. A.M. phytopathol. Soc. pp 8. St. Paul, Minn, 1982.

Singh, U.D. 1932. Studies on *Cercospora indica* N. sp. parasitic on *Cajanus indicus* spreng, Imperial Institute of Agricultural Research, Pusa, Bihar.

Smith, D.H. (1971). A simple method for producing *Cercospora arachidicola* conidial inoculum. Phytopath. 61: 1414.

Sobers, E.K. 1969. The relationship of conidia morphology to colony characteristic in species of *Cercospora*. Phytopath. 59:118.

Solel, Z. and G. Minz, 1971. Infection process of *Cercospora beticola* in sugarbeet in relation to susceptibility. Phytopath. 61: 463-466.

Sowell, G.; D.H. Smith and R.D. Hammons, 1976. Resistance to peanut introductions to *Cercospora arachidicola*. Pl. dis. Reptr. 60:494 - 498.

Sridharan R. and G. Rangaswami. 1968. Studies of two *Cercospora* leafspots of *Abelmoschus Esculentus*. Indian phytopath. 21:37–41.

Stavely, J.R. and J.A. Nimmo, 1968. Effects of temperature upon growth and sporulation of *Cercospora nicotianae*. Phytopath. 59:496-498.

Sturtevant, E.L. 1972. Edible plants of the world pp 3-6. Edited by U.P. Hedrick. Dover Publications, Inc. New York.

Sussman, A.S. 1966a. Dormancy and spore germination.In (Insworth, G.C., and A.S. Sussman, Eds). 2:733-764, The fungi–an advanced treatise. Academic Press, New Yórk.

Sussman, A.S. 1966b. Types of dormancy as represented by conidia and ascoscospores of *Neurospora*. In (Madelin, M.F.,Ed.,) The fungus spore. pp. 235–257. Butterworth & Co., Ltd.,London .

Sussman A.S. and H.O. Halvorson. 1966. Spore– their dormancy and germination. Harper & Row, Pub., Inc. New York. 354 pp.

Sztejnberg, A. and I. Wahl, 1977. Mechanisms and stability of slow rusting resistance in *Avenae sterilis*. Phytopath 67: 74 - 80.

Teri, J.M.; H.D. Thurston and C.J. Lozano, 1980. Effect of brown leafspot and *Cercospora* leaf blight on cassava productivity. Trop. Agric. Trinidad 57: 239-242.

Thothathri K. and S.K. Jain. 1980. Taxonomy of the Genus *Cajanus* DC. In Proceedings of the International Workshop on Pigeonpeas Vol. 2. ICRISAT Patancheru, India, 15–19 December 1980.

Umearus, V. 1970. Studies on field resistance to *Phytophthora infestans*. 5. mechanism of resistance and application in potato breeding. Z. pflanzenziicht 63: 1-23.

Vakili, N.G. 1977. Field screening of cowpeas for *Cercospora* leafspot resistance, Trop. Agric. (Trin), 54: 69-76.

Van der Vossen, R.T.A. Cook and G.N.W. Murakaru, 1976. Breeding for resistance to coffee berry disease caused by *Colletotrichum coffeanum* Noack (Sensu Hindorf). In *Coffea arabica* L.T. Methods of preselection for resistance. Euphytica 25:733 - 745.

Vathakos, M.G. and H.J. Walters, 1979. Production of conidia by *Cercospora kikuchii* in culture. Phytopath. 69: 832-839.

Verma, P.R.; S.R.S. Dange, and P.N. Patel. 1968. Host range and epidemiology of *Cercospora* leafspot disease of spinash–beet. Indian Phytopath 21: 428–433.

Verma, P.R. and Patel, P.N. 1969. Host range a varietal resistance and epidemiological observation of *Cercospora* leafspot disease of cowpea. Indian Phytopath 22: 61-66.

Vestal, E.G. 1933. Pathogenicity, host response and control of *Cercospora* leafspot of sugar beets. Iowa Agr. Exp. Sta. Res. Bull.168: 41–72.

Wallin J.R. and D.V. Loonan, 1971. Effect of leaf wetness duration and air temperature on *Cercospora beticola* infection of sugarbeet. Phytopath 61: 546–549.

Walls, S.B. 1984. The evaluation of resistance to *Cercosporidium personatum* in early and late generation *Arachis hypogaea* breeding lines. Ms. thesis, pp 55. North Carolina State University, Raleigh.

Walls, S.B.; J.C. Wynne and M.K. Beute, 1985. Resistance to late leafspot of peanut of progenies selected for resistance to early leafspot. Peanut Sci. **12**: 17-22.

Warren, R.C.; J.E. King and J. Colhoun, 1971. Reaction of potato leaves to infection by *Phytophthora infestans* in relation to position on the plant. Trans Br. Mycol. Soc. 57:501-514.

Williams, R.J. 1974. Comparison of varietal reactions to cowpea anthracnose in field plot and detached petiole inoculations. Proc. A.M. Phytopath. soc. **1**: 87 (abstr.).

Wyllie, T.D. and L.F. Williams, 1965. The effects of temperature and leaf age on the development of lesions caused by *Peronospora manshurica* on soybeans. Phytopath 55: 166-170.

Zeven, A.C. and P.M. Zhukovsky. 1975. Dictionary of cultivated plants and their centres of diversity. pp. 90-92. Wageningen, Netherlands: Centre for Agricultural Publishing and Documentation.

Appendix 1.

CULTURE MEDIA AND THEIR INGREDIENTS.

Unless otherwise indicated the term autoclave means 15 minutes at 121ºC

1. PPLDA – pigeonpea leaf decoction agar

300 g of freshly picked leaves of pigeonpea were finely ground in a blendor. 1 litre of water was added and then autoclaved. The solution was strained and 12 g of agar added. The solution was then autoclaved again.

2. CDA– Carrot leaf decoction agar

Finely chopped carrot leaves	300 g
Agar	12 g
Water	1 l

Leaves were finely ground and autoclaved in 500 ml water. The resulting suspension was strained through two layer cheese cloth. 500 ml of melted agar was added. Modification of a method described by Kilpatric, R. A. and H.W. OJohnson 1956. Phytopath 46: 180–181

3. PDA– Potato dextrose agar

Potato extract	4 g
Dextrose	20 g
Agar No. 1	20 g
pH 5.6	(approx.)

4. Potato carrot agar

Potato	20 g	sliced tissue was steamed in water for 1 hr
Carrot	20g	then boiled for 5 minutes. Agar was added to the
Agar	20 g	the filtrate and autoclaved
Water	1l	

5. CA – Carrot agar

Carrot tissue was soaked in water for 1 hr then boiled for 5 minutes.The suspension was then filtered through two layer cheese cloth.

Carrot (gratted)	20 g
Agar	20 g
Water	1 l

6.PMA – Pigeonpea meal agar

Pigeonpea seeds	15 g
Agar	20 g
Water	1 l

Pigeopea seeds were ground to fine flour and 15 g weighed. 1 l distilled water and agar were added and then autoclaved.

Appendix 2. Mean squares

(i) Effect media and incubation period on sporulating capacity (conidia ml-1 x 10'6) of Mycovellosiella cajani

Source	df	Ms	
Media	5	235.8	**
Time	3	43.2	**
Time x Media	15	13.1	**
Reps	2	5.3	
Error	46	2.2	

** significant at probability 1 percent
ns not significant

(ii) Effect of conidial concentration used for plate inoculation and incubation period on sporulation capacity (conidia ml-1 x 10'6) of Mycovellosiella cajani

Source	df	Ms	
Inoc. conc.	5	68.4	**
Period	3	52.1	**
Conc. x Period	15	8.9	**
Reps	2	0.1	
Error	46	1.3	

** significant at probability 1 percent

(iii) The effect of temperatures and incubation period on sporulation of Mycovellosiella cajani (conidia ml-1 x 10'6)

Source	df	Ms	
Temperature ºC	6	105.6	**
Period	3	5.8	**
Temp x Period	18	9.5	**
Reps	2	0.3	
Error	54	0.4	

** significant at probability 1 percent

Appendix 3.

(i) Mean squares

Effect of pH and incubation period on the sporulation of Mycovellosiella cajani (conidia ml-1 x 10'6)

Source	df	ms	
pH	6	63.0	**
period	3	10.8	**
pH x period	18	3.6	**
Reps	2	1.1	
Error	54	0.7	

(ii) Table of means

Effect of three light regimes and incubation period on spolulation capacity of Mycovellosiella cajani (conidial ml-1 x 10'6)

	Incubation period (days)				
Light regime	10	14	18	22	Mean
Dark 24 hrs	2.6	4.2	4.1	3.1	3.5
Light 24 hrs	6.6	5.4	3.4	3.0	4.6
Dark 12 hrs/ Light 12 hrs	4.5	5.8	2.6	1.7	3.7
Incub. period Mean	4.6	5.1	3.2	2.8	3.9

	Light	Period	Light x period
LSD (0.05)	0.9	0.8	1.5
LSD (0.01)	1.2	1.2	2.1

(iii) Mean squares

Effect of three light regimes and incubation period on sporulation of Mycovellosiella cajani (conidia ml-1 x 10'6)

Source	df	Ms	
Light reg. (A)	2	4.3	*
period (B)	3	11.8	**
Interaction (A X B)	6	4.4	**
Reps	2	1.3	
Error	22	0.8	

* significant at probability 5 percent

** significant at probability 1 percent

cont.....Appendix 3.

Appendix 3(iv). Effect of subculturing on sporulating capacity of Mycovellosiella cajani (conidia ml-1x10'6)

No. of subculturing	Mean
2	2.5
5	5.6
7	6.2
9	9.7
Mean	6.0

LSD (0.05) 1.94
LSD(0.01) 2.93

(3v). Mean squares of the effect of subculturing on sporulating capacity of Mycovellosiella cajani

Source	df	Mean squares
Number of times subcultured	3	26.26**
Reps	2	0.2
Error	6	0.9

(i) Mean squares

The effect of conidial concentration on germination of Mycovellosilla cajani

Source	df	ms	
Suspension concentration	3	443.8	**
period	6	9595.4	**
conc. x period	18	80.4	**
Reps	2	2.2	
Error	54	13.4	

** significant at probability 1 percent

(ii) Table of means.

Effect of eight different temperatures and incubation period on conidia germination of Mycovellosiella cajani

	Incubation period (hr)							
Temperature	3	4	8	12	24	48	72	Mean
	Percent conidial germination							
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
15	0.0	0.0	0.8	8.0	17.1	26.1	29.9	11.7
20	0.0	28.5	63.7	72.5	79.3	80.1	81.7	58.0
25	0.0	45.3	73.6	79.3	83.5	86.2	86.5	64.9
30	0.0	0.3	1.0	1.5	5.6	6.7	11.0	3.7
35	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mean	0.0	9.3	17.4	20.2	23.2	24.9	26.2	17.3

	Temp	period	Temp. x period
LSD (0.05)	1.2	0.5	3.4
LSD (0.01)	1.6	1.7	4.5

(i). Mean squares. Effect of different temperatures and incubation period on spore germination (% germination)

Source	df	ms		F
Temperature	7	16001.9	**	11537.0
Period	6	2170.7	**	1565.0
Temp. x Period	42	633.5	**	456.7
Reps	2	2.9		2.1
Error	110	1.4		

** significant at probability 1 percent

(ii) Table of means

Effect of inoculum concentration on the infection of pigeonpea plants by Mycovellosiella cajani

	Period after inoculation (Days)					
Conidial conc.	15	19	23	27	34	Mean
2 x 10'4	0.2	0.3	0.4	0.5	0.9	0.5
5 x 10'4	0.3	0.4	0.7	0.9	1.3	0.3
2 x 10'5	0.5	1.0	1.6	2.4	2.8	1.7
2 x 10'6	1.0	1.3	1.4	2.1	2.6	1.7
Mean	0.5	0.8	1.1	1.5	1.9	1.1

	concentration	Period	Conc. x Period
LSD (0.05)	0.3	0.2	0.5
LSD (0.01)	0.3	0.3	0.7

(iii). Mean squares. Effect of pigeonpea leaf plant extract from15 and 120 day old plant on spore germination (% spore germination)

Source	df	ms	
Genotype (A)	8	970.2	**
Age (B)	1	10.4	ns
A x B	8	921.0	**
Reps	3	20.8	
Error	51	64.4	

Appendix 6

(i) Mean squares

Effect of inoculum concentration on the infection level of eight pigeonpea genotypes by Mycovellosiella cajani

Source	df	Ms	
Concentration	3	4.0	**
period after inoc.	4	2.4	**
Conc. x period	12	0.2	**
Reps	1	0.9	
Error	19	1.1	

(ii) Mean squares

Mycovellosiella leafspot incidence in different stages of growth of eight pigeonpea genotypes

Source	df	Ms	
Genotypes	7	408.2	*
Age (days)	3	1401.7	**
genotypes x age	21	298.6	*
Reps	2	1243.7	
Error	62	141.9	

(iii) Mean squares

Seedling reaction of eight pigeonpea genotypes to infection by Mycovellosiella cajani

Source	df	Ms	
genotypes	7	9.8	**
period after ionc.	5	37.1	**
genotype x period	35	0.6	**
Reps	2	0.1	
Error	94	0.0	

* significant at probability 5 percent

** significant at probability 1 percent

Appendix 7.

(i) Table data

Disease progress on eight pigeonpea genotypes in the glasshouse

	Days after inoculation					
Genotype	15	19	23	25	27	Mean
	percent disease severity					
TK-3	1.5	6.4	3.0	3.6	4.6	3.8
ICPL 295	13.7	16.3	9.9	11.8	14.6	13.2
Katheka	1.9	3.5	4.0	7.1	10.0	5.3
KB 91/1	4.2	12.7	8.8	6.8	7.8	8.0
KZ 13/2	2.6	3.7	6.6	6.8	13.4	6.6
NPP 673/3	2.7	3.3	6.4	14.0	21.3	9.6
KO 71/2	2.3	2.4	4.2	6.1	8.2	4.7
NPP 670	0.6	1.3	2.1	3.6	5.0	2.5
Mean	3.7	6.2	5.6	7.5	11.4	6.9

(ii)Mean squares

Reaction of eight pigeonpea genotypes to Mycovellosiella cajani at flowering stage

Source	df	Ms	
Genotype	7	4.7	**
Period after inoc.	4	31.3	**
Genotype x Period	28	0.3	**
Reps	3	0.2	
Error	117	0.1	

(iii). Mean squares

The effect of leaf position on the infection level of pigeonpea genotypes by Mycovellosiella cajani

Source	df	Ms	
Genotype (A)	7	3.0	**
Leaf position (B)	2	36.0	**
A x B	14	0.6	**
Reps	3	0.1	
Error	69	0.2	

Appendix 8.

(i) Table of means

Progress of Mycovellosiella leafspot on eight pigeonpea genotypes at Kiboko

	Period after inoculation (days)				
Genotypes	15	21	28	42	Mean
	percent disease severity				
TK-3	15.1	29.0	24.8	20.1	20.1
ICPL 295	11.5	33.7	27.2	21.2	21.2
Katheka	20.1	30.2	17.8	20.8	20.8
KB 91/1	18.0	29.1	26.9	20.9	20.9
KZ 13/2	9.2	26.3	28.5	9.2	18.3
NPP 673/3	22.1	34.1	18.0	22.8	24.3
KO 71/2	12.3	28.2	29.8	12.7	20.8
NPP 670	23.1	34.8	33.0	11.5	25.6
Mean	16.4	30.7	25.8	13.1	21.5

	Genotype	Period	Genotype x Period
LSD (0.05)	1.9	2.7	5.3
LSD (0.01)	2.5	3.5	7.1

Mean square: Progress of Mycovellosiella leafspot on eight pigeonpea genotypes at Kiboko

Source	df	Mean square	
genotype	7	86.8	**
sampling date (period)	3	2117.3	**
genotype x period	21	83.5	**
Reps	3	40.5	
Error	93	29.0	

Appendix 9

(i) Mean squares for infection level on eight pigeonpea genotypes grown at Kiboko and Kabete

		Mean squares			
Source	df	Kiboko		Kabete	
Genotype	7	1.0	**	2.0	**
Period	3	15.0	**	3.7	**
Genotype x Period	21	0.3	*	0.1	ns
Reps	3	0.3		0.0	
Error	93	0.2		0.1	

(ii) Effect of leaf position on leafspot infection level on eight pigeonpea genotypes in the field at Kiboko

Source	df	Ms	
Genotype (A)	7	0.7	**
Leaf position (B)	2	31.7	**
A x B	14	0.7	**
Reps	3	0.2	
Error	69	0.1	

* significant at probability 5 percent

** significant at probability 1 percent

ns– not significant

Appendix 10.

(i) Mean squares

Infection level on four pigeonpea genotypes evaluated at Kabete and Kiboko

Source	df	Mean squares	
Genotype (A)	3	4.7	**
Location (B)	1	12.7	**
A xB	3	0.6	*
Days after inoc. (C)	3	6.5	**
A x C	9	0.1	ns
A x B	3	1.8	**
A x B x C	9	0.2	ns
Reps	3	0.0	
Error	93	0.2	

(ii)Anova

The effect of leaf position on Mycovellosiella leafspot infection level four pigeonpea genotypes in the field at Kabete and Kiboko

Source	df	Mean squares	
Genotype (A)	3	3.5	**
Leaf post. (B)	2	39.1	**
A xB	6	0.8	**
Location (C)	1	9.2	**
A x C	3	0.5	**
A x B	2	2.9	**
A x B x C	6	0.6	**
Reps	3	0.0	
Error	69	0.1	

* significant at probability 5 percent

** significant at probability 1 percent

ns–not significant

Appendix 11.

Correlation coefficient between genotypic infection mean grades for six pigeonpea genotypes evaluated in the glasshouse and field at mature stage and seedling stage in the glasshouse (n = 6)

Variable	Field vs seedling	Field vs glasshouse	Glasshouse vs seedling
Infection mean grade	0.1	0.3	-0.4

Appendix 12.

(i). Meteorological data: Kabete 1990

Month	Rainfall	Temperature ºC			RH	
	(mm)	Max	Min	Mean	0600(9a.m.)	1200(3pm)
Jan	51.6	23.0	12.0	17.5	81.0	54.0
Feb	47.8	24.9	14.0	19.4	80.0	48.0
March	199.7	23.3	14.3	18.8	86.0	62.0
April	275.2	22.9	14.7	18.8	88.0	67.0
May	309.3	22.7	13.9	18.4	86.0	61.0
June	6.5	21.8	11.6	11.7	88.0	59.0
July	13.6	21.5	10.4	15.8	88.0	57.0
Aug	21.0	20.3	11.5	15.9	88.0	65.0
Sept	31.8	23.6	11.3	17.5	81.0	48.0
Oct	90.0	23.8	13.3	18.5	82.0	49.0
Nov	126.0	22.0	12.8	17.8	87.0	62.0
Dec	74.6	22.3	13.2	17.8	83.0	58.0

(ii). Meteorological data: Kiboko 1990

Month	Rainfall	Temperature (ºC)			RH	
	(mm)	Max	Min	Mean	0600 (9 am)	1200 (3 pm)
Jan	2.8	28.2	16.5	22.3	85.4	64.5
Feb	3.6	31.1	18.3	24.7	77.1	67.3
March	5.3	29.1	18.8	24.2	83.7	65.5
April	4.0	28.6	18.7	23.6	79.3	66.6
May	-	-	-	-	-	-
June	-	-	-	-	-	-
July	-	-	-	-	-	-
Aug	-	-	-	-	-	-
Sept.	-	-	-	-	-	-
Oct.	-	-	-	-	-	-
Nov.	-	-	-	-	-	-
Dec.	-	-	-	-	-	-