# BIOLOGY AND EPIDEMIOLOGY OF *SPIIACELOMA* sp. The PATHOGEN OF COWPEA SCAB.

BY

CATHERINE MOUKOU MUNGO

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## DECLARATION

I hereby declare that this thesis has been written by me and that it is a record of my own research work. It has not been presented before in any previous application for higher degree.

Mungo — 4/2/97

CATHERINE MOUKOU MUNGO — Date

(Ph.D. Candidate)

The above declaration is confirmed

A. M. Emechebe — Feb. 04, 1997

PROFESSOR A. M. EMECHEBE — Date

(Major Supervisor)

# CERTIFICATION

This thesis entitled "BIOLOGY AND EPIDEMIOLOGY OF *SPHACELOMA* SP. THE PATHOGEN OF COWPEA SCAB" by Catherine Moukou Mungo meets the regulations governing the award of the degree of DOCTOR OF PHILOSOPHY of Ahmadu Bello University, and it is approved for its contribution to knowledge and literary presentation.

Feb. 04, 1997

PROFESSOR A. M. EMECHEBE — Date

(Major Supervisor)

5/2/97

PROFESSOR I. D. Erinle — Date

(Member, Supervisory Committee)

4-2-97

Dr. M. D. Alegbejo — Date

(Member, Supervisory Committee)

05/02/97

Dr. I. Onu

(Head of Department)

Date

24/10/97

Professor J.Y. Olayemi — Date

(Dean, Postgraduate School)

# DEDICATION

This thesis is dedicated to God the Father Almighty for his earnest love, care and protection throughtout the course of the study and to my late mother (Mrs Juliana Ajoacha Mungo) whose words of academic inspirations echo daily in my ears.

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# ABSTRACT

Transverse sections of scab infected cowpea pods revealed the presence of conidia and conidiophores in the acervuli of *Sphaceloma* sp. on the pods and peduncles. The pathogen colonized the epidermis in which it produced stromatic tissue from which the conidiophores and conidia developed.

*Sphaceloma* sp. was isolated either by plating out intact young pod or leaf lesions or by grinding these (lesions) to a paste which was subsequently dispersed on media containing cowpea extracts or on potato dextrose agar (PDA) amended with streptomycin sulphate and rose bengal. Cowpea pod agar (CpdA) and PDA amended with streptomycin sulphate (1.5 g/1) and rose bengal (0.0025 g/1 of agar) were the best media for isolation of *Sphaceloma* sp., when intact lesions were plated on the agar surface. The dispersal of a paste of ground lesions on agar plates was less tedious and less time consuming than plating individual lesions.

Scab symptoms were induced earlier on younger cowpea plants than on older plants after plants were sprayed to run-off with a conidial suspension of *Sphaceloma* sp. The incubation periods for the leaf, peduncle and floral cushion were 5, 9 and 8 days, respectively, regardless of the age of plants at inoculation. However, incubation period varied from 7-10 days on the stem, depending on the age of the plant at the time of inoculation.

In agar culture, the fungus grows vertically, producing very little or no radial growth. Colonies readily come off agar plates when picked with forceps; consequently the mycelial mass colonies of *Sphaceloma* sp. was used as measure of fungal growth. These colonies, each about 2 mm in diameter and weighing approximately 0.00lg, were incubated at temperatures of 15, 20, 26, 30, and 35 °C for 3 to 15 days on corn meal agar plus rose bengal (CMAR), malt agar plus rose bengal (MAR), and potato dextrose agar plus rose bengal (PDAR). The fungus grew at 15-26°C on all three media, but no growth occurred at 30°C. Colony weight loss was recorded on MAR and PDAR at 35°C. Among the three

media, PDAR was the best for growth of *Sphaceloma* sp., with maximum growth occurring at 26° C. On CMAR and MAR the fungus flattened out somewhat instead of growing vertically as it does on PDAR. By the 15th day at 26°C, a gelatinous matrix coated MAR colonies. Streaking this matrix on PDAR and MAR and incubating the plates at 26°C produced many colonies of the fungus.

Attempts to isolate the fungus from infected cowpea seeds were unsuccessful. In view of this, an indirect method was used to determine if scab is seed transmitted in cowpea. In the first year of the study, the incidence of scab on seedlings grown from seeds produced in a region where little cowpea is grown and scab does not occur was significantly lower than on those from seeds produced in a scab-infected field, despite the fact that seedlings were probably affected by inoculum from an adjacent, older cowpea field. In the first and second year, seed treatment with benomyl drastically reduced incidence of scab in seedlings grown from seeds produced in infected fields. The data strongly suggest seed-transmission of cowpea scab.

The time taken for scab symptoms to appear on all the fields with different cowpea rotations, irrespective of mulching treatment, was 25-27 days in 1993 and 1994. In both years the incidence of scab in fields last planted with cowpea a year before the trial was the highest compared to the fields last planted with cowpea 2 or 3 years before the trial. Polyethylene mulching on cowpea plots reduced rain splashing of soil onto healthy plants and probably reduced inoculum levels of the pathogen, resulting in low disease incidence for the mulched plots. More than 75% of the first symptoms of scab in cowpea seedlings under natural inoculation occurred on the epicotyl. No scab symptoms were observed on the primary leaves. The evidence suggests that the pathogen is soil-borne either in infected trash or as perennating structures (probably chlamydospores) in the soil.

Disease incidences and apparent infection rates in both 1992 and 1993 were lower in later plantings ($P = 0.05$). In 1992 there were higher disease incidences on all the affected plant parts than in 1993.

In 1992, the grain yields obtained in the early sown crop of the variety susceptible to scab (IT 85F-1517) were the lowest (306.0 kg/ha) though there were no significant differences between these yields and those of the last planting date. In 1993, the late sown crop of IT 85F-1517 had a lower grain yield (340.0 kg/ha) which was not significantly different (P= 0.05) from that of the earliest sown crop (346.0 kg/ha). The second planting date yielded the highest grain yield (1390.0 kg/ha). The earliest sown crop of the cowpea variety resistant to scab IT 83S-720-2 in 1992 and 1993 had higher grain yields (2220.0 and 2580.0 kg/ha respectively) which were not significantly different from the grain yields obtained in the second planting dates (2067.0 and 2050.0 kg/ha). The last sown crops had the lowest yields (1670.0 and 685.0 kg/ha in 1992 and 1993, respectively).

Weekly applications of benomyl or carbendazim + maneb reduced the development of scab symptoms on peduncles and gave good control of the disease on flowering cushions and pods, compared to mancozeb and untreated control plants treatments. Regardless of the plant part on which disease incidence was recorded, the areas under the disease progress curves were almost zero when benomyl or carbendazim + maneb were used to control scab. Mancozeb treated plants had significantly higher disease severity values on all aerial plant parts in comparison with plants treated with benomyl or carbendazim + maneb. However, the disease severities in plants treated with mancozeb were similar to those in the untreated (check) plants.

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# CHAPTER ONE

## INTRODUCTION

Cowpea belongs to the botanical species, *Vigna unguiculata* (L.) Walp., but experts still debate the classification and nomenclature of the taxa at the intraspecific level (Maréchal and Ng, 1985). Cowpea also known in the USA as the black-eye pea or southern pea, is a protein-rich crop with 24% protein content (Bressani, 1985). Cowpea in Africa is cultivated by small-scale farmers as a subsistence crop in semi-arid ecologies, usually in association with millet and sorghum (Ashraf, 1985). Cowpea is a valuable warm season grain legume in tropical and subtropical zones of Africa, Asia and the Americas (Nielson and Hall, 1985). It is drought tolerant in areas of low rainfall (Hall *et al.*, 1979). According to Rachie and Rawal (1976), cowpea appears to have originated in West Africa, very likely in Nigeria, where a profusion of wild and weedy species abound. However, more recent studies on the genetic exploration of cowpea in Africa (Padulosi S.S. - personal communication, 1993) suggested that Swaziland may be the primary center of origin of wild progenitors because this country has a higher species diversity than others in Africa and, indeed, the world. On the other hand, Congo may be a secondary center of diversity because of the presence of perennial types of cowpea there. The relatively high diversity in Congo is related to the number of taxa present in that country. Nigeria has been considered by Steele (1972) as the country with the highest diversification for domesticated cowpea.

According to Purseglove (1974), cowpea is important as a food throughout West Africa, being second only to groundnut as a cultivated legume. Unlike many other legumes, cowpea may be consumed at various stages of its development as green leaves, dried leaves, green pods, green peas and dry seeds. The dry seeds take comparatively less time to cook than many other grain

legumes, an important consideration in most developing countries where cooking fuel is scarce and expensive. Cowpea is also important as fodder for animals in the Sahelian regions (IITA GLIP Research Monograph No. 1, 1989).

There are several advantages that cowpea enjoys over *Phaseolus* beans according to Rachie and Rawal (1976). Nutritionally, cowpea has excellent protein, varying from 23 to 30 per cent depending on genotypes and environmental factors (Bliss, 1975). It is also free of metabolic inhibitors and flatulence factors. The true digestibility of cowpea protein was found to be 85%, with 95% of the biological value of egg albumen. An important agricultural advantage is that cowpea is able to fix nitrogen in the soil efficiently at about 30 to 70 kg/ha.

Cowpea is predominantly a hot weather crop well adapted to the semi-arid and humid savannas as well as to the margins of humid forests in tropical lowlands. According to Rachie and Rawal (1976), it is also deep rooted, has drought escaping abilities, and some varieties mature quickly in 45 to 60 days, but most normally in 85 days. Ojomo (1967) observed that in Nigeria, only 50-127 mm of rainfall per month is required for seed ripening in the drier north of the country. The growing season (June to November) provides adequate rain and sunlight to allow sufficient production of photosynthates (Ebong, 1965). Hence, more cowpea is grown in the north than in the south of Nigeria. These conditions, along with crop protection measures against insect pests, diseases, weeds and nematodes encourage the production of high quality seeds.

Despite the importance of cowpea in the Nigerian diet and economy, its production has been in the hands of small-scale farmers whose traditional methods do not produce enough cowpea for the country's needs. According to IITA GLIP Research Monograph No. 1 (1989), world cowpea production has been estimated at about 2.3 million tons from 7.7 million hectares. However, these figures are considered to be underestimates of the real production levels and total area cultivated because cowpeas are grown as multipurpose subsistence crops

on small farm holdings and much of the small-scale production is neither quantified nor included in crop production statistics. Rachie and Rawal (1976) states that real production levels may approach or exceed 2.5 million tons of dry seeds on about 9.0 million hectares. Nigeria produces about 0.9 million tons annually on 4.0 million hectares, most of which are in Kano, Jigawa, Sokoto, Katsina, Kebbi, Yobe and Borno States (Emechebe and Shoyinka, 1985). Other major producers in Africa include Benin, Botswana, Burkina Faso, Cameroon, Ghana, Kenya, Malawi, Mali, Mozambique, Niger, Senegal, Somalia, Togo and Uganda. In Asia, cowpeas are increasingly becoming part of the rural diet. This has led to increased local production (IITA GLIP Research Monograph, No 1, 1989).

In Nigeria, six major agro-ecological zones are distinguished on the basis of rainfall (IITA, GLIP Research Monograph 1989):

| Zone | Rainfall (mm) |
|---|---|
| Sahel | 300 - 600 |
| Dry savanna | 600 - 900 |
| Moist savanna | 900 - 1300 |
| Transition zone | 1300 - 1600 |
| Humid forest | 1600 - 2500 |
| Mid-altitudes | 500 - 1200 |

Cowpea varieties cultivated in these different zones vary a great deal, being influenced by the climatic conditions and cropping systems.

The constraints to cowpea production vary to some extent according to the above mentioned agro-ecological zones in Nigeria. Diseases, insect pests, drought and heat have been considered by farmers and researchers as major constraints in the countries where cowpea is produced.

According to Emechebe and Shoyinka (1985), the most important fungal and bacterial diseases of cowpea in the savannas are scab, *Septoria* leaf spot, bacterial blight, brown blotch web blight *Pseudocercospora/Cercospora* leaf spot and *Macrophomina* blight. Viral diseases of cowpeas are: those incited by cowpea (yellow) mosaic virus (Chant, 1959), cowpea aphid-borne mosaic virus (Ladipo and Allen, 1976; Rossel, 1977), Cowpea mottle virus (Robertson, 1963), cowpea mild mottle virus (Thouvenel *et al.*, 1982) and cowpea golden mosaic virus (Anno-Nyako *et. al.*, 1983) and blackeye cowpea mosaic virus (Thottappilly and Rossel, 1985).

Parasitic seed plants such as *Striga gesnerioides* and *Alectra vogelii* are of major concern (Emechebe *et al.*, 1991). Root knot nematodes are a problem in sandy soils (Emechebe and Shoyinka, 1985).

Scab induced by *Sphaceloma* sp. is a devastating disease of cowpea in the Guinea savanna ecologies. For example, in Samaru, Zaria yield losses of up to 71% have been reported (Mungo *et al.*, 1995). Scab is also a problem in East Africa (Kenya and Uganda) and in Southern Africa (A.M. Emechebe, personal communication, 1990). The sources of primary inoculum of scab are considered to include the seed, soil and infected cowpea debris. The survival of the fungus on infected cowpea crop residues is the basis for the use of cowpea residues of one season as inoculating materials for crops grown in the immediate succeeding season (Donli, 1983). The conidia of *Sphaceloma* sp. (imperfect or anamorphic state of *Elsinoe phaseoli* ) are responsible for inducing scab on cowpea plants.

Limited information is available on the biology of *Elsinoe phaseoli,* the perfect (teleomorphic) state. Consequently, the role of the perfect state in epidemiology of scab of cowpea has not been understood. No studies have been carried out on the teleomorphic state, even though the teleomorph may be important in increasing genetic diversity of the pathogen. Paucity of reports on both the imperfect and perfect states has called for a study of the pathogen's biology and the epidemiology of the disease. This study therefore addressed the

need to:

(1) develop easier methods for isolating and culturing the fungus,

(2) determine the sources of primary inoculum, and

(3) study some important epidemiological characteristics of the fungus under field and screen house environments.

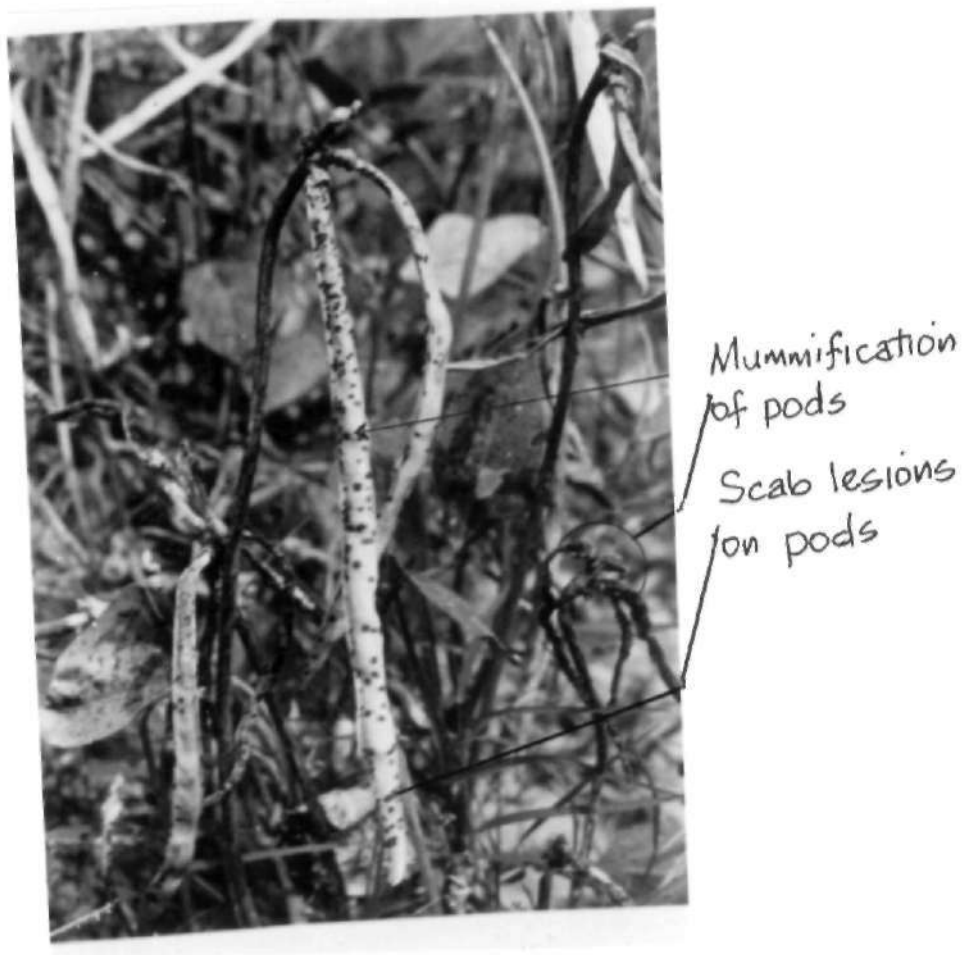



**Plate 2. Cowpea pods with scab lesions.**

## 2.4 Isolation of the Pathogen

Generally species of *Elsinoe* or *Sphaceloma* are difficult to isolate. Bitancourt and Jenkins (1937) had difficulty in isolating *Elsinoe fawcetti* (citrus scab). They reported that the fungus grew on agar medium and produced a visible colony from a single spore or small mycelial fragment only after 4 - 6 days and it was frequently contaminated by *Colletotrichum gloeosporioides*, *Fusarium* spp., *Curvularia* spp., *Choanephora* spp., *Cladosporium* spp. and yeasts. Surface-sterilization with ethanol or sodium hypochlorite reduced contamination only slightly. To isolate the fungus, Winston (1923) and Bitancourt and Jenkins (1937) scraped the surfaces of scab pustules into Petri dish and poured agar media over them. Also Whiteside (1978) cut thin tangential sections from scab pustules and mashed them in a sterile Petri dish with a bent steel spatula to produce small fragments that were dispersed in cooled melted water agar in a Petri dish. Within 3-4 days, before contaminants overran the plate, the fragments were examined with the aid of a microscope for any growth of *E . fawcettii* from their margins. These procedures were laborious and succeeded only if young pustules were available. Whiteside (1986) improved on the techniques by incorporating dodine, streptomycin and tetracycline into PDA to produce dodine-streptomycin-tetracycline (DST)-PDA medium on which he isolated *Sphaceloma perseae* Jenkins from avocado fruits and *Sphaceloma poinsettiae* Jenkins and Ruehl from poinsettia (*Euphorbia pulcherrima* Willd. ex Klotsch) leaves. Both of these pathogens tolerated relatively high concentrations of dodine.

The fungus was isolated on potato dextrose agar plus streptomycin and rose bengal by (Emechebe, 1980). Colonies appeared within 4-6 days when young scabbed leaves were plated on this medium. The fungus produces acervuli, which are initially dome-shaped and raised above the medium and with little or no lateral growth on the agar surface. Due to the slow growth of the fungus, colonies of *Sphaceloma* sp. are frequently overgrown by faster-growing micro-organisms if subculturing is delayed.

## 2.5 Effect of Temperature on the Growth of *Sphaceloma* species

For a disease to occur, three conditions must be met: a susceptible host plant must be present, the pathogen must be in an infective stage, and the environmental conditions must be favourable for disease development. For an epidemic to develope, numerous susceptible hosts and abundant inoculum of the pathogen are required and the environment must be favorable for a sufficient length of time. Meteorological conditions influence the development of an epidemic not only over a single growing season, but in subsequent seasons. Temperature and moisture conditions are the most frequently limiting factors of suscept-pathogen interactions (Coakley, 1987). According to Rache and Kuc (1970), temperature is one of the environmental parameters frequently reported as being of great importance in the alteration of disease epidemics in crop plants. A knowledge of the optimum temperature for growth and sporulation of a fungal pathogen is necessary in studies of diseases and genetics of disease resistance (Fakorede, 1976). Emechebe (1980) isolated *Sphaceloma* sp. from cowpea leaves at 27±2°C in Samaru, Nigeria. Whiteside (1986) isolated *S. perseae* Jenkins from avocado (*Persea americana*) and *S. poinsettiae* from poinsettia at 25°C using DST-PDA medium in Florida, USA.

Work on similar pathogens has shown that temperature is important for pathogenesis. Solanki *et al.* (1974) reported an optimum temperature of 25°C for germination of the conidia of the *Capsicum* isolate of *Colletotrichum capsici* while Fakorede (1976) reported that 25°C to 26°C was the optimum temperature for growth, sporulation and infection of cowpea by *C. lindemuthianum*.. Singh *et al.* (1977) found that the optimum temperature for growth and sporulation of the *Capsicum* isolate of *C. capsici* was 30°C and that no growth occurred below 12°C. Emechebe (1986) studied the effect of temperature on the interaction between cowpea genotypes and *C. capsici* and found that the higher the susceptibility of a variety the higher the maximum temperature conducive for brown blotch pathogenesis in that variety. Thus, for Ife Brown, which is highly

susceptible, the disease developed at both day / night temperature regimes of 35/26°C and 28/23°C but developed only at 28/23°C in TVx 3236 and IT 82E-16, which are less susceptible (Emechebe, 1986).

A study by Mazlan and Sariah (1980) on anthracnose of chilli induced by *C. capsici* showed that growth and sporulation of the fungus in vitro were maximum at 30°C and pH 5. On the other hand, Tu (1981) studied anthracnose of French bean (*C. lindemuthianum* ) and reported that temperatures above 25°C restricted disease development. Later, Sindhan (1983) showed that the optimum temperature for the development of *C. lindemuthianum* in French beans was 18-27°C, the maximum disease intensity occurring at 21°C. Also, Ghogre and Kodmelwar (1986) reported that maximum growth of *C. curcumae*, causal agent of leaf blotch of turmeric, occurred at 27°C. Similarly, Sartorato (1988) reported from Brazil that *C. lindemuthianum* developed at between 13 and 17 °C, the optimum temperature being 17°C; and high relative humidity favoured disease development.

**2.6 Effect of Age of the Suspect on Disease Development**

In most cases, the age of a plant influences the susceptiblity of the plant to diseases. Field observations in Samaru, Zaria (Nigeria) have shown that *Sphaceloma* sp. infects mostly the young tissues of the various plant parts. The older the plant tissue, the less susceptible it is to scab infection. Similarly Griffey and Leach (1965) found that "old" tissue (hypocotyl of a French bean plant, the second, non-cotyledonary node of which had fully developed leaves) of a susceptible variety of French bean had small anthracnose lesions subsequent to inoculations with *C. lindemuthianum* compared to larger lesions on younger tissue. This mature plant resistance in French bean to *C. lindemuthianum* has been corroborated by other workers. For example, Coronel and Chaves (1975) studied the interaction of age of Phaseolus bean and inoculum concentration on the susceptiblity of bean plants to races of *C. lindemuthianum* and showed that

the reactions of 3- to 15-day old plants were similar; by contrast, 35-day old plants were less susceptible even when inoculated with the highest inoculum concentration. It has also been found that if plants passed the seedling stage without heavy infection they were less likely to be severely attacked until perhaps the early stages of pod development when there is another phase of marked susceptibility, mostly of young pods (Wheeler, 1982). Centro Internacional de Agricultura Tropical (CIAT) also reported that when different ages of a susceptible bean variety (Ecuador 1056) were inoculated with the same inoculum of *C. lindemuthianum*, one-week old seedlings were very susceptible, but as the age of plants increased the amount of anthracnose symptoms decreased; consequently, plants inoculated at 3 weeks of age developed very little disease while those inoculated after 3 weeks of age had virtually no disease (CIAT, 1982).

### 2.7 Host Range of the Pathogen

Up till now it has not been ascertained whether *Sphaceloma* sp. pathogenic to cowpea is similar to the *Sphaceloma* stage of *E. phaseoli* or to any of the *Sphaceloma* spp. reported on common tropical food legumes.

According to Emechebe (1980) cowpea isolates of *Sphaceloma* sp. cause severe and mild scab symptoms on asparagus pea (*Vigna sesquipedalis* ) and hyacinth bean (*Lablab niger*) respectively; the fungus was not pathogenic on lima bean (*Phaseolus lunatus*) and French bean (*P. vulgaris*) which are known to be susceptible to *E. phaseoli* (Bruner and Jenkins, 1933; Bates, 1959), nor was it pathogenic on soybean and groundnut. Emechebe (1980) therefore concluded that the *Sphaceloma* sp. causing cowpea scab is pathogenically distinct from *S. glycines* Kurata and Kuribaysahi and *S. arachidis* Bitancourt and Jenkins, the pathogens of soybean and groundnut scabs, respectively. He suggested that the host range of cowpea scab pathogen may well be restricted to *Vigna* spp. and species of closely related genera and that it could be an undescribed species of

*Sphaceloma*. Donli (1983) carried out some host range studies and found out that Bambara nut, hyacinth bean, yardlong bean and *Cassia tora* (a weed) were affected by cowpea scab pathogen.

Species of both *Elsinoe* and *Sphaceloma* affect several other hosts apart from legumes. *Elsinoe thea* has been reported from tea in Sao Paulo, Brazil (Bitancourt and Jenkins, 1951). *Elsinoe batatas* induces scab on sweet potato (Floyd *et al*., 1988) while cassava is attacked by *S. manihoticola* in Brazil, Nicaragua, Guatemala and Dominican Republic (Bitancourt and Jenkins, 1951).

## 2.8 Screening Cowpea Genotypes for Resistance to Scab

In a study to identify cowpea lines that possessed multiple resistance to the four major cowpea diseases (scab, brown blotch, *Septoria* leaf spot and bacterial blight) of the Guinea savanna belt of Nigeria, Allen *et al* . (1979) found that Vita-4 was resistant to all of them. The reaction of the lines to scab was determined by placing scab infected cowpea tissue on the upper canopy of healthy plants. Those which had no disease symptoms on them were regarded as resistant while those with severe scabbing were susceptible. Further work carried out at Samaru, Zaria in 1982, using this method, revealed that IAR 81-37 and IAR 81-63 appeared to possess resistance to the four diseases (Emechebe, 1984). Donli (1983) reported TVu 851 as being resistant to the disease under field conditions. Emechebe, (1984) screened 16 IITA cowpea lines in search for resistance/susceptibility to scab and Mungo (1991) further confirmed resistance/susceptibility to the disease in a study using different inoculation techniques on the cowpea lines. Seven lines were found to be resistant to scab:
IT 83S-720-2, IT 85S-742-2, IT 86D-400, IT 86D-1057, IT 86D-1033, IT 81D-1137 and IT 83D-641.

## 2.9 Crop Loss Assessment and Grain Yields of Cowpea Lines in Relation to Their Reaction to Scab.

Scab is one of the important pod diseases of cowpea in Nigeria. Grain yields of cowpea lines in relation to their reaction to the disease were reported by Donli (1983). She showed that grain yields of the most susceptible varieties ranged from 650g to 750g per plot while those of the resistant lines yielded 950g to 1200g per plot. Emechebe and Shoyinka (1985) reported a crop loss of 60% in farmers' fields and experimental plots in Zaria while Mungo *et al.* (1995) working in the same area also reported a yield loss of 71% in experimental plots. Madden *et al.* (1981) defined crop loss as the measurable reduction in quantity and, or quality of yield. Crop loss estimates provide evidence that will justify or condemn the use of fungicides to control epidemics (James, 1974). This is because the loss caused by diseases in crops may not be apparent to a casual observer, unless it is a complete crop failure. Le Clerg (1964) has suggested that yield loss estimates are the best way to indicate to the grower and the consumer the opportunities for gain when sound control measures are applied. They also guide in the selection of priorities for research, in predicting crop production, and in establishing the need for the control of the disease. The primary reason for applying plant protection measures is to reduce crop loss to a level that is economically acceptable.

The measurement of disease and pathogen is fundamental to crop loss studies and to many types of disease prediction and management systems (Gaunt, 1987). Several terms have been used to define aspects of disease measurement including incidence, severity and intensity. Incidence, the proportion of plants or plant parts infected, is used more widely for both pathogen and disease monitoring for many purposes. Severity, the amount of tissue damaged, is the most demanding and the most diverse form of disease measurement. Disease intensity is used as a general term for the amount of disease present, often based on incidence and /or severity. The measurement of incidence is not sufficient for

many diseases, where there is a quantitative relationship between the degree of infection or severity and the amount of yield loss. However, methods have been developed for estimation of disease severity; these include a variety of approaches and may be carried out on individual plant parts, whole plants or at the whole crop level. In cowpea scab, an assessment scale described by Emechebe (1981b) takes into consideration damage to individual plant parts rather than the whole plant. Much of what is known about yield loss caused by individual plant diseases has been the result of field experimentation (Sah and Mackenzie, 1987). In order to study the effect of disease on yield in field experiments several methods have been developed to generate different levels of disease severity, such as the use of isogenic lines and fungicides (James, 1974). Perhaps the most common research tool used to effect different amounts of fungal diseases in field plots is the use of fungicides.

Research with fungicides has added a new dimension to the method of yield loss studies (Romig and Calpouzous, 1970; James *et al.*, 1972; James, 1974 and Schneider *et al.*, 1976). In studying the crop loss due to cowpea scab, Emechebe and Shoyinka (1985 ) and Mungo *et al.* (1995) used benomyl to generate different levels of disease intensities in field experimental plots and reported a crop loss of 60 and 71%, respectively. Anthracnose of French beans (*C. lindemuthianum* ) has been associated with heavy yield losses wherever susceptible bean cultivars are grown in locations with cool to moderate temperatures and high humidity or free moisture (Schwartz and Sanders, 1979). Yield losses of up to 100% have been reported in many countries ( Zaumeyr and Thomas, 1957; Guzman *et al.*, 1979; Mukunya and Keya, 1979 ). In Tanzania, Shao and Teri (1985) reported that yield losses of 86% occurred in highly susceptible variety, T8, and 27% in the moderately susceptible Mexican 142. Yield losses in susceptible cowpea varieties as a result of brown blotch attack have been estimated at between 75 - 100% (Emechebe, 1981a; and Okpala, 1981).

Models for describing disease-loss relationships use one or more epidemic

variables to predict loss. The common variables used include disease severity at one or more points in time, time or growth stage at which a certain disease level is reached, area under the disease progress curve (AUDPC), and slope of linearized disease progress curve (James, 1974; Mian, 1977 and Teng, 1987).

## 2.10 Survival and Secondary Spread of the Pathogen and Some Species of *Colletotrichum*.

Very little information is available on how *Sphaceloma* spp. survive in absence of their host. Donli (1983) investigated the survival of *Sphaceloma* sp. in cowpea in northern Nigeria and found that the pathogen was capable of over seasoning on infected cowpea residues in the field. Emechebe (1980) and Mungo (1991) observed from field inoculation studies that cowpea scab pathogen survives the non-crop season on infected plant residues in the field. Under field conditions in the Guinea savanna ecologies the pathogen produces conidia on infected plant residue. Wind driven moisture, rain splash and run-off water help the secondary spread of the pathogen in the field (Emechebe and Shoyinka, 1985). According to McCubbin (1933) the spores of *Elsinoe canavaliae* are wind borne. The spores are capable of surviving to the next cropping season on infected debris. He also reported that stormy weather increased the incidence of scab.

Survival of other members of the Melanconiales such as *Colletotrichum* spp. have been investigated. Chowdhury (1957) studied *C. capsici* in chilli (*Capsicum frutescens)* and reported that the fungus survived in the field in plant debris and that primary infection of its host took place from the spores of the fungus which were carried by wind from such sources and deposited on the host. Smith and Crossan (1958) found that *C. piperatum* could survive for at least 9 months in naturally infected chilli in or on soil surface. This was supported by the work of Grover and Bansal (1970) who found that conidia of *C. piperatum* survived for up to 10 months in dry chilli fruit kept in the laboratory on dry soil surface at 8 - 28°C, or buried in potted soil in the field to a depth of 10 - 15 cm.

Warren (1972) reported that the conidia of *C. graminicola*, the pathogen of maize anthracnose, lost their viability within 6 weeks when stored at 4°C. Farley (1976) found that the conidia of *C. coccodes* (Wallr.) Hughes, the pathogen of anthracnose of tomato fruits, could survive for up to one year in artificially infested moist and dry soil incubated at 4°C and 25°C, respectively. Okpala (1981) found that *C. capsici* can survive in naturally infected cowpea plant parts during the off season and that the residue acted as sources of primary inoculum for the following year's crop. Survival of *C. capsici* in cowpea seeds and seed transmission of the disease has also been reported by Emechebe (1981a).

## 2.11 Control of Cowpea Scab

Experiments carried out by Donli (1983) suggested the following control measures for cowpea scab: destruction of infected trash, i.e., collecting infected plant materials and burning them to ashes; crop rotation in which cowpea is followed by an unrelated host crop; and the development and use of scab resistant varieties. Emechebe (1981b) and Mungo *et al.* (1995) reported that a weekly spray with benomyl, starting at three weeks after planting, will control the disease resulting in higher yield and lower crop loss. Emechebe (1991) reported that seed treatment with benomyl reduced the incidence of scab.

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

### 3.1 Histopathological Study of Scab Lesions on Cowpea Plants

The cowpea variety used for the study was IT 85F-1517, a susceptible variety. Cowpea plant parts (leaves, stems, peduncles, flowering cushions and pods) were collected from field plots in Samaru (Zaria), Nigeria during the cropping seasons of 1992 and 1993. The plant parts were cut into 5-cm pieces and processed for microtome sections following the method described by Sass (1958). The transverse sections were 8 to 10 µm thick. Cut sections were mounted on slides with the aid of egg albumen adhesive. Staining of cut sections with safranin-fast green was as described by Sass (1958) given in Appendix 1. Slides were then examined for the presence of structures of *Sphaceloma* sp. and the damage caused by the pathogen under a compound microscope.

### 3.2 Isolation of *Sphaceloma* sp. from Four Cowpea Plant Parts Using Eight Media

A variety (IT 85F-1517) susceptible to cowpea scab was the source of infected plant materials used in this study which was carried out in 1992 and 1993. Four cowpea plant parts and eight culture media were used to determine the plant part from which *Sphaceloma* sp. can easily be isolated and the best medium for the isolation. The plant parts used for the isolation were leaves, stems, peduncles and pods. The media tested were: potato dextrose agar (PDA), malt agar (MA), corn meal agar (CMA), V-8 juice agar (V-8 juice), cowpea leaf agar (ClfA), cowpea stem agar (CstA), cowpea peduncle agar (CpedA), and cowpea pod agar (CpdA). The cowpea extract media were prepared from young cowpea plant parts. The composition of the media was based on Tuite (1969) and is shown in Appendix 2. All the media were amended with streptomycin sulphate (1.5g/l) and rose bengal (0.0025g/l of agar) to inhibit bacterial growth.

### 3.2.1 Method I- isolation by plating out of intact young scab lesions

Young scab lesions were carefully cut off from different cowpea plant parts with sterile surgical blades immediately after they were brought from the field. All lesions were surface-sterilised in 1% sodium hypochlorite (NaOCl) for 5 minutes and rinsed twice with sterile distilled water. The composition of media is presented in Appendix 2. Lesions of each cowpea part (leaf, stem, peduncle or pod) were plated on each of the eight media in Petri dishes (four lesions per plate) and incubated at 26°C for 5 days. The experimental design was a complete randomized block design in six replicates due to the large number of plates used. For each plant part, one replicate consisted of ten plates, making a total of 60 plates per plant part per medium, i.e., 480 plates for each plant part for the eight media. Because young lesions appeared at different times on the four parts of the cowpea studied, isolations from these parts were done at four different times. Three incubators set at the same temperatures were used in this experiment. In each incubator two replicates of a plant part plated on all media on the same day were placed on two shelves. From the third day of incubation, the Petri dishes were examined daily for the presence of colonies of *Sphaceloma* sp. The number of lesions on which colonies of the fungus were produced was determined to obtain the frequency of successful isolation from each plant part in each of the eight media.

To obtain cultures for subsequent use, pure colonies of the fungus were crushed in sterile distilled water in sterilized mortar using a sterilized pestle. The suspension was then spread on the media surfaces using a sterilized L-shaped glass rod and incubated at 26°C.

### 3.2.2 Method II- isolation by plating out macerated young scab lesions

The composition of the media was the same as in 3.2.1. The media were put in 9 cm-diameter Petri dishes. About 50 young lesions were cutout with sterile

surgical blades from each of the four cowpea plant parts. The lesions were surface sterilised with 1% NaOCl for 5 minutes and rinsed twice with sterile distilled water. The lesions were then ground in a sterilized porcelain mortar using a sterilized pestle until a smooth paste was obtained. Ten ml of sterile distilled water plus 200 mg of streptomycin sulphate were added to the paste to make a suspension. A sterile syringe was used to apply two drops of the suspension on each agar plate. Using sterile, L-shaped glass-rods, the suspension was then spread on the agar surface. The experimental design was similar to that described in 3.2.1. There were three replicates per plant part per medium and each replicate had six Petri dishes. For each plant part, one replicate consisted of ten plates, making a total of 30 plates per plant part per medium, i.e., 240 plates for each plant part for the eight media. The plates were incubated as described in 3.2.1.

### 3.3 Determination of Incubation Period of *Sphaceloma* sp. in Cowpea

A susceptible cowpea cultivar (IT85F-1517) was used in this study. Seeds from pods not affected by scab, produced in a scab-free zone at Ikenne, Ogun State, (Nigeria) were planted in sterilised soil in 11 cm (diameter) plastic pots in the screen house at Samaru. Plants of four different ages (14, 28, 42 and 56 days old) were used in order to obtain leaves, stem, flowering cushion, peduncle and pods for simultaneous inoculation with the same conidial suspension. There were seven replicates. Each replicate comprised three pots, each containing two plants. The pots were kept on benches in the screenhouse. The procedure for inoculum preparation is described below.

Thirty mls of potato dextrose (PD) broth containing streptomycin (1.5 g/l) and rose bengal (0.0025 mg/l of broth) were dispensed into sterilized test tubes of 25 x 150 mm. The PD broth in test tubes were autoclaved and inoculated with five colonies of a 6-day-old *Sphaceloma* culture and incubated at 26°C for conidia

production, with occasional manual agitation every 6 hours. After 4 weeks, the mycelial mat was separated from the conidia suspension by filtering through double layer of sterile cheese cloth. A haemocytometer was used to determine the concentration of conidia in the suspension. In one of the evenings of the month of September when the temperatures were cool, the plants were sprayed with sterile distilled water and covered with large polyethylene bags for 24 hours. After 24 hours, the bags were removed and the plants sprayed to run-off with a conidial suspension containing $7 \times 10^5$ conidia per ml. The plants were then covered with polyethylene bags for another 24 hours after which the bags were removed. The plant surfaces were sprayed daily with sterile distilled water to keep them moist. The plants were observed daily for appearance of symptoms on the various parts for 14 days. The experiment was repeated once but the data reported here is that of the second inoculation study since the results were similar to those of the first inoculation trial. Organ age in the various plant parts in this study was not considered.

### 3.4 Influence of Temperature and Type of Culture Medium on the Growth of Cowpea Isolate of *Sphaceloma* species

The isolate of the pathogen used in this study was obtained using the method described in section 3.2.2 above. Pure cultures of *Sphaceloma* sp. were maintained for subsequent use on potato dextrose broth and stored at 5°C (Plate 3).

On the agar surface, *Sphaceloma* sp. grows vertically in colonies comprising masses of mycelia. One of these colonies was crushed in a sterilized mortar with a sterilized pestle to obtain a paste to which 10 ml of sterile distilled water was added to obtain a conidial suspension. Using an L-shaped glass rod, two drops of the suspension were carefully spread on PDA containing rose bengal (PDAR) in Petri dishes. The Petri dishes were incubated at 26°C. On the sixth day of incubation when individual colonies were actively growing, 10 groups of

colonies, each group consisting of 10 colonies, were weighed to determine the average weight of 10 colonies. Ten colonies (weighing approximately 0.01 g) were obtained at random from 6-day old cultures and plated separately on agar in a 9-cm (diameter) Petri dish containing one of the following media: PDAR, corn meal agar plus rose bengal (CMAR), and malt agar plus rose bengal (MAR). The concentration of rose bengal was 0.0025 mg/l of agar in all media. Rose bengal was added to all media to aid in fungal identification since the fungus readily absorb the pinkish colour of rose bengal. Streptomycin sulphate (1.5 g/l) was added to all media to control bacterial growth. The Petri dishes were incubated at 15, 20, 26, 30 and 35°C. Fungal growth was measured by taking the weight of 10 colonies for each treatment at 3-day intervals up to 15 days of incubation. The colonies were discarded after weighing. The rate of growth (V) was calculated using the formula:

$V = \frac{X_2 - X_1}{t_2 - t_1}$ where:

$X_1$ = average growth weight at time $t_1$

$X_2$ = average growth weight at time $t_2$

Each of the 18 treatments was replicated six times (for each sampling period), each replicate consisting of one plate. Since plates sampled at 3-day interval were discarded and sampling was done five times, there were 90 plates for each treatment. At the end of 15 days the fungus on the plates incubated at 35°C appeared dead. In order to investigate if the fungus was actually dead the plates were transferred to 26°C for two weeks. The experiment was done insplit plot design with temperature as the main plot and agar medium as the subplot.

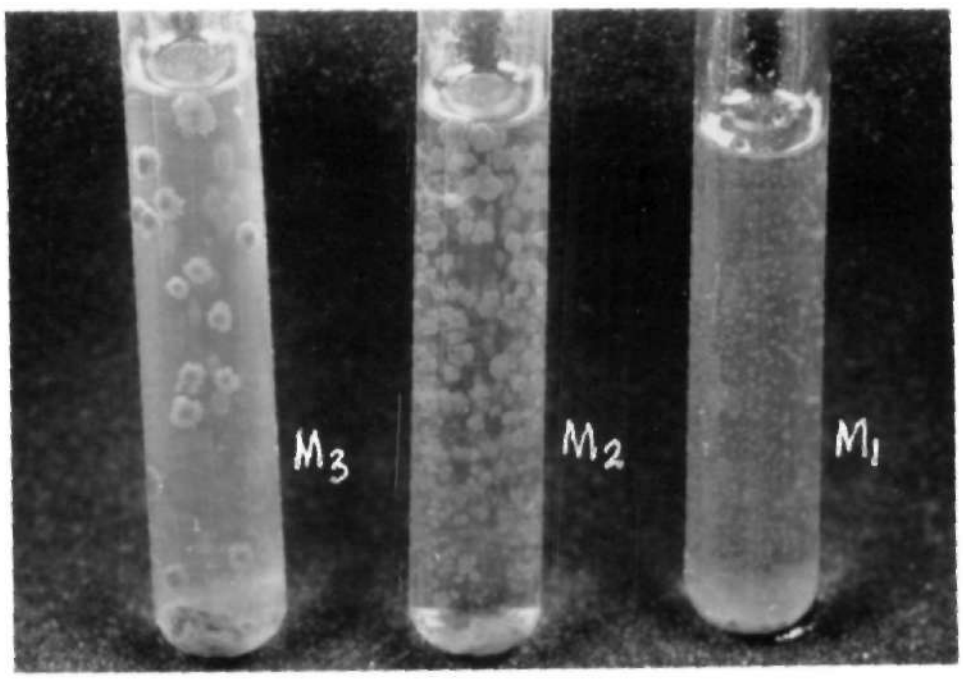



Plate 3. 1- to 4-week-old cultures of *Sphaceloma* sp. in potato dextrose broth at 26°C. $M_1$ = 1-week-old culture, $M_2$ = 2-week-old culture, $M_3$ = 3-week-old culture at 25x magnification.

### 3.5 Seed as Possible Source of Primary Inoculum

Seeds of a cowpea variety (IT 85F-1517) susceptible to scab grown either at Samaru (where scab occurs) and or at Ikenne (where scab does not yet occur) were planted in Samaru to see if *Sphaceloma* sp. is seed transmitted. Seeds from Samaru were obtained from pods with scab lesions. The seeds were planted in plastic pots containing sterilized soil and the treatments were as follows:

(i) Seeds from Samaru from pods heavily infected by scab but treated with benomyl at the rate of 1.5 g of commercial product (Benlate$^R$ 50 WP) / 1kg of seed.

(ii) Untreated seeds from Samaru from pods heavily affected by scab.

(iii) Seeds from Ikenne treated with benomyl at the rate of 1.5 g of commercial product of Benlate$^R$ 50 wp / 1kg of seed.

(iv) Untreated seeds from Ikenne, from the same seed lot as that of seeds in (iii).

Plastic pots (diameter 13 cm and height 12 cm) were filled with sterilized soil. The soil surface was protected against incoming inoculum by covering with polyethylene sheet held firmly onto pots with a rubber band. Planting of seeds was done by piercing through the polyethylene sheets. In 1993 the pots were placed on top of polyethylene sheet laid out in the open air close to older cowpea fields. In 1994, however, the pots were placed on top of polyethylene sheet laid out in the open air far from any cowpea fields to avoid background contamination. Immediately after germination the open space surrounding the hypocotyls of the plants was filled with sterilized sawdust to minimize soil to aerial plant part contact. The total number of plants per pot was recorded. There were ten replicates for each treatment. Each replicate had 20 pots. The experiment was laid out in a randomized complete block design. The experiment was repeated in 1994 but there were only two treatments (Samaru infected seeds: untreated and Samaru infected seeds: treated) because of the shortage of seeds from Ikenne.

Observations on the number of plants with scab were recorded when the

plants were three weeks old after sowing.

### 3.6 Soil as Possible Source of Primary Inoculum (for Cowpea Scab)

Three different field sites were chosen for this study which was carried out in both 1993 and 1994:

Field 1. Last planted with cowpea a year before the trial,

Field 2. Last planted with cowpea two years before the trial,

Field 3. Last planted with cowpea three years before the trial.

The experiment was laid out in each of these fields. The different fields served as three locations of the experiment. In each location, the treatments comprised soil covered with plastic mulch and soil without plastic mulch. There were six replicates of treatment within each field. Each plot consisted of four, 6-m-long ridges spaced 75 cm apart. In mulched plots, the ridges were covered with clear polyethylene mulch. In 1993 cowpea seeds were planted on July 29, while in 1994 the planting was done on July 15. Cowpea seed was planted only on the central 3 m of each ridge, leaving 1.5 m on both sides which were planted to soybean. The soybean was intended to reduce soil splash and interplot inoculum drift. For the same reason, two ridges on each side of the plot and 1.5 m at the end of each plot and between all plots were planted with soybean.

Seeds of IT 85F-1517 were obtained from Ikenne, Ogun State (Nigeria) which at present, is a scab free zone. Seeds were planted in mulched plots through a hole made on the polyethylene mulch. Two seeds were sown per hole at 20 cm spacing along the ridge. Immediately after germination, the area around the plant base in mulched plots was filled with sterilized sawdust to reduce soil splash. The furrows of all mulched plots were covered with saw-dust to minimize the chances of soil being splashed at the plants. Stones and cut branches of trees were laid over the sawdust to minimize its being blown away by the wind. The plants were protected from insect damage by applying forthnightly with insecticidal spray (a tank mixture of cypermethrin and dimethoate each at 1

litre/ha) starting at 2 weeks after sowing.

Plots were observed daily for disease development, for 12 days, starting from 14 days after sowing. The following data were collected:

(i) Presence of symptoms on primary leaves, epicotyl and hypocotyl.

(ii) Disease incidence on the affected plant parts. (Disease incidence on affected plant parts is the number of plant parts expressed as a percentage of the total number of plant parts).

(iii) Disease severity on the affected plant parts.

(iv) Overall disease incidence. (The overall disease incidence was defined as the number of plants with scab symptoms, irrespective of the plant parts on which the symptoms were found).

The rating scale below, modified from Emechebe (1981b), was used to assess disease severity on affected plant parts.

1 - No symptoms

2 - Few pin point lesions

4 - Few lesions 3-5 mm in diameter with well defined borders

6 - Many lesions some larger than 5 mm diameter with darker brown or white centers

8 - Coalescing stem lesions

10 - Stem severely damaged with marked stem stunting.

### 3.7 Effect of Planting Date on Incidence and Apparent Infection Rate of Scab and on Cowpea Yield in the Field in 1992 and 1993

In 1992 a field experiment was conducted at the IAR fields in Samaru-Zaria (Nigeria) to determine the effect of planting date on the disease incidence, and apparent infection rate on the affected cowpea parts. The experiment was repeated at the same location in 1993. Three cowpea varieties (IT 85F-1517, IT 81D-1137 and IT 83S-720-2) were chosen because they differ in their susceptibility to scab; thus, IT 85F-1517 is very susceptible, IT 81D-1137 is moderately susceptible while IT 83S-720-2 is resistant. The field layout was a randomized complete block design with four replicates. Each plot consisted of three, 75-cm wide ridges, each ridge being 3 m long. The plots were separated by buffer rows consisting of two ridges along each of the lengths and 2 m along the widths. There were three planting dates spaced 10 days apart. In 1992 the planting dates were July 16, July 26 and August 5. This is because cowpea is usually planted in mid July in the northern Guinea savanna after the establishment of the rains . In 1993 there was a delay in planting because of the late rainfall. The planting dates were July 24, August 4 and August 14. Seeds were sown at the rate of two seeds per hole at 20 cm spacing. Plant stand establishment was taken at 21 days after planting. The plants were naturally infected with the scab pathogen during the growing season. The plants were protected from insect damage by spraying forthnightly with insecticidal spray (tank mixture of cypermethrin and dimethoate each at 1 litre / ha) starting at 4 weeks after sowing.

Records of disease incidence were taken for the different plant parts. The disease incidence ratings were determined every 7 days, starting 4 weeks after sowing.

The apparent infection rate was determined using Van der Plank's (1963) formula:

$$r = \frac{2.3}{t_2 - t_1}\left(\log_{10}\frac{x_2(1-x_1)}{x_1(1-x_2)}\right)$$

where $x_1$ and $x_2$ are the proportions of disease on dates $t_1$ and $t_2$, respectively. The pods were harvested, threshed and grain weight recorded.

### 3.8 Effect of Fungicides on Development of Scab Disease

In both 1992 and 1993 a study was carried out at the experimental farms of the Institute for Agricultural Research (IAR), Samaru, Zaria (Nigeria) to determine the effect of fungicides on the development of scab.

In both years the development of scab in various aerial parts of three cowpea varieties with different levels of reaction to the disease (namely, IT 85-1517, susceptible; IT 81D-1137, moderately susceptible and IT 83S-720-2, resistant) was studied in the field under different fungicidal spray regimes.

The following fungicides were used:

1) Benomyl (Benlate 50 W.P., E.I. Du Pont de Nemours & Co. Inc. Wilmington, Delaware USA).
2) Carbendazim + maneb (50 W.P., Sumitomo Chemical Co. Ltd, Japan).
3) Mancozeb (Dithane M—45, Rhom and Haas Co., Philadelphia, USA).

All compounds were used at the recommended rates.

The field layout was a split-plot design with four replicates. The main plots consisted of the three cowpea varieties while the subplots consisted of the fungicide treatments. Each subplot consisted of five ridges, each ridge being 5 m long and 75 cm wide. Seeds were sown at the rate of two seeds per hole, with an inter-stand spacing of 20 cm. The subplots were separated from each other by one ridge on which soybean (SAMSOY-2) was planted to reduce interplot

interference due to spray drift and splash-borne inoculum. The main plots were separated from each other on all four sides by a 2 m space on which SAMSOY-2 was also planted. In 1992 planting of the trial was carried out on July 24 while in 1993 it was done on July 28. Plant stand establishment was taken at 3 weeks after planting.

Inoculum of *Sphaceloma* sp. for field inoculation was obtained by growing a susceptible variety (IT 85F-1517) in a disease garden sown at the onset of rains in May of each year. Plants in the disease garden were inoculated with scabbed cowpea residues of the previous year. When the test plants were 3 weeks old , the scabbed plant parts from the disease garden were used for field inoculation. The field inoculation was carried out by placing the inoculum in the leaf axils of the upper part of the plant's canopy. Fungicidal sprays commenced a week after inoculation until 50% pod maturity (i.e., eight weeks after sowing)

Commercially available fungicides were applied at the recommended rates. The four treatments applied each year included: 1) an untreated check, 2) one treatment of each of three fungicides commonly used commercially: Benlate (benomyl) at the rate of 0.6 kg commercial product per 400 litres of water; Dithane M -45 (mancozeb) at rate of 2.5 kg of commercial product per 400 l; and Delsene M (carbendazim+maneb) at rate of 2.4 kg of commercial product per 400 l. Fungicide application (with knapsack sprayer) commenced at 4 weeks after sowing and continued on a weekly interval until 50% pod maturity in each year. The insecticide, cypermethrin, was also applied to control insects.

Data were collected from the three middle ridges in each plot. Disease incidence was recorded per plot for each variety. Scab severity in the field on leaves, stems, peduncles, flowering cushions and pods was measured using the assessment scale of Emechebe (1981b), namely:

**Leaf scab**

1. No symptoms
2 Few, small spots on leaves
4 Many, large spots on leaves
6 Extensive, large spots on leaves
8 Many, large coalescing spots with shot hole spots.
10 Many large coalescing spots with marked leaf distortion.

**Stem scab**

1 No symptoms
2 Few, scattered pin point lesions on lower stem.
4 Few, scattered small lesions spreading up stem
6 Extensive scabbing of young stem.
8 Stem lesions coalescing covering also the branches.
10 Stem severely damaged with marked stem stunting.

**Peduncle scab**

1 No symptoms
2 Few scattered pin point lesions on lower peduncle.
4 Scattered small lesions spreading up peduncle
6 Extensive scabbing of young peduncle
8 Peduncle lesions coalescing, covering flowering cushion.
10 Peduncle severely damaged with marked peduncle stunting.

**Flowering cushion scab**

1 No symptoms
2 Few scattered lesions on flowering cushions
4 Numerous, small lesions on flowering cushions
6 Large lesions on older flowering cushions

8 Large lesions on young cushions not associated with floral abortion.

10 Large coalescing lesions with abortion of flowers and /or pods.

**Pod scab**

1 No symptoms

2 Few scattered minute lesions on older pods

4 Scattered small lesions on most pods

6 Extensive discrete scabbing of young pods

8 Large, numerous coalescing lesions on pods

10 Severe scabbing of pods with shriveling and mummification of old pods and /or abscission of young pods.

Areas under the disease progress curves (AUDPC) were calculated from disease ratings according to the formula of Bowen *et al.* (1994) :

$$AUDPC = \Sigma[(1/2)(y_{i+1} + y_i)(t_{i+1} - t_i)] - h$$

in which; y = the disease ratings at time t; i = the day of the assessment from the first date of assessment; and h = correction factor for disease rating "1" being 0 disease. AUDPC values were used as comparative measures of disease throughout the season in plots within years. Analysis of variance (ANOVA) was used to determine differences among fungicide treatments in each year of study. Treatment means were compared with Fisher's protected least significant difference (SAS, 1990). Except where indicated, treatment differences were determined to be significant at the 0.05 level of probability. AUDPC has unit in proportion-days.

### 3.9 Statistical Analyses

All analyses were made with the statistical soft ware, SAS (statistical analysis system, Institute, Inc., Cary, NC). Means were tested. Duncan's multiple range test (DMRT) was done where required to differentiate between means. Orthogonal contrasts analysis were done where necessary and the contrasts were used to test hypotheses. In orthogonal contrasts there is no overlap of contrasts. For example, two contrasts with coefficients $\{c_1\}$ and $\{d_1\}$ are orthogonal if

$$\sum_{i=1}^{n} c_i d_i = 0$$

or, for an unbalanced design, if

$$\sum_{i=1}^{n} n c_i d_i = 0$$

According to Montgomery (1984) contrasts coefficients must be chosen prior to running the experiment and examining the data. The reason he gave for this is that if comparisons are selected after examining the data, most experimenters would conduct tests that correspond to large observed mean differences. These large differences could be the result of the presence of real effects or they could be the result of random error. The contrasts give the sums of squares associated with the estimate statements. The estimate statements compute the sequential coefficients using the orthogonal polynomial. In isolation studies, media were compared orthogonally in the following sequence:

- Media composed from plant extract versus commercial media
- Cowpea stem agar versus the other cowpea extract media
- Cowpea peduncle agar versus cowpea pod agar
- PDA versus cowpea extract media
- PDA versus commercial media

The least square means (Meredith *et al.*, 1985) were used in the tables for adjustment of treatment means for residual effects in isolation studies. The type III sum of squares was used in all analysis of variance because it approximates more to the normal sum of squares calculation.

Since isolation from different plant part was done at different times, it is

not possible to compare across plant parts to determine the best medium that could be used for isolating *Sphaceloma* sp. from any plant part. To enable comparison, therefore, a principal component analysis (PCA) was done to generate eigen vectors for the plant part. PCA is concerned with combining variables. The strategy of PCA was to construct new variables, called vectors, which are composites of the original variables. Such factors present a more concise description of the trends in the data than do the original variables. All variables used in PCA should be quantitative rather than qualitative. The initial result from PCA of a data set is the vector matrix. The number of factors calculated is always equal to the number of original variables. The eigen value under each factor column indicates the variability attributed to the vector. The eigen values sum to a value equal to the number of variables in the analysis. In most analysis, factors with eigen values of less than one are usually not considered any further. In PCA the hope is that the major trends in the data can be explained with only a few vectors, so-called principal components denoted as PRIN1, PRIN2, etc. (Shane, 1987).

In the study of the incubation period of scab, plant parts were handled as single entities and thus the standard error of the difference between means was calculated to enable comparison between interaction least square means.

Repeated measure analysis was carried on the data obtained from the study of the growth of *Sphaceloma* sp. at various temperatures and in different media. Repeated measures analysis indicates that there is a repeated measurement on a variable name over time. Repeated measures design the method of creating several observation from one (Cody, 1987). Repeated measures analysis was conducted to test whether media or temperature changed the rate of growth of the

fungus over time (S. Nokoe, 1995 personal communication,). Means (for day by temperature by media) were separated using their standard error of the difference. A regression analysis was run using Genstat to determine the shape of

the growth curves obtained by plotting the change in weight over time for each temperature and for each medium.

Independent comparison of slopes for the linearized transformed data was done using the formula:

$$t = \frac{\overline{x_1} - \overline{x_2}}{SE = \sqrt{sex_1 + sex_2}}$$

where $x_1$ = slope 1, $x_2$ = slope 2.

S.E. = standard error of both slopes $x_1$ and $x_2$.

For analysis of the data from the study of soil as a possible source of primary inoculum, plant parts were handled as separate entities.

# CHAPTER FOUR

## 4.0 RESULTS

### 4.1 Histopathological Study of Scab (*Sphaceloma* sp.) Lesions on Cowpea Plants

Transverse sections of scabbed pods and peduncles revealed the presence of acervuli of the fungus in the epidermal tissue. Conidiophores were very short and aggregated in the acervulus (Plate 4). Two shapes of hyaline conidia were observed in the acervulus, namely oblong and spherical conidia. The oblong spores measured, on the average, 2.4–4.7 x 1.8–2.3 µm while the spherical ones measured 1.8 µm (diameter).

### 4.2 Isolation of *Sphaceloma* species from Four Cowpea Plant Parts Using Eight Media

#### 4.2.1 Method I: Isolation by plating out intact young scab lesions

The mean number of colonies isolated in 1993 and 1994 from the different plant parts using different media are presented in Tables 1 and 2. On the 4$^{th}$ day of incubation, colonies of *Sphaceloma* sp. from leaf, stem, peduncle and pods could be seen at 40x magnification on cowpea pod extract agar (CpdA), cowpea leaf extract agar (ClfA), cowpea stem extract agar (ClfA), cowpea peduncle extract agar (CpedA) and potato dextrose agar (PDA). However, it took one more day for these colonies to be seen on culture plates with the unaided eye. The colonies were sticky and pale brown up to the 5$^{th}$ day of incubation. They gradually changed to dark brown and to black by the 9$^{th}$ day (Plate 5). The fungus could not be isolated from any tissue plated on cornmeal agar (CMA), malt agar (MA) or V8 juice agar.

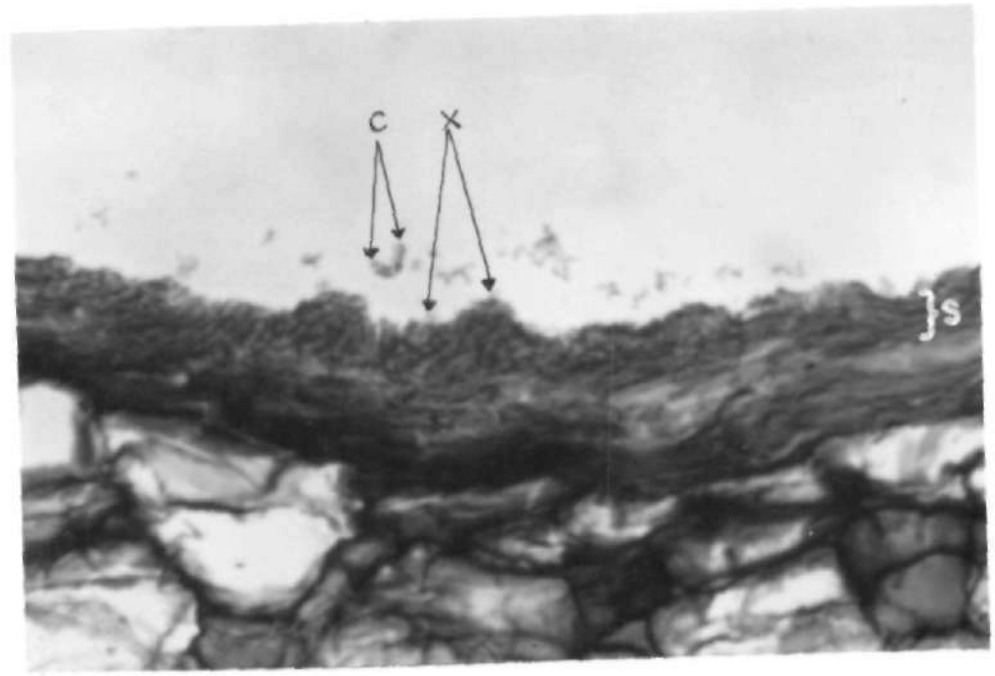



Plate 4. Transverse section of scabbed cowpea pod showing acervuli within the epidermal cells at 40x magnification. S = stromatic tissue of the acervulus from which conidiophores are produced, X = short conidiophores of *Sphaceloma* species and C = conidia of *Sphaceloma* species.

Among the commercial media tested in this study, only PDA yielded isolates arising from lesions from the four plant parts. Statistical comparison of frequency of isolation from different plant parts was not possible (because the isolation was done on different days).

In 1993, pod lesions gave the highest number of isolates when plated on ClfA, CpdA and PDA while CstA and CpedA yielded significantly lower number of isolates compared to the other media when leaf lesions were used (Table 1). PDA and agars amended with cowpea extracts yielded significantly more isolates per pod lesion plated than CMA, MA or V8 juice. ClfA and CstA yielded lowest number of isolates when stem lesions were used. The number of isolates yielded by PDA and CpdA from peduncle lesions were not significantly different from each other but were significantly (P= 0.05) higher than the numbers yielded by the other media. The orthogonal contrasts and linear estimates for the five media that yielded isolates for the different plant parts in 1993 are presented in Tables 3 and 5 respectively. The orthogonal contrasts for cowpea plant extract media compared to non-extract media on the one hand and PDA compared to other commercial media on the other hand were highly significant (P= 0.01). Compared to other non-extract media, cowpea plant extracts media yielded four times the number of isolates from pod lesions but twice the number from leaf lesions. The numbers of isolates on CstA compared to other media were not significantly different except when peduncle lesions were used (Table 3). PDA yielded as much as five times the number of isolates from pod lesions compared to other commercial media.

In 1994, CpdA yielded significantly the highest number of isolations from pod lesions but not from the lesion of other plant parts. Compared to other extract media ClfA yielded significantly (P= 0.05) lowest number of colonies from stem lesions.

The contrasts and estimates for different media comparisons are presented

in Tables 5 and 6. All pairs of media comparisons, except for CstA compared to other extracts in respect of leaf and pod lesions shown on Table 5 were highly significant (P= 0.01). The estimates for extracts media compared to non-extracts media were highest for all the plant parts, compared to the other pairs of comparisons shown in Table 6. Thus lesions from the leaves, stems and pods plated on extract media yielded more than two times more isolations than those plated on non-extract media.

In order to tell the best medium for isolating the fungus using any plant part, principal component analysis was done to generate eigen vectors for the four plant parts. In 1993 and 1994, 78% and 66% of the entire data was explained by PRIN1 and PRIN2 together (Tables 7 and 8). The eigen values of PRIN2 were less than 1; therefore media were compared using the eigen values generated by PRIN1 (Table 9). The principal component analysis (Table 9) showed therefore that CpdA medium was the best medium in 1993 and 1994 but it was not significantly (P =0.05) different from ClfA or CstA and PDA in 1993.

Table 1. Number of isolations of *Sphaceloma* sp. per four lesions from four different cowpea plant parts using eight different media in 1993.

| Medium for isolation | Number of colonies per plate isolated from lesions on[2]: | | | |
|---|---|---|---|---|
| | Leaf[1] | Stem[1] | Peduncle[1] | Pod[1] |
| Cowpea leaf agar | 1.0±0.1 a | 0.4±0.1 b | 0.7±0.1bc | 1.6±0.1a |
| Cowpea stem agar | 0.9±0.1 b | 0.4±0.1 b | 0.6±0.1c | 1.5±0.1a |
| Cowpea peduncle agar | 0.7±0.1 b | 0.5±0.1 b | 0.7±0.1bc | 1.3±0.1a |
| Cowpea pod agar | 0.9±0.1 a | 0.7+0.1 a | 0.9±0.1ab | 1.5±0.1a |
| Potato dextrose agar | 0.9±0.1 a | 0.9±0.1 a | 1.0±0.1a | 1.7±0.1a |
| Corn meal agar | 0.0±0.0 b | 0.0±0.0 b | 0.0±0.0 c | 0.0±0.0d |
| Malt agar | 0.0±0.0 b | 0.0±0.0 b | 0.0±0.0 c | 0.0±0.0d |
| V-8 juice agar | 0.0±0.0 b | 0.0±0.0 b | 0.0±0.0 c | 0.0±0.0d |

[1] Isolations from different plant parts were done on different days.
[2] Least square means±standard errors were calculated from six replications of 10 plates each. Means followed by the same letter within a column are not significantly different ($P<0.05$) using Duncan's multiple range test (Gomez and Gomez, 1984).

Table 2. Number of isolations of *Sphaceloma* sp. per four lesions from four different cowpea plant parts using eight different media in 1994.

| Medium for isolation | Number of colonies per plate isolated from lesions on[2]: | | | |
|---|---|---|---|---|
| | Leaf[1] | Stem[1] | Peduncle[1] | Pod[1] |
| Cowpea leaf agar | 1.2±0.1 ab | 0.2±0.1 de | 0.7±0.1 a | 0.8±0.1b |
| Cowpea stem agar | 0.9±0.1 b | 0.8±0.1 ab | 0.9±0.1 a | 0.7±0.1b |
| Cowpea peduncle agar | 0.8±0.1 b | 0.6±0.1 bc | 0.8±0.1 a | 0.7±0.1b |
| Cowpea pod agar | 1.3±0.1 a | 0.9±0.1 a | 1.0±0.1 a | 1.6±0.1a |
| Potato dextrose agar | 0.8±0.1 b | 0.4±0.1 cd | 0.7±0.1 a | 0.9±0.1b |
| Corn meal agar | 0.0±0.1 c | 0.0±0.1 e | 0.0±0.1 b | 0.0±0.1c |
| Malt agar | 0.0±0.1 c | 0.0±0.1 e | 0.0±0.1 b | 0.0±0.1c |
| V-8 juice agar | 0.0±0.1 c | 0.0±0.1 e | 0.0±0.1 b | 0.0±0.1c |

[1] Isolations from different plant parts were done on different days.
[2] Least square means±standard errors were calculated from six replications of 10 plates each. Means followed by the same letter within a column are not significantly different ($P<0.05$) using Duncan's multiple range test (Gomez and Gomez, 1984).

Table 3. Contrast analysis of different culture media using different plant parts as individual entities in 1993.

| | | Plant part from which lesions were obtained*: | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Leaf | | Stem | | Peduncle | | Pod | |
| Source | DF | SS | Pr > F | SS | Pr > F | SS | Pr > F | SS | Pr > F |
| **Orthogonal Contrasts** | | | | | | | | | |
| Extracts vs Non extracts | 1 | 52.0083 | 0.0001 | 9.0750 | 0.0001 | 25.6688 | 0.0001 | 132.30 | 0.0001 |
| CstA vs Other extracts | 1 | 0.1680 | 0.5931 | 0.8681 | 0.0909 | 1.7014 | 0.0401 | 0.2347 | 0.6117 |
| CpedA vs CpfA | 1 | 1.6333 | 0.0962 | 1.6333 | 0.0206 | 1.4083 | 0.0618 | 1.6333 | 0.1809 |
| PDA vs Other commercial media | 1 | 32.5125 | 0.0001 | 32.5125 | 0.0001 | 45.0000 | 0.0001 | 132.61 | 0.0001 |
| **Additional contrast of interest**** | | | | | | | | | |
| PDA vs Extracts | 1 | 0.0208 | 0.8508 | 6.3075 | 0.0001 | 3.9675 | 0.0018 | 2.7075 | 0.0852 |

* Isolations from different plant parts were done on different days.

** Significance tested with Scheffe's test. (Snedecor and Cochran, 1980).

Table 4. Contrasts analysis of different culture media using different plant parts as individual entities in 1994.

| | | Plant part from which lesions were obtained*: | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Leaf | | Peduncle | | Pod | | Stem | |
| Source | DF | SS | Pr > F | SS | Pr > F | SS | Pr > F | SS | Pr > F |
| **Orthogonal Contrasts** | | | | | | | | | |
| Extracts vs Non extracts | 1 | 82.50 | 0.0001 | 32.03 | 0.0001 | 50.05 | 0.0001 | 58.10 | 0.0001 |
| CstA vs Other extracts | 1 | 2.01 | 0.0902 | 2.01 | 0.003 | 0.01 | 0.7765 | 5.51 | 0.0006 |
| CpedA vs Cpdag | 1 | 9.08 | 0.0007 | 2.70 | 0.0007 | 1.88 | 0.0013 | 22.53 | 0.0001 |
| PDA vs Other com- | | | | | | | | | |
| mercial media | 1 | 27.61 | 0.0001 | 6.05 | 0.0001 | 20.00 | 0.0001 | 39.20 | 0.0001 |
| **Additional contrast of interest**** | | | | | | | | | |
| PDA vs Extracts | 1 | 2.80 | 0.0469 | 2.80 | 0.0006 | 1.02 | 0.0141 | 0.00 | 0.9633 |

* Isolations from different plant parts were done on different days
** Significance tested with Scheffe's test. (Snedecor and Cochran, 1980).

Table 5. Level of estimates (linear contrasts) for five contrasts showing the effect of culture media and plant parts in the isolation study in 1993.

| | Plant part from which lesions were obtained*: | | | |
|---|---|---|---|---|
| Source | Leaf | Stem | Peduncle | Pod |
| **Orthogonal Contrasts** | | | | |
| Extracts vs Non extracts | 2.63± 0.28 | 1.10±0.20 | 1.85± 0.23 | 4.20±0.35 |
| CstA vs Other extracts | -0.18± 0.34 | 0.42± 0.25 | 0.58± 0.28 | -0.22±0.43 |
| CpedA vs CpdA | 0.23± 0.13 | 0.23± 0.10 | 0.22± 0.12 | 0.23± 0.17 |
| PDA vs Other com- | | | | |
| mercial media | -2.55± 0.34 | -2.55± 0.25 | -3.00± 0.28 | -5.15± 0.43 |
| **Additional contrast of interest**** | | | | |
| PDA vs Extracts | 0.08± 0.44 | -1.45± 0.32 | -1.15± 0.37 | -0.95± 0.55 |

* Isolations from different plant parts were done on different days
** Significance tested with Scheffe's test. (Snedecor and Cochran, 1980).
Estimate±Standard error

Table 6. Level of estimates (linear contrasts) for five contrasts showing the effect of culture media and plant parts in the isolation study in 1994.

| | Plant part from which lesions were obtained*: | | | |
|---|---|---|---|---|
| *Source* | *leaf* | *Peduncle* | *Pod* | *Stem* |
| **Orthogonal Contrasts** | | | | |
| Extracts vs Nonextracts | 3.32±0.33 | 2.58±0.31 | 2.78±0.31 | 2.07±0.25 |
| CstA vs Other extracts | 0.63±0.40 | 0.05±0.37 | 1.05±0.38 | -0.63±0.31 |
| CpedA vs Cpdag | 0.55±0.16 | 0.25±0.15 | 0.87±0.15 | 0.30±0.13 |
| PDA vs Other com- | | | | |
| mercial media | -2.35±0.40 | -2.00±0.37 | -2.80±0.38 | -1.10±0.31 |
| **Additional contrast of interest**** | | | | |
| PDA vs Extracts | 0.97±0.52 | 0.58±0.48 | -0.02±0.48 | 0.97±0.40 |

* Isolations from different plant parts were done on different days
** Significance tested with Scheffe's test. (Snedecor and Cochran, 1980).
Estimate±Standard error

Table 7. Eigen values of the covariance matrix for 1993 isolations.

| Principal components | Eigen value | Difference | Proportion |
|---|---|---|---|
| PRIN1 | 1.84522 | 1.20923 | 0.58325 |
| PRIN2 | 0.63599 | 0.23911 | 0.20103 |
| PRIN3 | 0.39688 | 0.11128 | 0.12545 |
| | Eigen vectors | | |
| Plant parts | PRIN1 | PRIN2 | PRIN3 |
| LEAF | 0.36959 | 0.83119 | -0.3941 |
| PEDUNCLE | 0.33445 | 0.20038 | 0.85236 |
| POD | 0.83278 | -0.4945 | -0.2389 |
| STEM | 0.24088 | 0.1562 | 0.24718 |

Table 8. Eigen values of the covariance matrix for 1994 isolations.

| Principal components | Eigen value | Difference | Proportion | Cumulative |
|---|---|---|---|---|
| PRIN1 | 1.399 | 0.5784 | 0.4183 | 0.4183 |
| PRIN2 | 0.8207 | 0.2059 | 0.2454 | 0.6636 |
| PRIN3 | 0.6148 | 0.1045 | 0.1838 | 0.8474 |

Eigen vectors

| Plant parts | PRIN1 | PRIN2 | PRIN3 |
|---|---|---|---|
| LEAF | 0.6279 | -0.777 | -0.009 |
| PEDUNCLE | 0.4663 | 0.3529 | 0.7266 |
| POD | 0.5918 | 0.4825 | -0.643 |
| STEM | 0.195 | 0.1953 | 0.2417 |

Table 9. Means separation of the eight media using the Eigen vectors generated for PRIN1 in the principal component analysis in 1993 and 1994.

| | Eigen vector value for: | |
|---|---|---|
| | Least square means for PRIN1 | |
| Medium | 1993 | 1994 |
| Cowpea stem agar | 0.713 ab | 0.437 b |
| Cowpea leaf agar | 0.961 a | 0.531 b |
| Cowpea peduncle agar | 0.420 b | 0.333 b |
| Cowpea pod agar | 0.990 a | 1.367 a |
| Potato dextrose agar | 0.930 a | 0.403 b |
| Malt agar | -1.334 c | -1.024 c |
| Corn meal agar | -1.340 c | -1.024 c |
| V-8 juice agar | -1.340 c | -1.024 c |
| % of variance explained by PRIN1: | 58% | 42% |

Means in a column with the same letters are not significantly different (P=0.05) using Duncan's multiple range test

### 4.2.2 Method: II Isolation by plating out macerated young scab lesions

*Sphaceloma* sp. was isolated on six of the eight media namely, all cowpea extracts agars, PDA and V-8 juice agar. Cornmeal agar and malt agar yielded no isolate (Table 10). The highest number of successful isolations were obtained from pod lesions on CpdA although this was not significantly different (P=0.05) from the number of isolations got from other lesions on other media.

Table 10. Mean number of isolations (number of colonies/plate) of *Sphaceloma* sp. obtained by plating a suspension of macerated lesions from four cowpea plant parts on eight different media.

| | Mean colony count (colonies/plate)* | | | |
|---|---|---|---|---|
| *Lesions obtained from:*[1] Medium for isolation | *Leaf* | Stem | Peduncle | Pod |
| Cowpea leaf agar | 0.71±0.14 | 0.54±0.22 | 0.47±0.30 | 0.63±0.22 |
| Cowpea stem agar | 0.64±0.15 | 0.52±0.14 | 0.60±0.15 | 0.67±0.04 |
| Cowpea peduncle agar | 0.58±0.17 | 0.51±0.05 | 0.70±0.07 | 0.66±0.10 |
| Cowpea pod agar | 0.73±0.09 | 0.53±0.12 | 0.65±0.25 | 0.74±0.13 |
| Potato dextrose agar | 0.67±0.10 | 0.61±0.13 | 0.60±0.13 | 0.60±0.15 |
| Corn meal agar | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| Malt agar | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| V-8 juice agar | 0.58±0.21 | 0.62±0.08 | 0.57±0.07 | 0.61±0.25 |

* Means were not significantly different (P=0.05) from each other.

### 4.3 Determination of Incubation Period of *Sphaceloma* sp. in Parts of Cowpea

Five days after inoculation, only young leaves showed symptoms of scab, irrespective of plant age at the time of inoculation. After 7 days, scab symptoms appeared on stems of plants aged 14 or 28 days at the time of inoculation, but it took 9 days for symptoms to appear on stems of plants aged 42 or 56 days old at time of inoculation (Table 11). It took 8 days for symptoms to appear on the peduncles of plants aged 42 or 56 days on inoculation day. The 14- and 28- day old plants had no peduncles and flowering cushions at the time of inoculation. Lesions appeared 10 days after inoculation on the pods of plants aged 56-days at inoculation; younger plants had no pods on the day of inoculation.

Generally, there was no difference in incubation period for the various plant part ages (within a plant part) except for the stems were incubation period increased with age. This relationship is shown in Figure 1.

Table 11. Time of appearance scab symptoms on cowpea after foliar spraying of plants of various ages with inoculum suspensions of *Sphaceloma* sp.

| Plant part | Plant age at inoculation | Incubation period ± S.E.[a] |
|---|---|---|
| Leaf | 14 | 5.0±0.1 |
| | 28 | 5.0±0.1 |
| | 42 | 5.0±0.1 |
| | 56 | 5.0±0.1 |
| | S.E.D | 0.0 |
| Stem | 14 | 7.0±0.1 |
| | 28 | 7.0±0.1 |
| | 42 | 10.0±0.1 |
| | 56 | 10.0±0.1 |
| | S.E.D | 1.9 |
| Peduncle | 14 | NA |
| | 28 | NA |
| | 42 | 8.0±0.2 |
| | 56 | 8.0±0.2 |
| | S.E.D | 0.0 |
| Flowering cushion | 14 | NA |
| | 28 | NA |
| | 42 | 9.0±0.2 |
| | 56 | 9.0±0.2 |
| | S.E.D | 0.0 |
| Pod | 14 | NA |
| | 28 | NA |
| | 42 | NA |
| | 56 | 11.0±0.2 |
| | S.E.D | 0.0 |

S.E.D. Standard error of the difference between means within plant parts
[a]: NA = not applicable since organ had not been produced at time of inoculation.

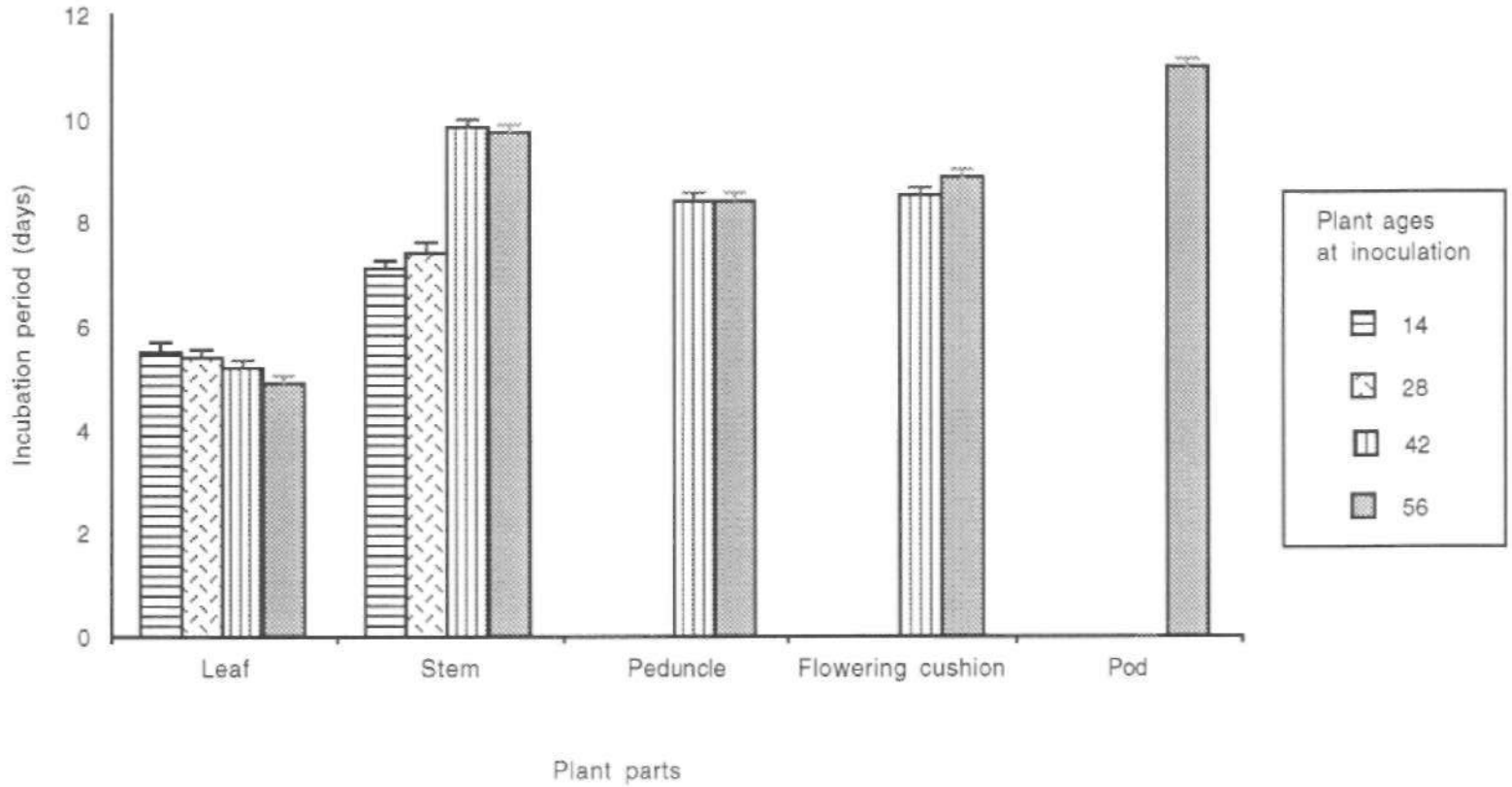



**Figure 1: Incubation period of cowpea scab in different plant parts spray-inoculated with conidial suspension of Sphaceloma sp.**

N.B.: Error bars represent standard error of the means (n = 2)

### 4.4 Influence of Temperature and Type of Culture Medium on the Growth of a Cowpea Isolate of *Sphaceloma* species

During the 15-day experimental period, there was a significant interaction (P= 0.05) between the type of medium, the incubation temperature and period of incubation. The greatest increase in colony weight for all three media occurred at 26°C (Table 12). At this temperature the greatest growth occurred in PDAR. At 30°C, no growth occurred on cornmeal agar plus rose bengal (CMAR) and malt agar plus rose bengal (MAR) whereas the colonies doubled in weight on potato dextrose agar plus rose bengal (PDAR) by the 6th day of incubation but there was no further change in weight throughout the experimental period. No growth occurred at 35°C regardless of the culture medium. In fact, the colonies lost weight between the 12th and the 15th day of incubation so that by the 15th day, the colony weight was not detectable with the aid of the weighing scale used in the present study. Colony growth rates between different assessment times on CMAR, MAR and PDAR at the different temperatures are given in Table 13. The highest growth rate was achieved between 12 and 15 days of incubation at 20°C on PDAR. At 15°C the growth rates in all media were low. At higher temperatures (30-35°C) there was little or no growth. Figures 2-4 show the relationship between colony weight and incubation period of the colony. Regression analyses of the mean colony weight over the period of incubation are shown in Table 14. At 20°C the growth rate was exponential in all media, while at 30°C, the growth rate was linear. The linear regression equations from the transformed data are presented in Table 15.

When Petri dishes that were originally incubated at 35°C were transferred from 35°C to 26°C, fungal growth of colonies resumed after 9 days.

Marked differences in colony morphology of *Sphaceloma* sp. were noted on different media in this study (Plate 5). On CMAR and MAR the fungus tended to grow radially compared to the upward massive growth noted on PDAR. On MAR by the 15th day of incubation at 26°C, a sticky gel-like substance (Plate 6)

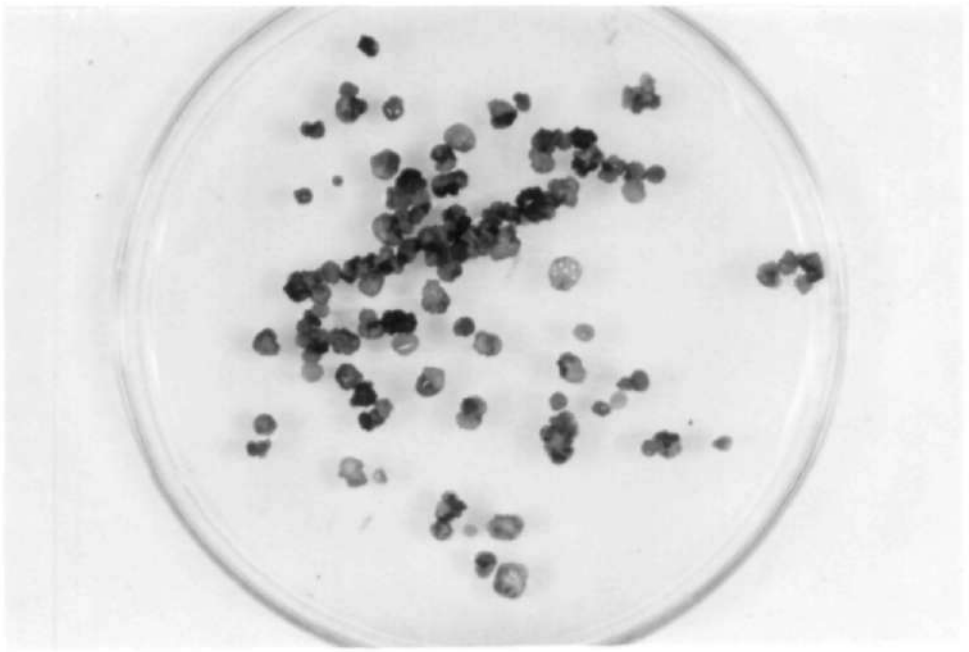
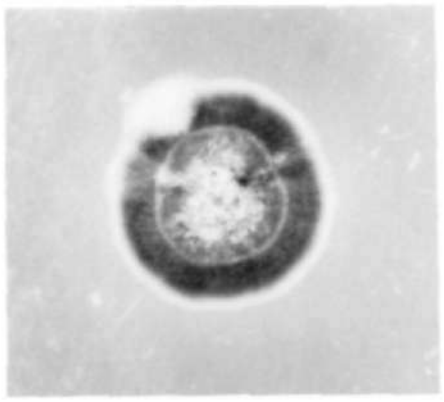
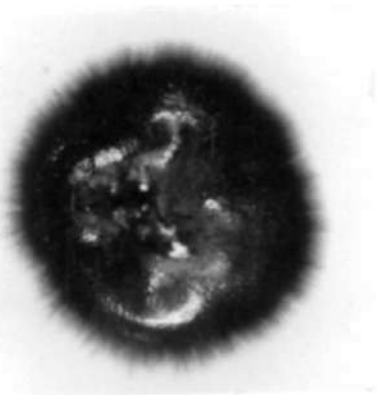


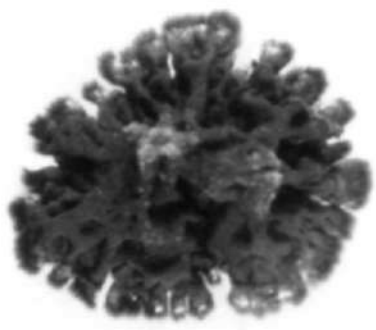
**Plate 5. Colonies of 9-day-old culture of *Sphaceloma* sp. showing variation in colour from light brown to dark and finally to black at 25x magnification.**



A



B



C

**Plate 6. Gross morphology of 15-day-old colonies of *Sphaceloma* sp. incubated at 26°C at 25x magnification on (A) cornmeal agar, (B) malt extract agar and (C) potato dextrose agar.**

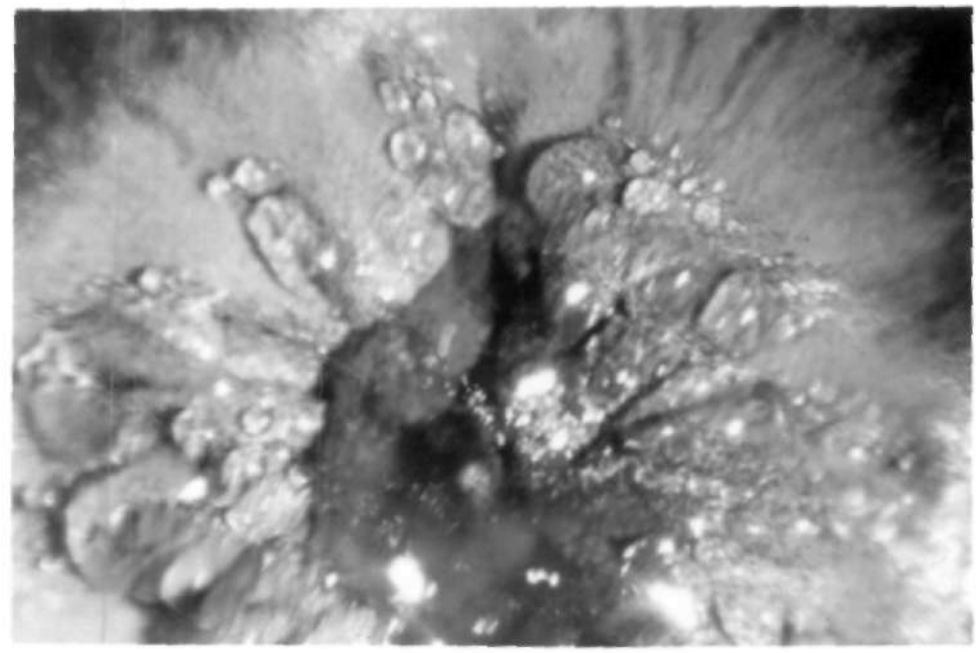


Plate 7. A sticky gel-like substance oozing out of the mycelia of *Sphaceloma* sp. mass on malt agar at 26°C at 40x magnification.

Table 12. Effect of temperature and culture media on the growth of *Sphaceloma* sp. at 3, 6, 9, 12 and 15 days of incubation.

| Temperature (°C) | Culture medium[a] | Days of incubation | | | | |
|---|---|---|---|---|---|---|
| | | 3 | 6 | 9 | 12 | 15 |
| | | Weight of *Sphaceloma* sp. colony (g) | | | | |
| 15 | CMAR | 0.01 | 0.02 | 0.03 | 0.04 | 0.06 |
| 15 | MAR | 0.01 | 0.02 | 0.03 | 0.04 | 0.06 |
| 15 | PDAR | 0.01 | 0.01 | 0.03 | 0.07 | 0.23 |
| 20 | CMAR | 0.02 | 0.03 | 0.11 | 0.22 | 0.46 |
| 20 | MAR | 0.02 | 0.03 | 0.11 | 0.19 | 0.43 |
| 20 | PDAR | 0.02 | 0.06 | 0.23 | 0.41 | 0.69 |
| 26 | CMAR | 0.03 | 0.06 | 0.23 | 0.41 | 0.60 |
| 26 | MAR | 0.04 | 0.07 | 0.25 | 0.62 | 0.55 |
| 26 | PDAR | 0.04 | 0.20 | 0.45 | 0.80 | 1.02 |
| 30 | CMAR | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| 30 | MAR | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| 30 | PDAR | 0.01 | 0.02 | 0.02 | 0.02 | 0.02 |
| 35 | All | 0.01 | 0.01 | 0.01 | 0.01 | 0.00 |
| S.E.D.: | | 0.001 | 0.003 | 0.002 | 0.008 | 0.058 |

a: CMAR = cornmeal agar plus rose bengal; MAR = malt agar plus rose bengal; PDAR = potato dextrose agar plus bengal.
S.E.D. Standard error of the difference between means (days of incubation)

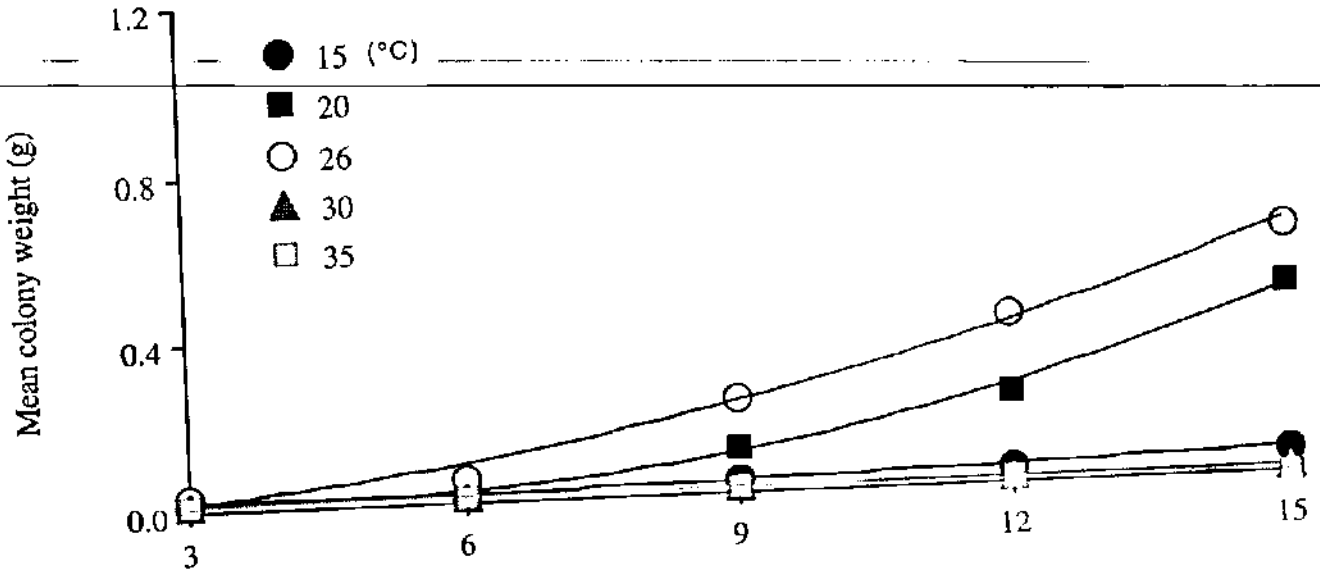



Figure 2: Growth in weight of Sphaceloma sp. at different temperature on cornmeal agar amended with streptomycin plus rose bengal. in weight of Sphaceloma species.

N.B.: For regression equations see Table 14

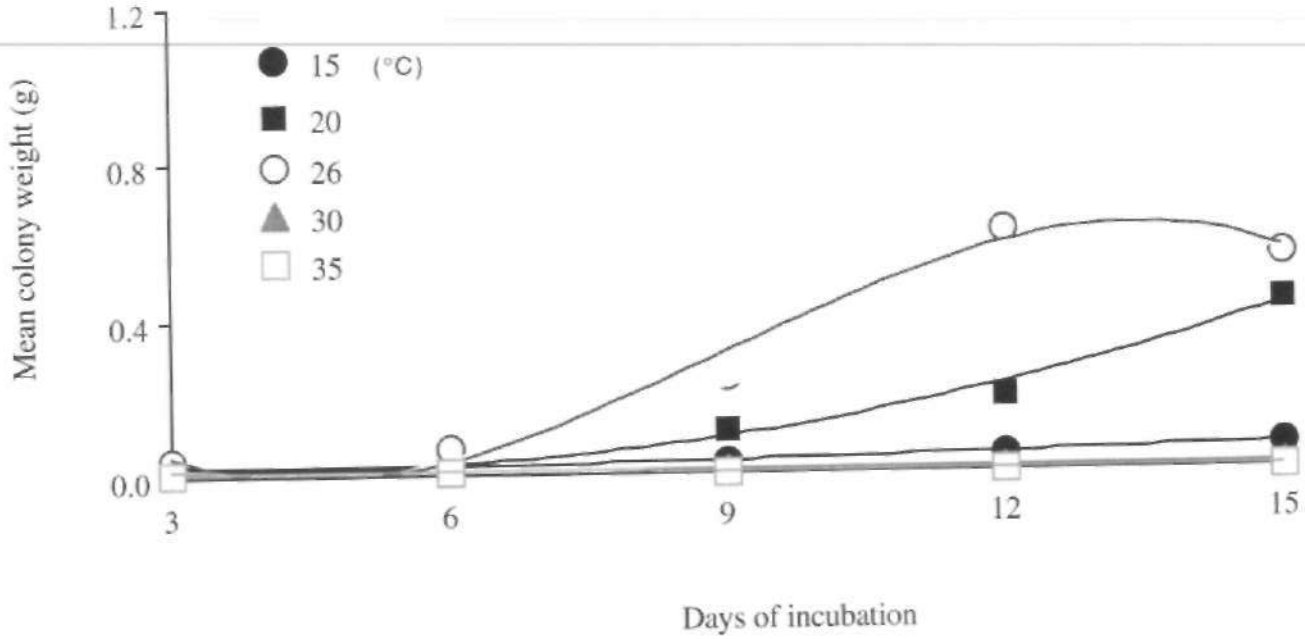



Figure 3: Growth in weight of Sphaceloma sp. at different temperatures on malt agar amended with streptomycin plus rose bengal.

N.B.: For regression equations see Table 14

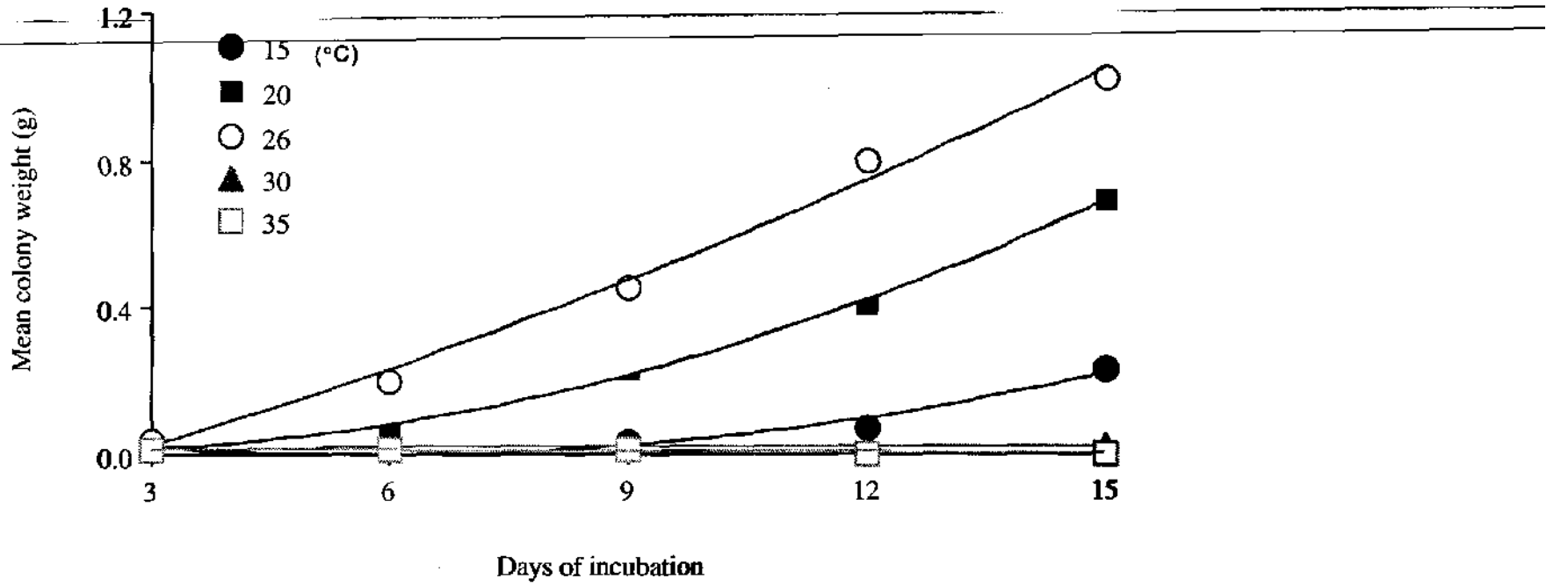



Figure 4: Growth in weight of Sphaceloma sp. at different temperatures on potato dextrose agar amended with streptomycin plus rose bengal.

N.B.: For regression equations see Table 14

Table 13. Rate of growth of *Sphaceloma* sp. between various dates of incubation at different temperatures on three media.

| Temperature (°C) | Culture medium | Rate of growth between the following days of incubation: | | | |
|---|---|---|---|---|---|
| | | 3 & 6 | 6 & 9 | 9 & 12 | 12 & 15 |
| | | Rate of growth of mycelia (mg/day) | | | |
| 15 | CMAR | 2 | 3 | 4 | 6 |
| 15 | MAR | 2 | 3 | 6 | 6 |
| 15 | PDAR | 0 | 6 | 14 | 51 |
| 20 | CMAR | 3 | 28 | 33 | 78 |
| 20 | MAR | 3 | 26 | 27 | 76 |
| 20 | PDAR | 13 | 58 | 57 | 88 |
| 26 | CMAR | 9 | 56 | 59 | 59 |
| 26 | MAR | 11 | 58 | 12 | -23 |
| 26 | PDAR | 53 | 80 | 11 | 71 |
| 30 | CMAR | 0 | 0 | 0 | 0 |
| 30 | MAR | 0 | 0 | 0 | 0 |
| 30 | PDAR | 3 | -1 | 1 | 0 |
| 35 | CMAR | 0 | 0 | 0 | -3 |
| 35 | MAR | 0 | -2 | -1 | 0 |
| 35 | PDAR | 0 | 0 | -3 | 0 |

Table 14. Regression equations of mean colony weight of *Sphaceloma* sp. in three media at five different temperatures.

| Media | Temperature | Linear | Non-linear |
|---|---|---|---|
| PDAR | 15 | | $y = 0.0075 + 0.0004e^{4.59x}$ $R^2 = 0.99$ |
| | 20 | | $y = -0.1740 + 0.119e^{3.131x}$ $R^2 = 0.98$ |
| | 26 | | $y = -1.91 + 1.73e^{2.818x}$ $R^2 = 0.98$ |
| | 30 | | $y = 0.0196 - 5778.3e^{1.01x}$ $R^2 = 0.95$ |
| | 35 | $y = 0.0015 - 0.001x$ $R^2 = 0.67$ | |
| CMAR | 15 | | $y = -0.0077 + 0.0129e^{3.04x}$ $R^2 = 0.99$ |
| | 20 | | $y = -0.0187 + 0.0149e^{3.527x}$ $R^2 = 0.99$ |
| | 26 | | $y = -0.287 + 0.219e^{2.99x}$ $R^2 = 0.97$ |
| | 30 | $y = 0.01 + 0.0x$ $R^2$ = no estimate | |
| | 35 | $y = 0.01 - 0.0007x$ $R^2$ = no estimate | |
| MAR | 15 | $y = -0.0062 + 0.0041x$ $R^2 = 0.95$ | |
| | 20 | | $y = -0.0038 + 0.0097e^{3.623x}$ $R^2 = 0.99$ |
| | 26 | $y = -0.171 + 0.0527x$ $R^2 = 0.81$ | |
| | 30 | $y = 0.01 + 0.0x$ $R^2$ = no estimate | |
| | 35 | $y = 0.01 - 0.001x$ $R^2$ = no estimate | |

Table 15. Linear regression coefficients for the change overtime in mean colony weight of *Sphaceloma* sp. on three media at five temperatures.

| Media | Temperature | Intercepts | Slopes | $R^2$ |
|---|---|---|---|---|
| CMAR | 15 | -0.004 | 0.004 | 0.98 |
| | 20 | -0.123 | 0.029 | 0.95 |
| | 26 | -0.132 | 0.039 | 0.99 |
| | 30 | 0.010 | 0.0 | not estimable |
| | 35 | 0.014 | -0.001 | 0.90 |
| MAR | 15 | -0.006 | 0.004 | 0.97 |
| | 20 | 0.111 | 0.027 | 0.93 |
| | 26 | -0.120 | 0.041 | 0.96 |
| | 30 | 0.010 | 0.0 | not estimable |
| | 35 | 0.014 | -0.001 | 0.85 |
| PDAR | 15 | -0.070 | 0.015 | 0.85 |
| | 20 | -0.159 | 0.043 | 0.98 |
| | 26 | -0.143 | 0.058 | 0.99 |
| | 30 | 0.012 | 0.001 | 0.97 |
| | 35 | 0.015 | -0.001 | 0.90 |

Data transformed by log(x+1) Gomez and Gomez (1984)

**4.5 Seed as Possible Source of Primary Inoculum for Cowpea Scab**

In 1993, seedlings grown from untreated seeds from Samaru (Table 16) had the highest disease incidence (10.1%) which was significantly (P= 0.05) higher (2.7%) than seedlings from seeds obtained from Samaru but treated with benomyl. By contrasts seedlings raised from seeds from Ikenne treated with benomyl had the lowest disease incidence (0.5%) but this was not significantly different from that in seedlings of seeds from Ikenne that were not treated with benomyl (2.4%). Seedlings grown from untreated seeds from Samaru in 1994 had significantly (P = 0.05) higher scab incidence of 37% compared to those grown from seeds treated with benomyl which had scab incidence of 0.7% (Table 17).

Table 16. Scab incidence on potted seedlings from treated and untreated seeds from Ikenne and Samaru in 1993.

| Location where seed was produced | Seed treatment | Seedling scab incidence (%)[a] |
|---|---|---|
| Samaru (infected seed) | Untreated | 10.1 a |
| Samaru (infected seed) | Treated with benomyl | 2.7 b |
| Ikenne (clean seed) | Untreated | 2.4 b |
| Ikenne (clean seed) | Treated with benomyl | 0.53 b |

[a]: Incidence recorded at 3 weeks after sowing
Means with the same letters are not significantly different (P=0.05) using DMRT

Table 17. Scab incidence on potted seedlings from treated and untreated seeds from Samaru in 1994.

| Location where seed was produced | Seed treatment | Seedling scab incidence (%)[a] |
|---|---|---|
| Samaru (infected seed) | Untreated | 37.9 a |
| Samaru (infected seed) | Treated with benomyl | 0.7 b |

[a]: Incidence recorded at 3 weeks after sowing.
Means with the same letters are not significantly different (P=0.05) using DMRT.

**4.6 Soil as Possible Source of Primary Inoculum for Cowpea Scab**

The first scab symptoms were visible 27 and 25 days after sowing in 1993 and 1994, respectively when cowpea plants were at their five-leaf stage of growth. No symptoms were observed on the primary leaves in any of the three locations for both years. In both 1993 and 1994, the effect of mulching on incidence of scab, regardless of the plant part on which scab appeared, depended upon the location of the field (Appendices 16 and 17 cfr. interaction location x treatment). In location 1 in 1993 where cowpea had been planted the previous year and in location 2 where cowpea had last been planted 2 years previously, scab incidence was higher in plots with no mulch while in location 3 where cowpea was last planted 3 years before, however, scab incidence was higher in mulched plots (Table 18). A similar trend was obtained in 1994 except that higher disease incidence was obtained in plots with no mulch of location 2 but not in location 3 (Table 19).

In 1993, at 29 days after sowing (DAS), the maximum number of plants with scab (overall incidence) was obtained in location 1 in plots with no mulch (5.8%) while the minimum overall incidence was obtained in location 3 in plots with no mulch (0.2%). At 43 DAS, in location 1, plots with no mulch had the highest overall scab incidence of 39.1%, a six fold increase compared to the crop at 29 DAS.

In all three locations, at 29 DAS, in both plots with mulch and no mulch, the number of plants with scab on the epicotyl was significantly (P= 0.05) higher as compared to plants with scab on their hypocotyls. The same trend was observed as the plants grew older (Table 18).

At 43 DAS, location 1 recorded the highest scab incidence on both the epicotyl and hypocotyl. However, the scab incidence on the epicotyl (39.1%) was significantly (P= 0.05) higher than that on the hypocotyl (19.2%). Location 3 had the lowest scab incidence on the hypocotyl (3.8%) (Table 18).

For severity ratings, there was no interaction between location x treatment (Appendix 17). Mulching did not affect severity in 1993 while in 1994, severity on epicotyl was significant at P = 0.054 (Appendix 18). The maximum scab severity (8.0) was obtained at 43 DAS in location 2 on the epicotyl while the minimum scab severity was obtained in location 1 on both the epicotyl and hypocotyl (3.3%). Mulching did not have a significant effect (P=0.05) on scab severity on either the epicotyl or hypocotyl, except in location 1 at 29 DAS in 1993 where the severity of epicotyl scab was much higher than that on the hypocotyl.

In 1994, at 30 days after sowing (DAS), location 1 had the maximum overall incidence in plots with no mulch (5.0%) while the minimum overall incidence was obtained in location 1 in plots with mulch (0.3%). In location 1, at 44 DAS, the overall incidence in plots with no mulch had increased fifteen times (from 5.0 to 78.5). Generally, mulching significantly (P= 0.05) reduced scab incidence except at 30 DAS in location 2 where scab incidence was higher in mulched plots (2.5%) compared to plots without mulch (1.5%).

At 30 DAS, mulching had no consistent effect on scab severity both on the epicotyl and hypocotyl. Similarly, location did not consistently influence disease severity, especially at 44 DAS.

Table 18. Incidence and severity of scab on plants grown with and without mulch at Samaru, 1993.

| Days after sowing | Location[a] | Mulching treatment | Incidence (%) and severity of scab* | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Overall | On epicotyl | | On hypocotyl | | On hypocotyl and epicotyl | |
| | | | incidence | Incidence | Severity | Incidence | Severity | Incidence | Severity |
| 29 | 1 | Mulched | 2.8±2.9 | 2.3±2.9 | 4.1±3.2 | 0.0±0.0 | 1.0±0.0 | 0.0±0.0 | 1.0±0.0 |
| | 1 | No mulch | 5.8±4.1 | 5.1±3.5 | 4.9±2.4 | 2.9±2.8 | 3.9±3.0 | 2.5±3.0 | 3.0±3.3 |
| | 2 | Mulched | 1.5±1.3 | 1.5±1.3 | 4.0±3.5 | 1.5±1.3 | 3.3±3.1 | 1.5±1.3 | 3.7±3.2 |
| | 2 | No mulch | 2.1±1.9 | 1.9±1.7 | 4.0±3.1 | 0.6±0.7 | 2.0±2.2 | 0.4±0.7 | 1.8±2.8 |
| | 3 | Mulched | 0.3±0.7 | 0.3±0.7 | 1.3±0.5 | 0.0±0.0 | 1.0±0.0 | 0.0±0.0 | 1.0±0.0 |
| | 3 | No mulch | 0.2±0.6 | 0.2±0.6 | 1.0±0.0 | 0.2±0.6 | 0.7±1.6 | 0.2±0.6 | 0.8±2.0 |
| | | S.E.D | 0.009 | 0.001 | 0.009 | 0.006 | 0.762 | 0.006 | 0.081 |
| 36 | 1 | Mulched | 10.1±4.5 | 8.0±3.2 | 6.1±0.2 | 4.9±1.9 | 5.2±0.8 | 4.9±1.9 | 5.5±0.4 |
| | 1 | No mulch | 21.2±6.1 | 17.2±5.7 | 6.0±0.0 | 10.6±3.1 | 5.4±0.4 | 10.6±3.1 | 5.4±0.2 |
| | 2 | Mulched | 10.6±4.8 | 10.6±4.8 | 6.0±0.0 | 4.2±2.4 | 5.3±1.2 | 4.2±2.4 | 3.3±3.1 |
| | 2 | No mulch | 11.1±5.5 | 11.1±5.5 | 6.3±0.6 | 6.1±2.9 | 4.8±2.4 | 6.1±2.9 | 5.8±0.3 |
| | 3 | Mulched | 0.9±1.5 | 0.9±1.5 | 2.0±3.1 | 0.0±0.0 | 1.0±0.0 | 0.0±0.0 | 1.0±0.0 |
| | 3 | No mulch | 0.9±1.7 | 0.7±1.8 | 1.0±0.0 | 0.5±0.7 | 3.0±3.3 | 0.2±0.6 | 1.2±0.4 |
| | | S.E.D | 0.016 | 0.014 | 0.579 | 0.008 | 0.634 | 0.006 | 0.061 |
| 43 | 1 | Mulched | 21.2±6.9 | 17.7±5.5 | 7.5±0.9 | 9.0±3.8 | 5.9±0.2 | 10.6±5.0 | 5.9±0.2 |
| | 1 | No mulch | 39.1±9.6 | 33.9±9.2 | 7.4±0.7 | 19.1±4.1 | 6.1±0.2 | 19.1±4.1 | 6.0±0.0 |
| | 2 | Mulched | 27.0±14.2 | 26.5±14.6 | 8.0±0.0 | 20.2±8.0 | 6.0±0.0 | 19.6±7.8 | 6.7±0.8 |
| | 2 | No mulch | 22.9±9.3 | 22.8±9.3 | 7.7±0.8 | 14.7±6.9 | 6.3±0.8 | 14.7±6.9 | 6.3±0.8 |
| | 3 | Mulched | 4.7±7.6 | 4.7±7.6 | 3.3±3.7 | 3.8±6.2 | 3.3±3.7 | 3.8±6.2 | 3.3±3.7 |
| | 3 | No mulch | 7.6±8.0 | 7.3±7.9 | 5.3±2.7 | 4.6±3.8 | 5.3±2.7 | 4.3±3.4 | 4.1±3.2 |
| | | S.E.D | 0.033 | 0.031 | 0.783 | 0.020 | 0.0004 | 0.558 | 0.099 |

* Mean±Standard deviation.

a: Location 1 = last planted with cowpea 1 year before the experiment; location 2 = last planted with cowpea 2 years before the experiment; and location 3 = last planted with cowpea 3 years before the experiment.

S.E.D. Standard error of the difference between means for incidence and severity within days after sowing.

Table 19. Incidence and severity of scab on plants grown with and without mulch at Samaru, 1994.

| Days after sowing | Location[a] | Mulching treatment | Incidence (%) and severity of scab* | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Overall | On epicotyl | | On hypocotyl | | On hypocotyl and epicotyl | |
| | | | *incidence* | *Incidence* | *Severity* | *Incidence* | *Severity* | *Incidence* | *Severity* |
| 30 | 1 | Mulched | 0.3±0.5 | 0.3±0.5 | 2.0±3.1 | 0.3±0.5 | 1.3±2.1 | 0.3±0.5 | 1.3±2.1 |
| | 1 | No mulch | 5.0±2.6 | 4.8±2.6 | 5.0±2.6 | 1.5±2.3 | 3.3±2.7 | 1.5±2.3 | 3.3±2.7 |
| | 2 | Mulched | 2.6±3.0 | 1.7±2.2 | 4.0±3.3 | 1.3±1.8 | 2.3±2.5 | 1.3±1.8 | 2.3±2.5 |
| | 2 | No mulch | 1.5±1.5 | 0.9±1.5 | 0.7±1.6 | 0.6±1.4 | 0.7±1.6 | 0.6±1.4 | 0.7±1.6 |
| | 3 | Mulched | 0.5±0.5 | 0.3±0.5 | 1.6±0.9 | 0.0±0.0 | 1.0±0.0 | 0.0±0.0 | 1.0±0.0 |
| | 3 | No mulch | 1.01±1.0 | 0.6±1.2 | 2.0±3.1 | 0.6±1.0 | 0.7±2.1 | 0.6±1.0 | 1.3±2.1 |
| | | S.E.D | 0.006 | 0.005 | 1.052 | 0.004 | 0.858 | 0.004 | 0.858 |
| 37 | 1 | Mulched | 3.4±4.6 | 3.1±4.2 | 5.7±0.8 | 1.1±1.5 | 3.7±2.9 | 1.0±1.6 | 3.0±3.3 |
| | 1 | No mulch | 36.4±22.7 | 35.4±22.7 | 6.0±0.0 | 23.9±19.2 | 6.0±0.0 | 23.9±19.2 | 6.0±0.0 |
| | 2 | Mulched | 10.4±6.6 | 9.8±6.2 | 6.0±0.0 | 8.3±5.5 | 5.7±0.8 | 8.3±5.5 | 6.0±0.0 |
| | 2 | No mulch | 12.8±13.6 | 10.4±12.5 | 4.7±2.4 | 4.2±5.5 | 3.7±2.9 | 4.2±5.5 | 4.0±3.0 |
| | 3 | Mulched | 2.0±1.5 | 1.7±1.0 | 5.0±2.5 | 0.9±1.0 | 2.7±3.0 | 0.9±1.0 | 3.0±3.3 |
| | 3 | No mulch | 2.1±3.7 | 2.1±3.7 | 2.7±3.0 | 0.4±0.6 | 2.0±3.1 | 0.4±0.6 | 2.0±3.1 |
| | | S.E.D | 0.039 | 0.002 | 0.649 | 0.031 | 0.991 | 0.031 | 1.065 |
| 44 | 1 | Mulched | 27.2±18.5 | 24.1±17.0 | 8.0±0.0 | 20.2±14.2 | 8.0±0.0 | 20.2±14.2 | 7.3±1.0 |
| | 1 | No mulch | 78.5±8.3 | 71.9±9.8 | 8.0±0.0 | 50.3±8.8 | 8.0±0.0 | 50.3±8.8 | 7.5±0.6 |
| | 2 | Mulched | 41.7±21.6 | 33.4±17.7 | 8.0±0.0 | 27.4±17.1 | 8.0±0.0 | 27.4±17.0 | 7.7±0.5 |
| | 2 | No mulch | 47.3±23.4 | 34.1±16.6 | 8.0±0.0 | 22.6±11.5 | 8.0±0.0 | 22.6±11.5 | 7.5±0.6 |
| | 3 | Mulched | 20.0±6.9 | 17.3±7.2 | 8.0±0.0 | 9.1±3.3 | 8.0±0.0 | 9.1±3.4 | 7.8±0.5 |
| | 3 | No mulch | 21.9±12.0 | 18.7±9.5 | 8.0±0.0 | 13.8±9.7 | 8.0±0.0 | 13.8±10.0 | 7.8±0.5 |
| | | S.E.D | 0.057 | 0.052 | 0.000 | 0.037 | 0.000 | 0.037 | 0.303 |

*Mean±Standard deviation.

a: Location 1 = last planted with cowpea 1 year before the experiment; location 2 = last planted with cowpea 2 years before the experiment; and location 3 = last planted with cowpea 3 years before the experiment.

S.E.D. Standard error of the difference between means for incidence and severity within days after sowing.

**4.7 Effect of Planting Date on Incidence of Scab, and Apparent infection rate (r) of Scab and Yield of Three Cowpea Varieties**

The proportion of scab on leaves, stems, peduncles, flowering cushions and pods were observed for the first, second and third planting dates in respect of the susceptible (IT 85F-1517) and moderately susceptible (IT 81D-1137) varieties in 1992 and 1993. The variety resistant to scab (IT 83S-720-2) had the disease only on the pods of the earliest sown crop in both years. The rainfall, air temperatures, and relative humidities in July to September of 1992 and 1993 are presented in Appendices 51-56.

In 1992 at 42 and 48 DAS, the leaf scab incidence was significantly higher (P= 0.05) for the first planting date compared to the second and third planting dates in respect of the susceptible (IT 85F-1517) and moderately susceptible (IT 81D-1137) varieties (Table 20). The apparent infection rates for the leaf scab for both varieties decreased significantly (P= 0.05) from the first to the second planting date. There was no increase in the rate of diseased plants per unit per day in the third planting date of both varieties. The stem scab had maximum significant difference (P= 0.05) in disease incidence in the second planting date with the variety IT 81D-1137 while IT 85F-1517 recorded highest scab incidence during the third planting date. The apparent infection rates were significantly highest (P= 0.05) for the earliest sown crop for both IT 85F-1517 and IT 81D-1137 (Table 20).

At 54 and 60 DAS, (Table 21) the earliest sown crop of IT 81D-1137 had significantly (P= 0.05) the highest peduncle scab incidence while the second planting date had the lowest. At the same time interval of planting, the earliest sown crop of IT 85F-1517 had the highest peduncle scab incidence while the third planting date had the lowest incidence which was not significantly different from that of the second planting date. The apparent infection rate decreased from the first to third sown crop of both IT 81D-1137 and IT 85F-1517, although there were no significant differences between the first and second sown crops of IT

81D-1137 (Table 21).

Flowering cushion scab incidence was highest at the earliest sown crop at 54 and 60 DAS for both IT 81D-1137 and IT 85F-1517, although 100% of flowering cushions of IT 85F-1517 had been infected with scab by 54 DAS. The apparent infection rates for both varieties were higher during the second planting date. At 54 DAS of the third planting date treatment of IT 85F-1517, only 1% of the flowering cushion had been infected and there was no scab increase by 60 DAS (Table 21).

At 63 DAS, the three planting dates had no significant effect on pod scab on IT 81D-1137, while at 73 DAS the first planting date had the highest pod scab incidence and the third planting date the least. The effect of planting date on IT 85F-1517 was significant (P =0.05) for the earliest sown crop and the highest apparent infection rate was recorded during this time (Table 22).

In 1993 at 42 DAS, the effect of planting dates on IT 81D-1137 for the leaf and stem scabs were the same. The second planting dates recorded the highest stem scab incidences of 48% which was not significantly different from the first planting date. The 50% stem scab incidence was statistically the same as that recorded for the third planting date (Table 23). With IT 85F-1517, there were significant (P= 0.05) decreases in leaf scab incidences amongst the three planting dates. For the stem scab of IT 85F-1517, the third planting date had the highest incidence which did not differ significantly from the second sown crop. At 51 DAS, the later the planting date, the lower were leaf scab incidences for IT 81D-1137, IT 85F-1517 and stem scab incidences for IT 85F-1517.

The peduncle scab of IT 85F-1517 at 57 DAS had significantly the same scab incidence for the earliest and second crops, while at 66 DAS the disease incidences were significantly different (P= 0.05) for each planting date. Peduncle scab incidences for the first and third sown crops of IT 81D-1137 were significantly the same at 57 DAS. At 66 DAS, the scab incidences for the three planting dates were also significantly the same. The apparent infection rate was

highest for the earliest sown crop (Table 24). The incidence trend for the flowering cushion scab at 57 and 66 DAS was basically the same. The apparent infection rates for the earliest sown crop for the peduncle and flowering cushion scabs were significantly higher (P= 0.05) than those of the other planting dates (Table 24).

At 60 DAS the disease incidence for the pod scabs were significantly high in respect of the second and first planting dates for IT 81D-1137 and IT 85F-1517. The pod scab incidence significantly (P =0.05) decreased with later planting dates (Table 25). The apparent infection rates for the earliest sown crop of IT 85F-1517 was significantly higher (P= 0.05) than those of the other planting dates (Table 25). There was no increase in the apparent infection rate on pod of IT 81D-1137 for the three planting dates and for the second and third sown crops of IT 85F-1517. The first sown crop of IT 83S-720-2 also had no increase in pod scab (Table 25).

In 1992, the susceptible variety (IT 85F-1517) had the lowest grain yield while the resistant variety, IT 83S-720-2, recorded the highest grain yield regardless of the planting date. The second planting date of the moderately susceptible variety, IT 81D-1137, had the highest grain yield (1790.0 kg/ha) while the first planting date date had the lowest (510.0 kg/ha) (Table 22).

In 1993, the first and second sown crops of the resistant variety, IT 83S-720-2, compared to the other two varieties had the highest grain yields (Table 25). The susceptible variety IT 85F-1517 recorded significantly (P= 0.05) the highest grain yield in the second sown crop (1390.1 kg/ha), the yield of the earliest and last sown crop being significantly the same (346.3 and 340.5 kg/ha). The grain yields of the moderately susceptible variety, IT 81D-1137, decreased significantly (P= 0.05) with later plantings of the crop (Table 25).

Table 20. Effect of sowing date on the incidence and apparent infection rate of scab on leaves and stems in three varieties at 42 and 48 days after sowing (DAS) in 1992 at Samaru.

| Plant part | Sowing date | Cowpea variety | Proportion of plants with disease 42 DAS | 48 DAS | Apparent infection rate (plants/day) |
|---|---|---|---|---|---|
| Leaf | | | | | |
| | Jul-16 | IT 81D-1137 | 0.54a | 0.69a | 0.107a |
| | Jul-26 | IT 81D-1137 | 0.39b | 0.52b | 0.088b |
| | Aug-05 | IT 81D-1137 | 0.45c | 0.45c | 0.000*c |
| | Jul-16 | IT 85F-1517 | 0.92a | 0.99a | 0.358a |
| | Jul-26 | IT 85F-1517 | 0.43b | 0.60b | 0.114b |
| | Aug-05 | IT 85F-1517 | 0.46b | 0.46c | 0.000*c |
| | Jul-16 | IT 83S-720-2 | 0.00a | 0.00a | 0.000a |
| | Jul-26 | IT 83S-720-2 | 0.00a | 0.00a | 0.000a |
| | Aug-05 | IT 83S-720-2 | 0.00a | 0.00a | 0.000a |
| Stem | | | | | |
| | Jul-16 | IT 81D-1137 | 0.14c | 0.51a | 0.309a |
| | Jul-26 | IT 81D-1137 | 0.45a | 0.55a | 0.067b |
| | Aug-05 | IT 81D-1137 | 0.36b | 0.36b | 0.000*c |
| | Jul-16 | IT 85F-1517 | 0.17c | 0.52a | 0.277a |
| | Jul-26 | IT 85F-1517 | 0.29b | 0.51a | 0.156ab |
| | Aug-05 | IT 85F-1517 | 0.57a | 0.57a | 0.000*c |
| | Jul-16 | IT 83S-720-2 | 0.00a | 0.00a | 0.000a |
| | Jul-26 | IT 83S-720-2 | 0.00a | 0.00a | 0.000a |
| | Aug-05 | IT 83S-720-2 | 0.00a | 0.00a | 0.000a |

Figures with the same letters within a column for the same variety are not significantly different at (0.05) using DMRT .

* 0.000= No increase in disease incidence.

Table 21. Effect of sowing date on the incidence and apparent infection rate of sacb on peduncles and flowering cushions in three varieties at 54 and 60 days after sowing (DAS) in 1992 at Samaru.

| Plant part | Sowing date | Cowpea variety | Proportion of plants with disease 54 DAS | 60 DAS | Apparent infection rate (plants/day). |
|---|---|---|---|---|---|
| Peduncle | | | | | |
| | Jul-16 | IT 81D-1137 | 0.75a | 0.97a | 0.396a |
| | Jul-26 | IT 81D-1137 | 0.05c | 0.24c | 0.298ab |
| | Aug-05 | IT 81D-1137 | 0.45b | 0.46b | 0.0067c |
| | Jul-16 | IT 85F-1517 | 0.94a | 0.98a | 0.190a |
| | Jul-26 | IT 85F-1517 | 0.62bc | 0.64bc | 0.014b |
| | Aug-05 | IT 85F-1517 | 0.56c | 0.56c | 0.000*c |
| | Jul-16 | IT 83S-720-2 | 0.00a | 0.00a | 0.000a |
| | Jul-26 | IT 83S-720-2 | 0.00a | 0.09a | 0.000a |
| | Aug-05 | IT 83S-720-2 | 0.00a | 0.00a | 0.000a |
| Flowering cushion | | | | | |
| | Jul-16 | IT 81D-1137 | 0.47a | 0.66a | 0.130b |
| | Jul-26 | IT 81D-1137 | 0.04bc | 0.16b | 0.253a |
| | Aug-05 | IT 81D-1137 | 0.00c | 0.00c | 0.000 |
| | Jul-16 | IT 85F-1517 | 1.00a | 1.00a | 0.000*a |
| | Jul-26 | IT 85F-1517 | 0.41b | 0.46b | 0.034a |
| | Aug-05 | IT 85F-1517 | 0.01c | 0.01c | 0.000*ab |
| | Jul-16 | IT 83S-720-2 | 0.00a | 0.00a | 0.000a |
| | Jul-26 | IT 83S-720-2 | 0.00a | 0.00a | 0.000a |
| | Aug-05 | IT 83S-720-2 | 0.00a | 0.00a | 0.000a |

Figures with the same letters within a column for the same variety are not significantly different at (0.05) using DMRT .

* 0.000= No increase in disease incidence.

Table 22. Effect of planting date on the incidence and apparent infection rate of pod scab and on grain yield of 3 varieties at 63 and 73 days after sowing (DAS) in 1992 at Samaru.

| Sowing date | Variety | Plant part | Proportion of plants with disease | | Apparent infection rate (plants/day) | Grain yield (kg/ha) |
|---|---|---|---|---|---|---|
| | | | 60 DAS | 69 DAS | | |
| Jul-16 | IT 81D-1137 | Pod | 0.00b | 0.61a | 0.0b | 513.0c |
| Jul-26 | IT 81D-1137 | Pod | 0.04ab | 0.29b | 0.286a | 1790.0a |
| Aug-05 | IT 81D-1137 | Pod | 0.01b | 0.01c | 0.000*b | 978.0b |
| Jul-16 | IT 85F-1517 | Pod | 0.70a | 0.95a | 0.380a | 306.0b |
| Jul-26 | IT 85F-1517 | Pod | 0.20b | 0.26b | 0.057b | 617.0a |
| Aug-05 | IT 85F-1517 | Pod | 0.02c | 0.02c | 0.000*c | 440.0b |
| Jul-16 | IT 83S-720-2 | Pod | 0.04ab | 0.07a | 0.098a | 2216.0a |
| Jul-26 | IT 83S-720-2 | Pod | 0.00b | 0.00b | 0.000b | 2067.0a |
| Aug-05 | IT 83S-720-2 | Pod | 0.00b | 0.00b | 0.000b | 1670.0b |

Figures with the same letters within a column for the same variety are not significantly different at (0.05) using DMRT .
* 0.000= No increase in disease incidence.

Table 23. Effect of sowing dates on the incidence and apparent infection rate of foliar and stem scab in 3 varieties at 42 and 51 days (DAS) after sowing in 1993, at Samaru.

| Plant part | Sowing date | Cowpea variety | Proportion of plants with disease 42 DAS | 51 DAS | Apparent infection rate (plants/day) |
|---|---|---|---|---|---|
| Leaf | | | | | |
| | Jul-24 | IT 81D-1137 | 0.38ab | 0.73a | 0.247a |
| | Aug-04 | IT 81D-1137 | 0.48a | 0.52b | 0.027b |
| | Aug-14 | IT 81D-1137 | 0.12c | 0.12c | 0.000*c |
| | Jul-24 | IT 85F-1517 | 0.68a | 0.85a | 0.163a |
| | Aug-04 | IT 85F-1517 | 0.54b | 0.54b | 0.000*b |
| | Aug-14 | IT 85F-1517 | 0.31c | 0.31c | 0.000*b |
| | Jul-24 | IT 83S-720-2 | 0.00a | 0.00a | 0.000a |
| | Aug-04 | IT 83S-720-2 | 0.00a | 0.00a | 0.000a |
| | Aug-14 | IT 83S-720-2 | 0.00a | 0.00a | 0.000a |
| Stem | | | | | |
| | Jul-24 | IT 81D-1137 | 0.03c | 0.19c | 0.225a |
| | Aug-04 | IT 81D-1137 | 0.50a | 0.53a | 0.020b |
| | Aug-14 | IT 81D-1137 | 0.41ab | 0.43ab | 0.014b |
| | Jul-24 | IT 85F-1517 | 0.23c | 0.60a | 0.269a |
| | Aug-04 | IT 85F-1517 | 0.55a | 0.59a | 0.027b |
| | Aug-14 | IT 85F-1517 | 0.48ab | 0.48b | 0.000*c |
| | Jul-24 | IT 83S-720-2 | 0.00a | 0.00a | 0.000a |
| | Aug-04 | IT 83S-720-2 | 0.00a | 0.00a | 0.000a |
| | Aug-14 | IT 83S-720-2 | 0.00a | 0.00a | 0.000a |

Figures with the same letters within a column for the same variety are not significantly different at (0.05) using DMRT .

* 0.000= No increase in disease incidence.

Table 24. Effect of sowing dates on the incidence and apparent infection rate of peduncle and flowering cushion scab in 3 varieties at 57 and 66 days (DAS) after sowing in 1993 at Samaru.

| Plant part | Sowing date | Variety | Proportion of plants with disease 57 DAS | 66 DAS | Apparent infection rate (plants/day) |
|---|---|---|---|---|---|
| Peduncle | | | | | |
| | Jul-24 | IT 81D-1137 | 0.47b | 0.81ba | 0.261a |
| | Aug-04 | IT 81D-1137 | 0.55a | 0.55b | 0.000*b |
| | Aug-14 | IT 81D-1137 | 0.45b | 0.45b | 0.000*b |
| | Jul-24 | IT 85F-1517 | 0.72a | 0.97a | 0.421a |
| | Aug-04 | IT 85F-1517 | 0.72a | 0.72b | 0.000b |
| | Aug-14 | IT 85F-1517 | 0.24b | 0.24c | 0.000*b |
| | Jul-24 | IT 83S-720-2 | 0.00a | 0.00a | 0.000a |
| | Aug-04 | IT 83S-720-2 | 0.00a | 0.00a | 0.000a |
| | Aug-14 | IT 83S-720-2 | 0.00a | 0.00a | 0.000a |
| Flowering cushion | | | | | |
| | Jul-24 | IT 81D-1137 | 0.09a | 0.31a | 0.252a |
| | Aug-04 | IT 81D-1137 | 0.03b | 0.03b | 0.000*b |
| | Aug-14 | IT 81D-1137 | 0.03b | 0.03b | 0.000*b |
| | Jul-24 | IT 85F-1517 | 0.40a | 0.67a | 0.185a |
| | Aug-04 | IT 85F-1517 | 0.23b | 0.23b | 0.000*b |
| | Aug-14 | IT 85F-1517 | 0.25b | 0.25b | 0.000*b |
| | Jul-24 | IT 83S-720-2 | 0.00a | 0.00a | 0.000a |
| | Aug-04 | IT 83S-720-2 | 0.00a | 0.00a | 0.000a |
| | Aug-14 | IT 83S-720-2 | 0.00a | 0.00a | 0.000a |

Figures with the same letters within a column for the same variety are not significantly different at (0.05) using DMRT .

* 0.000= No increase in disease incidence.

Table 25. Effect of planting date on the incidence and apparent infection rate of pod scab and on grain yield of 3 varieties at 60 and 69 days after sowing (DAS) in 1993 at Samaru.

| Sowing date | Variety | Plant part | Proportion of plants with disease | | | Apparent |
|---|---|---|---|---|---|---|
| | Grain yield | | 60 DAS | 69 DAS | infection rate (plants/day) | (kg/ha) |
| Jul-24 | IT 81D-1137 | Pod | 0.00c | 0.44a | 0.000a | 1990.0a |
| Aug-04 | IT 81D-1137 | Pod | 0.26a | 0.26b | 0.000*b | 1546.0b |
| Aug-14 | IT 81D-1137 | Pod | 0.03bc | 0.03c | 0.000*b | 860.0c |
| Jul-24 | IT 85F-1517 | Pod | 0.63a | 0.77a | 0.113a | 346.0b |
| Aug-04 | IT 85F-1517 | Pod | 0.41b | 0.41b | 0.000*b | 1390.0a |
| Aug-14 | IT 85F-1517 | Pod | 0.20c | 0.20c | 0.000*b | 340.0b |
| Jul-24 | IT 83S-720-2 | Pod | 0.06a | 0.06a | 0.000*a | 2580.0a |
| Aug-04 | IT 83S-720-2 | Pod | 0.00a | 0.00a | 0.000a | 2050.0a |
| Aug-14 | IT 83S-720-2 | Pod | 0.00a | 0.00a | 0.000a | 685.0b |

Figures with the same letters within a column for the same variety are not significantly different at (0.05) using DMRT .

0.000* = No increase in disease incidence.

## 4.8 Effect of Fungicides on Development of Scab Disease

The ANOVA indicated that fungicides significantly affected scab development on leaves, stems, peduncles, flowering cushions and pods for the cowpea variety susceptible to scab (IT 85F-1517) and the variety moderately susceptible (IT 81D-1137). AUDPC values were significantly decreased by most fungicides on most plant parts (Tables 26 & 27, Appendices 57 & 58). In 1992 and 1993, AUDPC values were much lower on flowering cushions and pods of IT 85F-1517 and IT 81D-1137. In both years, the two varieties showed little or no significant differences in disease severity ratings and AUDPC values for the untreated control plants and mancozeb. In 1992, significant differences (P= 0.05) in disease severity ratings and AUDPC values were observed only on flowering cushion scab of IT 85F-1517 while in 1993, these differences were observed on flowering cushion scab of IT 81D-1137. Benomyl in both years significantly (P= 0.05) reduced scab severity on all plant parts especially on the flowering cushion and pod scabs compared to carbendazim + maneb and mancozeb. The low scab severity due to benomyl is reflected by its near to almost zero values for AUDPC (Tables 26 and 27).

The resistant variety (IT 83S-720-2) had scab symptoms only on the pods in both years. Treatment with benomyl significantly reduced scab development compared to mancozeb and untreated control plants.

Mancozeb was less effective than the other fungicides in reducing scab severities and consequently the AUDPCs. Plants treated with mancozeb were stunted and had thick waxy, leathery leaves like the untreated control plants. In 1992 the disease started much earlier than in 1993. In 1992, carbendazim + maneb significantly (P= 0.05) controlled scab better than mancozeb on the leaves stems, peduncles and flowering cushions of IT 81D-1137 and IT 85F-1517 with the exception of the leaf scab of IT 85F-1517 (Table 26). In 1993, carbendazim + maneb significantly (P= 0.05) controlled scab better than mancozeb on all plant parts of IT 81D-1137 while with IT 85F-1517 the control was effective only on

the peduncle, flowering cushion and pod scabs (Table 27). The curves obtained in both years are generally of sigmoid form (Figures 5 - 9) with the rate of disease progress at the beginning of the attack being slow on leaves and stems and slow or zero at the end of the epidemic on flowering cushions and pods. The AUDPC curves for the leaves, stems and peduncles of IT 85F-1517 and IT 81D-1137 show a staircase pattern. The time (49 and 52 days after sowing on July 24 1992) taken for the leaves and stems of unsprayed plants IT 85F-1517 and IT 81D-1137 to reach the maximum disease severity score of 10 was shorter in 1992 compared to 1993 when this occurred at 51 days after sowing in July 27 1993.

Table 26. Disease severity ratings at 64 days after sowing and areas under disease progress curves (AUDPC) in plots of three cowpea varieties sprayed weekly (beginning at 4 weeks after sowing) to control scab in 1992 at Samaru.

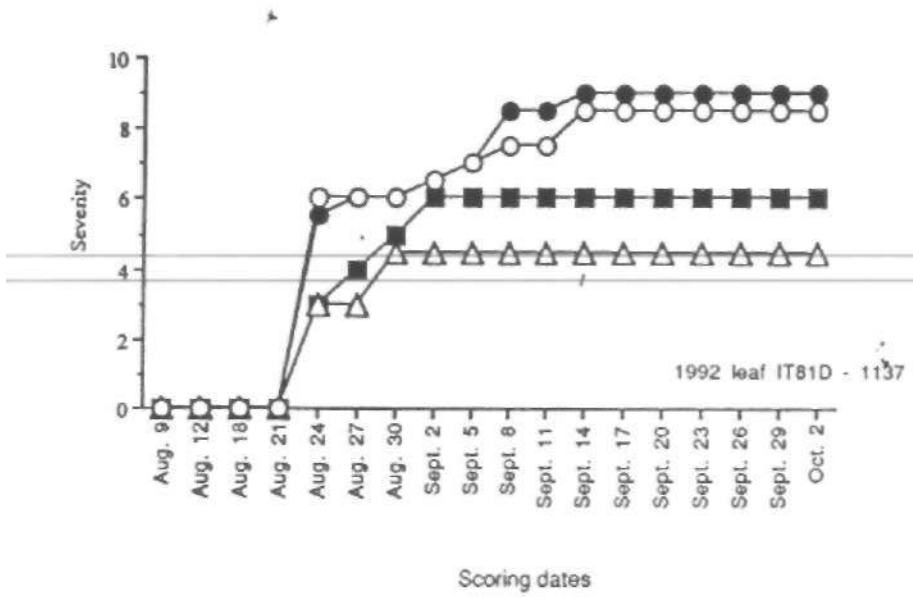
| Plant part/ Fungicide | IT 81D - 1137[a] moderately susceptible | | IT 85F - 1517[a] susceptible | | IT 83S - 720-2[a] resistant | |
|---|---|---|---|---|---|---|
| | DS | AUDPC | DS | AUDPC | DS | AUDPC |
| **Leaf** | | | | | | |
| Untreated control | 9.0 | 278.0 | 10.0 | 346.0 | 1.0 | 0.0 |
| mancozeb | 8.5 | 265.0 | 9.0 | 313.0 | 1.0 | 0.0 |
| carbendazim + maneb | 6.0 | 138.0 | 8.0 | 282.0 | 1.0 | 0.0 |
| benomyl | 4.5 | 185.0 | 1.0 | 0.0 | 1.0 | 0.0 |
| LSD (P= 0.05) | 2.0 | 74.6 | 1.7 | 60.6 | 0.0 | 0.0 |
| **Stem** | | | | | | |
| Untreated control | 10.0 | 322.0 | 10.0 | 295.0 | 1.0 | 0.0 |
| mancozeb | 9.0 | 260.0 | 9.0 | 289.0 | 1.0 | 0.0 |
| carbendazim + maneb | 5.0 | 131.0 | 7.0 | 239.0 | 1.0 | 0.0 |
| benomyl | 6.0 | 143.0 | 7.0 | 225.0 | 1.0 | 0.0 |
| LSD (P= 0.05) | 1.5 | 42.4 | 1.5 | 38.2 | 0.0 | 0.0 |
| **Peduncle** | | | | | | |
| Untreated control | 10.0 | 169.0 | 10.0 | 218.0 | 1.0 | 0.0 |
| mancozeb | 8.0 | 128.0 | 10.0 | 207.0 | 1.0 | 0.0 |
| carbendazim + maneb | 2.0 | 22.1 | 6.0 | 125.0 | 1.0 | 0.0 |
| benomyl | 2.0 | 30.4 | 6.0 | 135.0 | 1.0 | 0.0 |
| LSD (P= 0.05) | 2.6 | 56.6 | 3.8 | 19.2 | 0.0 | 0.0 |
| **Flowering cushion** | | | | | | |
| Untreated control | 9.0 | 81.8 | 10.0 | 182.0 | 1.0 | 0.0 |
| mancozeb | 8.0 | 82.5 | 8.0 | 136.0 | 1.0 | 0.0 |
| carbendazim + maneb | 1.0 | 0.0 | 5.0 | 68.3 | 1.0 | 0.0 |
| benomyl | 1.0 | 0.0 | 1.0 | 0.0 | 1.0 | 0.0 |
| LSD (P= 0.05) | 2.1 | 23.2 | 1.1 | 22.8 | 0.0 | 0.0 |
| **Pod** | | | | | | |
| Untreated control | 10.0 | 125.0 | 10.0 | 71.6 | 6.0 | 158.0 |
| mancozeb | 8.0 | 56.3 | 10.0 | 107.0 | 6.0 | 122.0 |
| carbendazim + maneb | 3.0 | 28.5 | 3.0 | 53.3 | 4.0 | 104.0 |
| benomyl | 1.0 | 0.0 | 1.0 | 0.0 | 1.0 | 0.0 |
| LSD (P= 0.05) | 3.6 | 48.8 | 1.8 | 66.6 | 3.2 | 66.6 |

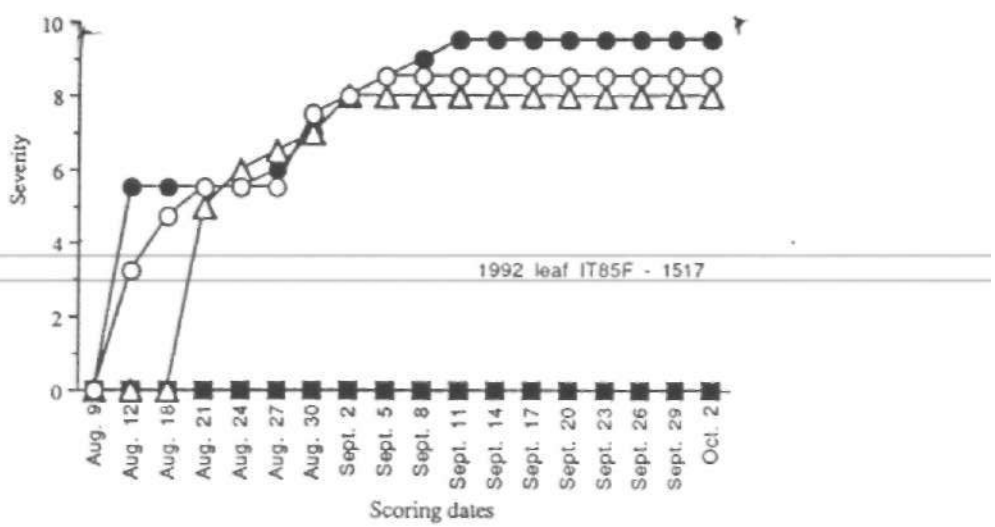
[a]; DS = disease severity rating (on scale of 1-10 in which 1 = no disease and 10 = maximum disease); AUDPC = Area under the disease progress curve points on the curve were the disease severity ratings from 28 DAS to 64 DAS.

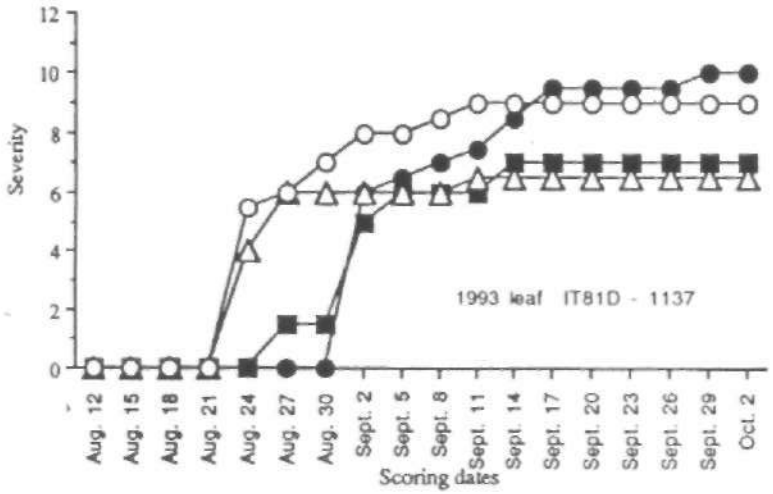
Table 27. Disease severity ratings at 60 days after sowing and areas under disease progress curves (AUDPC) in plots of three cowpea varieties sprayed weekly beginning at 4 weeks after sowing to control scab in 1993 at Samaru.

| Plant part/ Fungicide | IT 81D - 1137[a] moderately susceptible | | IT 85F - 1517[a] susceptible | | IT 83S - 720-2[a] resistant | |
|---|---|---|---|---|---|---|
| | DS | AUDPC | DS | AUDPC | DS | AUDPC |
| Leaf | | | | | | |
| Untreated control | 10.0 | 234 | 10.0 | 365.0 | 1.0 | 0.0 |
| Mancozeb | 9.0 | 291 | 9.0 | 306.0 | 1.0 | 0.0 |
| Carbendazim + maneb | 7.0 | 209 | 8.0 | 316.0 | 1.0 | 0.0 |
| Benomyl | 7.0 | 183 | 6.0 | 260.0 | 1.0 | 0.0 |
| LSD (P= 0.05) | 1.3 | 35.9 | 1.3 | 42.9 | 0.0 | 0.0 |
| Stem | | | | | | |
| Untreated control | 10.0 | 285 | 9.0 | 260.0 | 1.0 | 0.0 |
| Mancozeb | 10.0 | 281 | 9.0 | 226.0 | 1.0 | 0.0 |
| Carbendazim + maneb | 6.0 | 143 | 7.0 | 204.0 | 1.0 | 0.0 |
| Benomyl | 4.0 | 93.8 | 6.0 | 224.0 | 1.0 | 0.0 |
| LSD (P= 0.05) | 2.2 | 45.6 | 1.7 | 31.4 | 0.0 | 0.0 |
| Peduncle | | | | | | |
| Untreated control | 10.0 | 150 | 9.0 | 167.0 | 1.0 | 0.0 |
| Mancozeb | 9.0 | 145 | 7.0 | 212.0 | 1.0 | 0.0 |
| Carbendazim + maneb | 7.0 | 107 | 6.0 | 146.0 | 1.0 | 0.0 |
| Benomyl | 5.5 | 107 | 4.0 | 116.0 | 1.0 | 0.0 |
| LSD (P= 0.05) | 1.4 | 29.7 | 4.1 | 40.3 | 0.0 | 0.0 |
| Flowering cushion | | | | | | |
| Untreated control | 10.0 | 131 | 9.0 | 147.0 | 1.0 | 0.0 |
| Mancozeb | 8.0 | 93.8 | 9.0 | 154.0 | 1.0 | 0.0 |
| Carbendazim + maneb | 1.0 | 0 | 5.0 | 59.6 | 1.0 | 0.0 |
| Benomyl | 1.0 | 0 | 3.0 | 54.4 | 1.0 | 0.0 |
| LSD (P= 0.05) | 0.8 | 13 | 2.6 | 61.5 | 0.0 | 0.0 |
| Pod | | | | | | |
| Untreated control | 9.0 | 119 | 10.0 | 150.0 | 6.0 | 108.0 |
| Mancozeb | 8.0 | 119 | 9.0 | 182.0 | 6.0 | 93.0 |
| Carbendazim + maneb | 1.0 | 0 | 3.0 | 56.3 | 5.0 | 82.1 |
| Benomyl | 1.0 | 0 | 1.0 | 0.0 | 1.0 | 0.0 |
| LSD (P= 0.05) | 1.8 | 24.6 | 2.2 | 48.6 | 1.9 | 44.9 |

[a]: DS = disease severity rating (on scale of 1-10 in which 1 = no disease and 10 = maximum disease); AUDPC = Area under the disease progress curve points on the curve were the disease severity ratings from 28 DAS to 60 DAS.



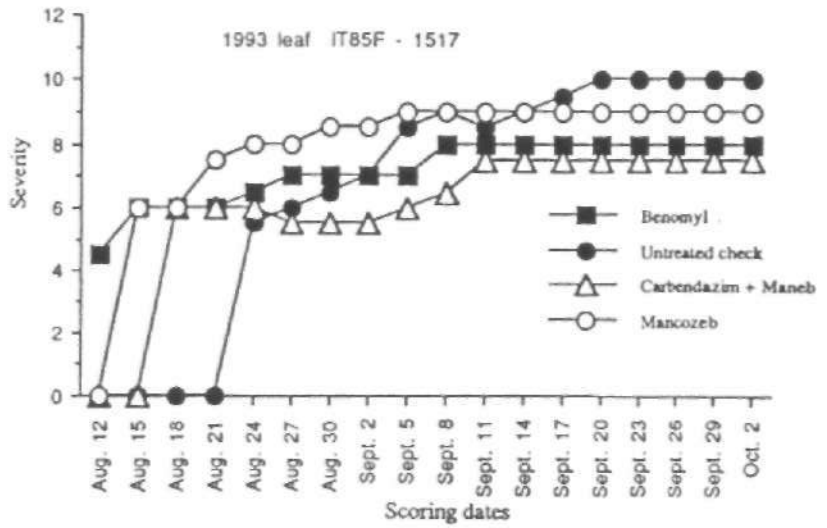
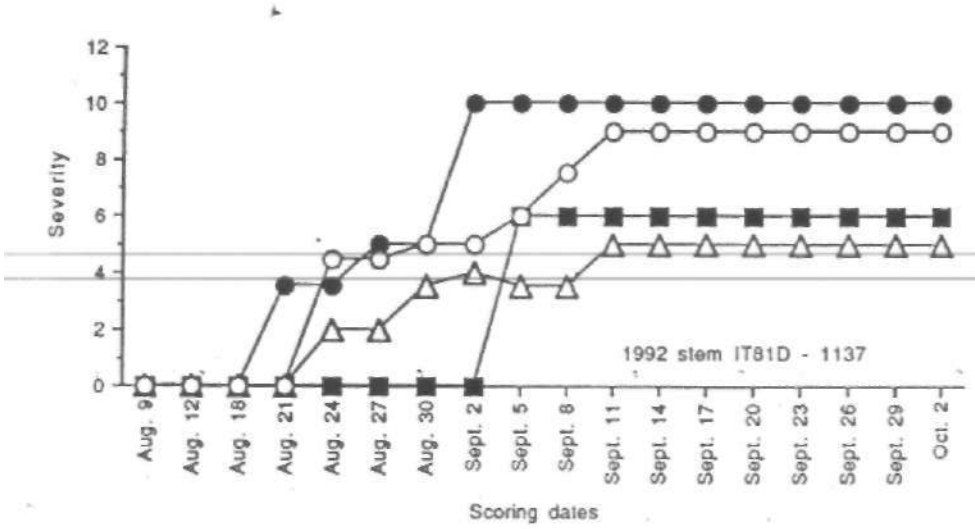
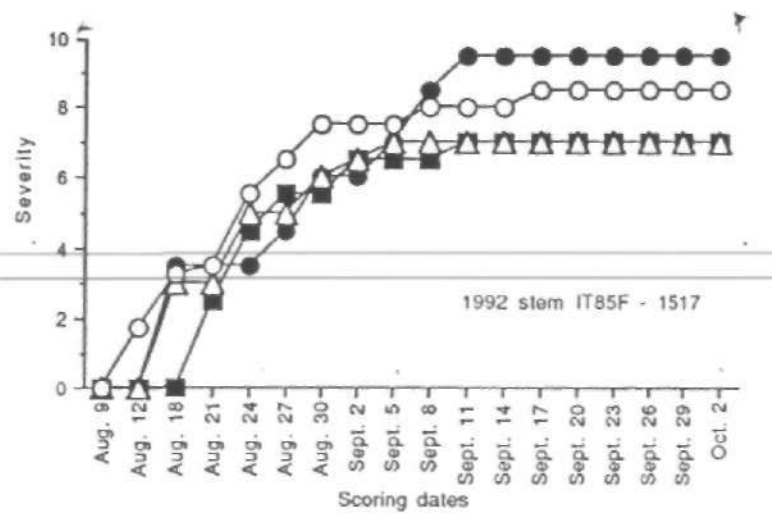
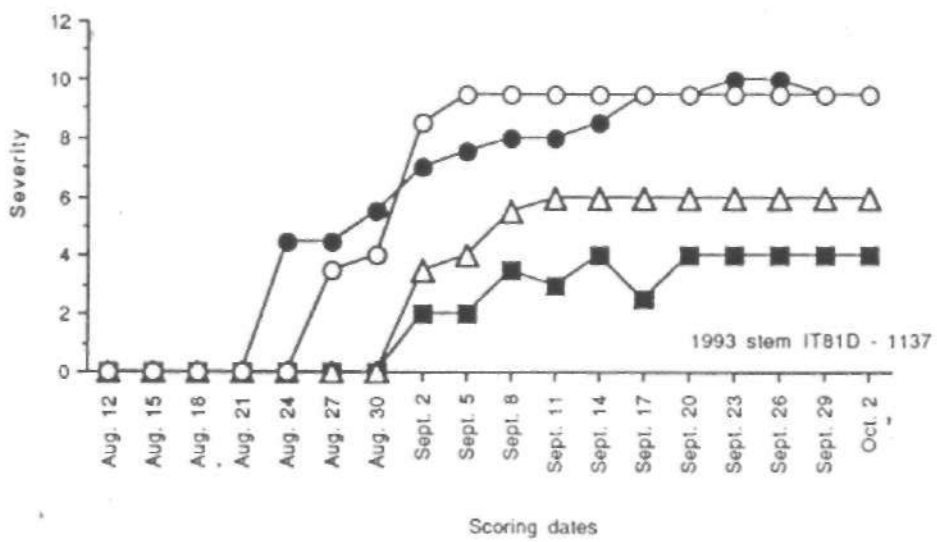




**Figure 5. Foliar scab severity and AUDPC values in IT - 1137D and IT 85F - 1517 treated with carbendazim + maneb, mancozeb, benomyl and untreated check in 1992 and 1993.**



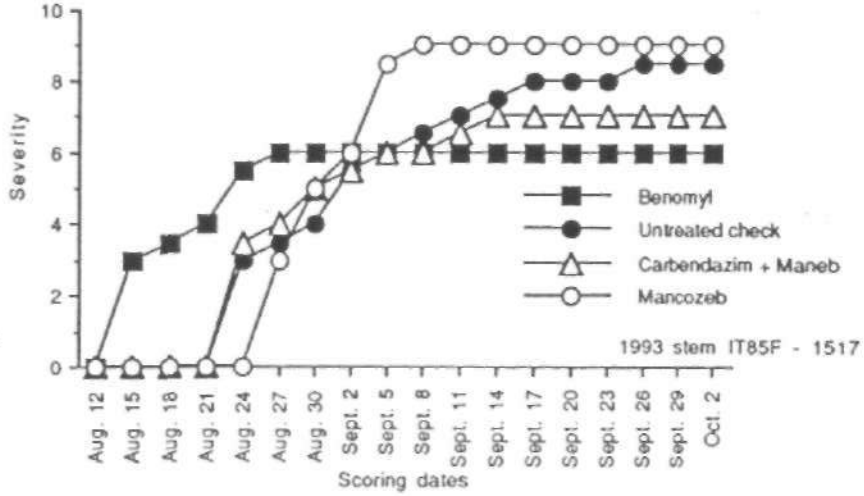




**Figure 6. Stem scab severity and AUDPC values in IT - 1137D and IT 85F - 1517 treated with carbendazim + maneb, mancozeb, benomyl and untreated check in 1992 and 1993.**

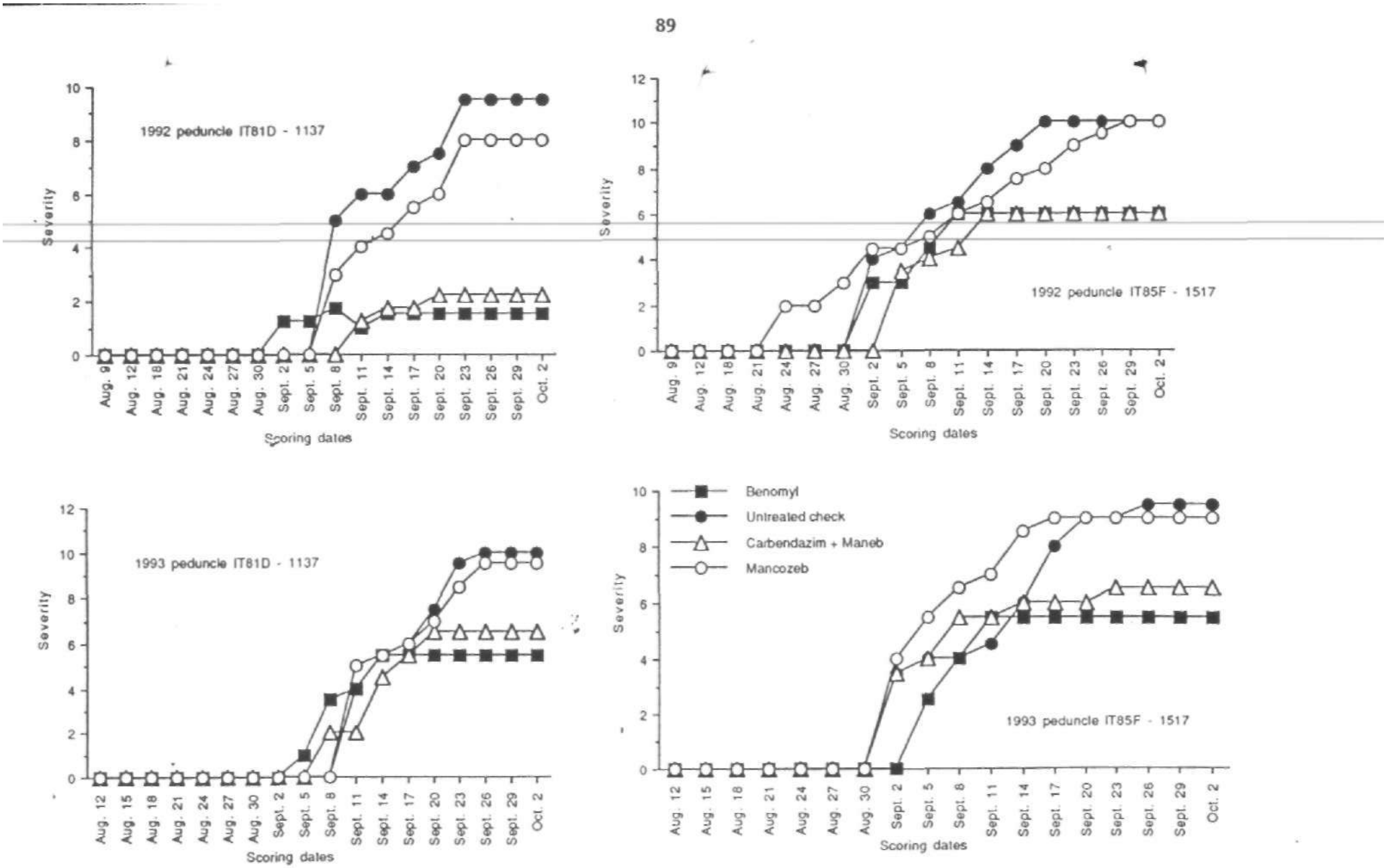
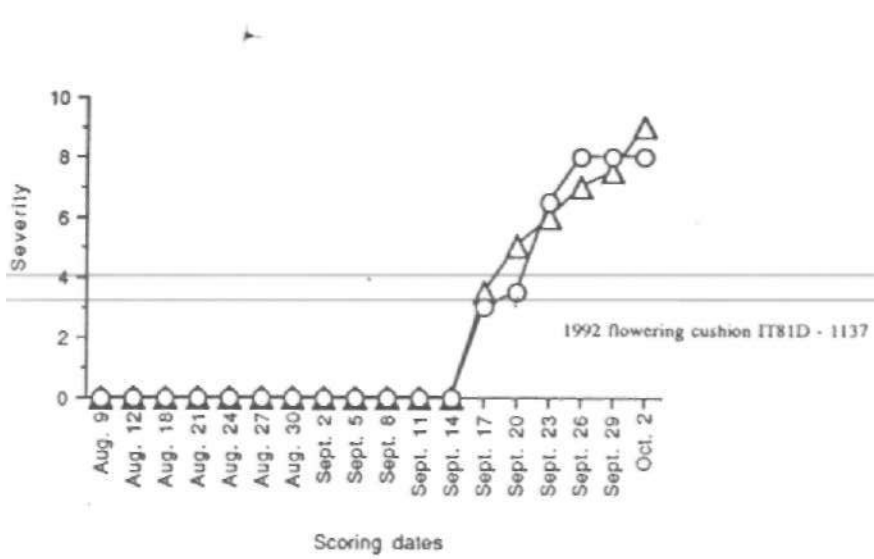
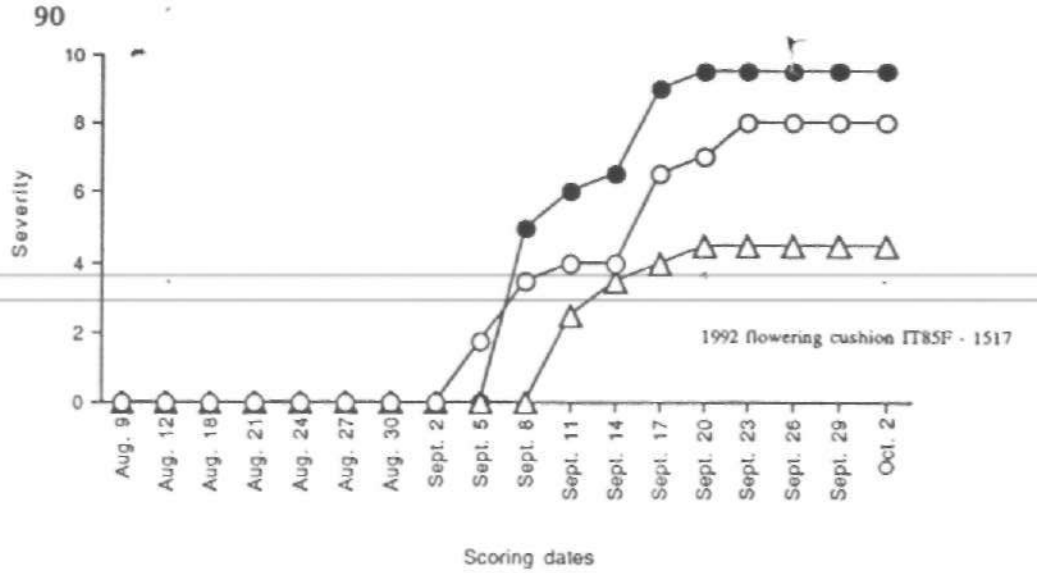
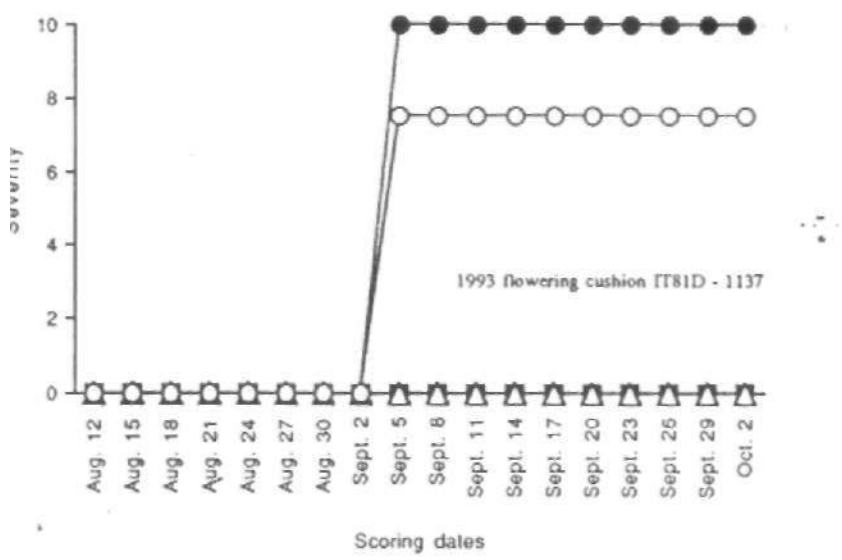



**Figure 7. Peduncle scab severity and AUDPC values in IT - 1137D and IT 85F - 1517 treated with carbendazim + maneb, mancozeb, benomyl and untreated check in 1992 and 1993.**



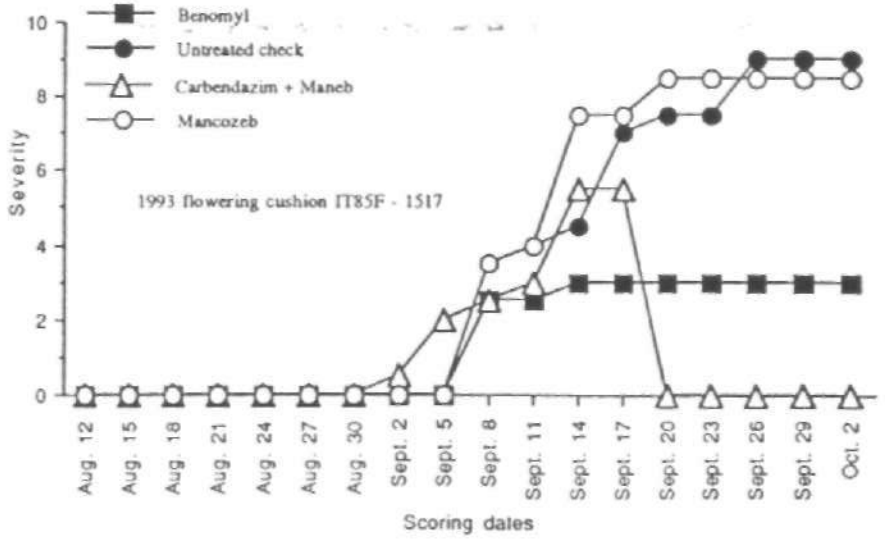




**Figure 8. Flowering cushion scab severity and AUDPC values in IT - 1137D and IT 85F - 1517 treated with carbendazim + maneb, mancozeb, benomyl and untreated check in 1992 and 1993.**

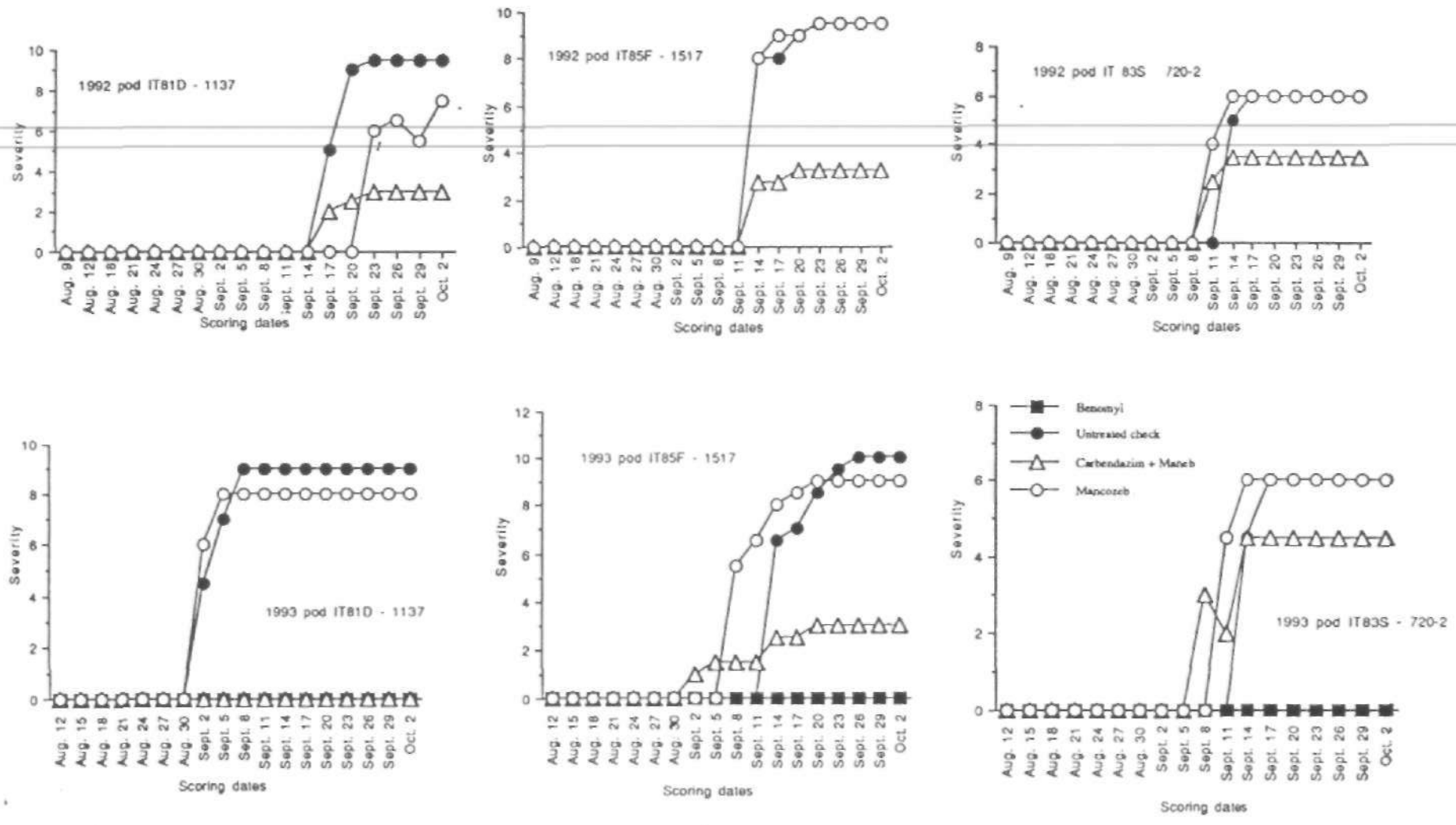



**Figure 9. Pod scab severity and AUDPC values in IT - 1137D and IT 85F - 1517 treated with carbendazim + maneb, mancozeb, benomyl and untreated check in 1992 and 1993.**

# CHAPTER FIVE

## DISCUSSION

Results of histopathological examinations have shown that penetration and colonization of naturally infected host tissue by *Sphaceloma* sp. appears to be restricted to the epidermal tissue. The apparent colonization of *Sphaceloma* sp. on the epidermal tissue of cowpea is important to consider when surface sterilizing infected tissue prior to isolating *Sphaceloma* sp. in artificial culture. Long surface sterilization will reduce chances of isolating *Sphaceloma* sp. Short surface sterilization will not kill other microorganisms found on the scab lesion. Therefore there will often be fast-growing microorganisms which over grow *Sphaceloma* sp. during attempts to isolate it in artificial culture. The sphercical and the oblong spores found in the epidermal layer fitted the description in size and shape given by Emechebe (1980) when he first isolated the pathogen. It was not determined if the fungus was physiologically too weak to invade other host tissues or if some barrier (chemical or/and mechanical) produced by host tissue as a reaction to infection inhibited further penetration beyond the epidermal cells. The symptoms of scab are too severe to be attributed to a fungus that is confined to the epidermal tissue. Such symptoms as the mummification of the young pods and the puckering and shot holes on the leaves are difficult to ascribe to a fungus located only in the epidermis unless that fungus produces toxins or extracellular enzymes that induce these symptoms. However, these results are in conformity with those of Jenkins (1933) who studied young *Sphaceloma* scab lesions in *Phaseolus lunatus* and reported that the conidiophores were aggregated in the centre of young lesions and that the acervuli were located in the epidermal layer. Further studies involving artificial inoculation of host tissues with pure cultures of the fungus under aseptic conditions are required to confirm the results of the present study.

Results of the study in to determine the easiest technique for isolation of the fungus from infected host tissue have shown that, in general cowpea pod extract agar (CpdA) tended to yield the highest number of isolations of the fungus from infected host tissue, irrespective of the plant part from which the lesions were obtained. Pod and leaf lesions also yielded the highest number of isolations irrespective of the media. These results may be related to the possible availability of growth promoting factors for the fungus in the pod but not in other plant parts. The pod being the site of grain filling with high concentration of photosynthates probably provides some nutrients for this fungus. The cowpea leaf extract agar (ClfA) was also a good medium for isolating the fungus. Again this may be associated with the fact that the leaves are the primary sites of photosynthesis and therefore, acting a source of nutrients for the fungus. However, PDA was also a good medium for the isolation of the fungus and compared favourably with CpdA and ClfA. Studies with most fungi have proved that PDA supports growth of most fungi. Emechebe (1980) first isolated *Sphaceloma* sp. using PDA amended with rose bengal and streptomycin. Whiteside (1986) easily isolated *Elsinoe fawcettii* from citrus leaf scab using PDA amended with streptomycin sulphate, tetracycline and dodine. In view of these and given the easy availability of PDA compared to CpdA or ClfA, PDA will serve as a good medium for the isolation of the fungus.

The study of the incubation period of scab reported in this dissertation showed that irrespective of plant age at time of inoculation, young leaves on plants of various ages showed symptoms within 5 days. While stems of younger plants aged 14–28 days at time of inoculation had symptoms within 7 days, stems of plants aged 42–56 days at inoculation manifested symptoms within 10 days. Hence incubation period for stems increased with age of the stem. The peduncles, flowering cushions and pods tended to exhibit symtoms much longer compared to leaves and stems. If stems are ignored then there is a trend to a longer incubation period for each of the plant parts which appears progressively as the plant is

maturing. In an earlier study, Emechebe (1980) spray-inoculated 4 to 6 weeks old plants and observed young foliar symptoms within 4-6 days after inoculation. The results of the present study corroborate Emechebe's (1980) report. The longer incubation period on older tissues may be a manifestation of mature plant resistance. Wheeler (1982) reported mature plant resistance in French bean anthracnose (*Colletotrichum lindemuthianum*). It has also been reported that young green fruits of *Capsicum annum* were more susceptible to anthracnose of chili pepper (*Colletotrichum capsici*) than mature fruits (Astuti and Suhardi, 1986).

Results of the present study have also shown that growth of *Sphaceloma* sp. in agar culture varies with type of culture medium and temperature. The fungus grew well at 26°C but did not grow at 35°C although incubation for up to 15 days at 35°C was not lethal to the fungus because the cultures resumed growth on transfer to 26°C. These results agree with those of Fakorede (1976) who reported that maximum growth of cowpea isolate of *C. lindemuthianum.* occurred on agar culture at 25-26°C and no fungal growth occurred at 34°C. Alabi (1994), working on *Colletotrichum capsici,* which induces cowpea brown blotch, found that 25°C was the optimum temperature for radial growth of the fungus on PDA.

The difficulty of isolating *Sphaceloma* sp. from seeds showing symptoms of scab infection, called for an indirect method used to determine if the pathogen is seed transmitted. Results obtained in 1993 showed that the incidence of infection in seedlings grown from untreated seeds from Samaru (where the disease is endemic) was 10.1% compared to 2.4% for seedlings from untreated seeds from Ikenne (where the disease does not occur). Therefore 7.7% seedling scab in untreated Samaru infected seeds is attributed to seedborne infection. The 2.4% seedling scab in clean Ikenne seeds is likely to be attributed airborne spores blown from an older, adjacent cowpea field that was attacked by scab in 1993 (background contamination). The 2.4% scab incidence was suppressed when

clean Ikenne seed was treated with benomyl. The primary infection therefore was airborne. When infected Samaru seeds were treated with benomyl, the 10.1% seedling scab was reduced to 2.7%. Benomyl therefore suppresses seedborne plus airborne infection. Seed treatment with benomyl reduced scab incidence to 2.7% (for seedlings grown from seeds from Samaru) and 0.5% (for seedlings raised from seeds from Ikenne). Consequently these results are consistent with seed-borne transmission of cowpea scab. The 1994 data, which showed that seed treatment with benomyl reduced seedling scab incidence from 37.9% to 0.7%, support this conclusion, especially as there was little chance for background contamination in 1994. This suggestion is in consonance with the report of Sivanesan and Holliday, (1971) who detected *Elsinoe phaseoli* within the testa of *Phaseolus lunatus*.

The pathogen (*Sphaceloma* sp.) on cowpea debris was able to survive in the soil as indicated by mulched and plots without mulch yielding disease on test plants. Studies by Donli (1983) showed that *Sphaceloma* sp. over-seasoned on cowpea debris. The appearance of scab, at varying incidences, in the fields 25-27 days after planting, regardless of the cowpea cropping history of the field in both 1993 and 1994 is expected. This is because availability of inoculum (even if at varying levels), and the incubation period of the disease on the same plant parts under similar environmental conditions would be the same. The increase in scab incidence over time (DAS) in mulched and plots without mulch can be attributed to a gradual release of conidia from the debris which carried the initial inoculum. The higher disease incidence recorded in the plots with no mulch compared to mulched plots is due to rainsplashing making it easy for the inoculum to be released to infect cowpea in plots without mulch. The scab pathogen is capable of over-seasoning on debris of infected plants. Cowpea fields last planted with susceptible cowpea varieties a year before sowing of another crop of cowpea should yield more infected debris than a field where cowpea was last grown three years previously. Hence, at the onset of a new cowpea growing season, disease

incidence should be higher in fields with fresher cowpea debris which would act as a source of inoculum. The higher incidence of scab on fields last sown to cowpea a year earlier than that last planted with cowpea 3 years suggests that part of soil-borne inoculum is associated with infected trash as this would have decomposed after two rain-fed, cowpea-free seasons. The presence of inoculum in soil last planted to cowpea 3 years earlier indicates the existence of inoculum in a form not associated with infected crop residue. *Sphaceloma* sp. is known to produce chlamydospores under natural conditions on infected stems, peduncles, pods and in artificial culture (Emechebe, 1980, Emechebe and Shoyinka, 1985). Thus soil survival outside infected tissue probably occurs in the form of chlamydospores. Further studies need to be conducted to confirm or refute this hypothesis.

The pod stage of the early sown susceptible and moderately susceptible cowpea varieties generally had the highest disease incidence, apparent infection rate and consequently lowest yield compared to cowpea sown on later dates. This may be due to the fact that there was higher inoculum load and abundant host tissue available for infection by the pathogen. Young pods prior to grain filling were infected by the pathogen and became mummified. The mummification and high disease incidence on the pods led to little or no grain yield. Pods in the first planting date that escaped the early pod infection may have been infected with scab during grain filling stage resulting in high disease incidence. The overall appearance for plants sown at the first planting date was stuntedness with waxy leaves and sometimes abscission of the flowers. These corroborate with the results of Emechebe (1984) who reported that early sown cowpea had higher scab incidence and disease severity than later sown cowpea. The high and even distribution of rainfall in late July creates conducive environment (high relative humidity and relatively cool temperatures) for the development of primary lesions and subsequent spread of scab on cowpea plants. Studies on brown blotch of cowpea by Emechebe (1984) and Alabi (1994) showed that disease incidences

and apparent infection rates were highest in the early sown cowpea compared to later planting dates. Work carried out by CIAT (1976) with French beans showed high incidence of anthracnose (*C. lindemuthianum*) on early sown French beans and reduced yield compared to the later sown crop.

The grain yields of the early sown cowpea (July 16 1992 and July 24 1993) were lower in quantity and quality. The early sown cowpea had scab symptoms on their seed coat, thereby reducing the quality of the cowpea. However, the grain yields of the late sown cowpea were also of poor quality. This may be due to poor grain filling in the late sown cowpea resulting in lack of adequate photosynthates. The appearance of visible symptoms of scab on the seed coat of the early sown cowpea may be responsible for the seedborne transmission of the pathogen. The onset of the dry periods at the last sown crop probably renders the pathogen less infective. This may be an explanation why there was no increase in disease incidence in the late sown crop. Moisture and rainfall is therefore important for infection by the pathogen and spread of the disease. Rain probably facilitates the spread of conidia which induces scab symptoms on cowpea. *Sphaceloma* sp. of cowpea therefore falls under the characteristic group of splash-borne inoculum (Emechebe and Shoyinka, 1985). Splash-borne inoculum pathogens comprise those pathogens that are dispersed from the host in splash droplets because the mucilage surrounding their spores prevents dispersal by wind. Generally they are not present in the air except during the rain. Wind may also be involved in the dispersal of typically splash-borne pathogens. The fact that IT 83S -720-2, a variety resistant to scab had symptoms only on the pod stage of the earliest sown crop suggests the high susceptibility of cowpea pods to scab. Cowpea sown during the second planting date gave the highest grain yield. This may be due to the fact that there was limited host tissue available for infection at this time compared to the earliest sown crop which had produced abundant tissue for infection by the pathogen. In order to escape heavy yield loss due to scab and to obtain scab free seeds from cowpea varieties susceptible to

scab, early planting of susceptible varieties should be avoided.

Benomyl has been tested and recommended for the control of cowpea scab (Emechebe, 1981b). The effect of benomyl on scab development in resistant, moderately, and susceptible cowpea varieties was compared with that of carbendazim+maneb and mancozeb; the latter two are not currently being used for the control of scab. Foliar-applied benomyl sprays reduced the disease severity as well as area under the disease progress curve in all three varieties in 1992 and 1993. The peduncles had little scabbing while the flowering cushion and pod escaped the disease. In 1992 with IT 81D-1137 leaf scab for example, plots sprayed with benomyl had a disease severity of 4.5 and AUDPC value of 185.0 proportion days while untreated control had a disease severity of 9.0 and AUDPC value of 278 proportion days. The disease severity on the peduncle was 2.0 and AUDPC value was 30.4 while untreated control had a disease severity of 10.0 and AUDPC value of 169.0 proportion days. The pod escaped the disease with a severity of 1.0 and AUDPC value of 0.0 proportion days. The untreated control plants with pod had a disease severity of 10.0 and AUDPC value of 125.0 proportion days. This was followed by carbendazim+maneb which had no significant difference with benomyl for disease severity and AUDPC. This is probably due to the fact that carbendazim, one of the active ingredients of carbendazim+maneb, belongs to the same class of systemic fungicides - benzimidazole carbamates - as that present in benomyl which effectively controls scab. Scab severity and AUDPC values for plots sprayed with mancozeb were not significantly different from those of untreated control plots.

In 1993 the trends in the effect of the fungicides on scab development were almost the same as those observed in 1992. The work done by Bowen *et al.* (1994) also showed that foliar application of the fungicide, Tebuconazole, for the control of Entomosporium leaf spot of photinia resulted in the lowest disease severity and low AUDPC value as compared to the other fungicides and the unsprayed treatment. The little or no scab lesions on the peduncles, flowering

cushions, and pods of benomyl treated plants suggests that by the time these plant parts were produced, the disease levels in the weekly sprayed plots had been reduced to such low levels that there was little development of the disease on them, regardless of the susceptibility of the variety; it also implies effective protection of these organs, formed after commencement of the weekly sprays, by benomyl. The slow progress at the end of the season is probably due to the unavailability of fresh host tissue for infection. The stair-case pattern of the curves suggest a relatively long interval between disease assessments and alternation of favourable and unfavourable climatic conditions (especially rainfall and relative humidity) for disease development. Periods of dry weather conditions affect infection process of the pathogen on the host and subsequent inoculum production. Epidemiological study of diseases of other crops have also shown similar stair-case pattern. For example, Large (1945), working on late blight disease of potato, realised that outbreak of dry weather checks sporulation of the fungus resulting in a curve which is drawn out towards the end of the season. The highest disease scale of 10 obtained for IT 85F-1517 on all plant parts in 1992 is probably due to the well distributed rains in the period, July to September, 1992 compared to the same period in 1993.

## CONCLUSION

The apparent localization of colonization by *Sphaceloma* sp. of cowpea host tissue to the epidermal tissue has implication in surface sterilization of the infected tissue prior to isolation in artificial culture. This is because the infected tissue is likely to be colonised by many fast growing micro-organisms which usually out - grow *Sphaceloma* sp. in artificial culture However, the present results are tentative and further histopathological study is required to confirm the extent of host invasion by the pathogen.

A knowlege of the best infected host organ and best media for isolation of the pathogen will help in rapid and abundant production of inoculum for use in

screening varieties for their reaction to scab in laboratory/screenhouse studies.

The incubation period determined in the screenhouse for *Sphaceloma* sp. on the various plant organs varied with the plant age. The incubation period of *Sphaceloma* sp. in young leaves was 5 days. The incubation periods on other organs (stems, peduncles and pods) on plant aged 14-56 days varied from 7-10 days. For disease control purposes, however, a more useful parameter to measure would have been the latent period but this was not determined in the present study because of the difficulty in detecting acervuli on infected tissue. A method of determining the occurrence of sporulation by the fungus in nature should receive future research attention. The knowlege will assist in developing effective fungicidal spray schedules and in comparing mechanisms of resistance in cowpea cultivars.

*Sphaceloma* sp. grew best on PDAR although it grew well on CMAR and MAR. The fungus grew between 15–26°C but no growth occurred at 30° and higher temperatures. In 1994, the mean minimum and maximum air temperatures in Samaru during the growing season (July to early October) were 20° and 26°C respectively. These air temperatures are conducive for growth of the fungus in artificial culture; thus, this temperature range is also probably conducive for scab development under field conditions during a normal growing season.

An indirect method was used to determine if cowpea seed is a possible source of primary inoculum for the scab pathogen. Seed treatment with benomyl reduced incidence of seedling scab in seedlings grown from seeds from infected plants compared to untreated seeds from the same source. A lower incidence of seedling scab was obtained in seedlings grown from seeds from a scab-free zone compared to seedlings from scab-endemic zone. Planting seeds from a scab free zone or seeds from healthy pods from scab-endemic zone will reduce seedling scab incidence provided that contamination is avoided during threshing.

The soil-borne cowpea debris as a source of primary inoculum was

investigated using different fields with varying lengths of cropping history. Fields which were last planted with cowpea a year before the trial and which had no mulch had higher disease incidence than those that were last planted to cowpea 2 or 3 years before the trial. This is a strong indication that the pathogen is capable of surviving in the soil, probably on infected host debris. Hence, burning of such debris at the end of the cropping season would be a good control strategy. Fields which were last planted with cowpea three years before the trial still produced infected plants, although the incidence of scab was the lowest. From the results of this study it can be concluded that *Sphaceloma* sp. is capable of surviving in the soil after decomposition of cowpea residue. Hence a longer period of cowpea free period is needed for good control of scab by crop rotation.

The effects of three sowing dates on the incidence of infection, apparent infection rate and grain yield of three cowpea varieties were studied in 1992 and 1993. Generally, early sowing of cowpea resulted in higher incidence of infection and higher apparent infection rates of scab in both years. Late sowing resulted in low disease incidence and a low apparent infection rate. The grains of the earliest sown cowpea had scab symptoms, suggesting seed-borne transmission of the pathogen.

Benomyl, carbendazim + maneb and mancozeb were assessed for their effects on scab development in resistant, moderately resistant, and susceptible varieties of cowpea in 1992 and 1993. Different levels of scab development were obtained on leaves, stems, peduncles, flowering cushions and pods in the three varieties sprayed with the fungicides. Benomyl drastically lowered the disease severity on all plant parts and consequently reduced further scab development which was reflected in the lowest AUDPCs in comparisons to the other fungicides. Carbendazim + maneb was also effective in reducing disease severity on cowpea plants and producing low AUDPCs. Benomyl or carbendazim + maneb are therefore recommended as good fungicides for control of scab in cowpea. Mancozeb treated plots had the highest disease severities and the largest

AUDPCs. It appears that mancozeb had little effect on scab development; it should not be used as a protectant against *Sphaceloma* scab of cowpea.

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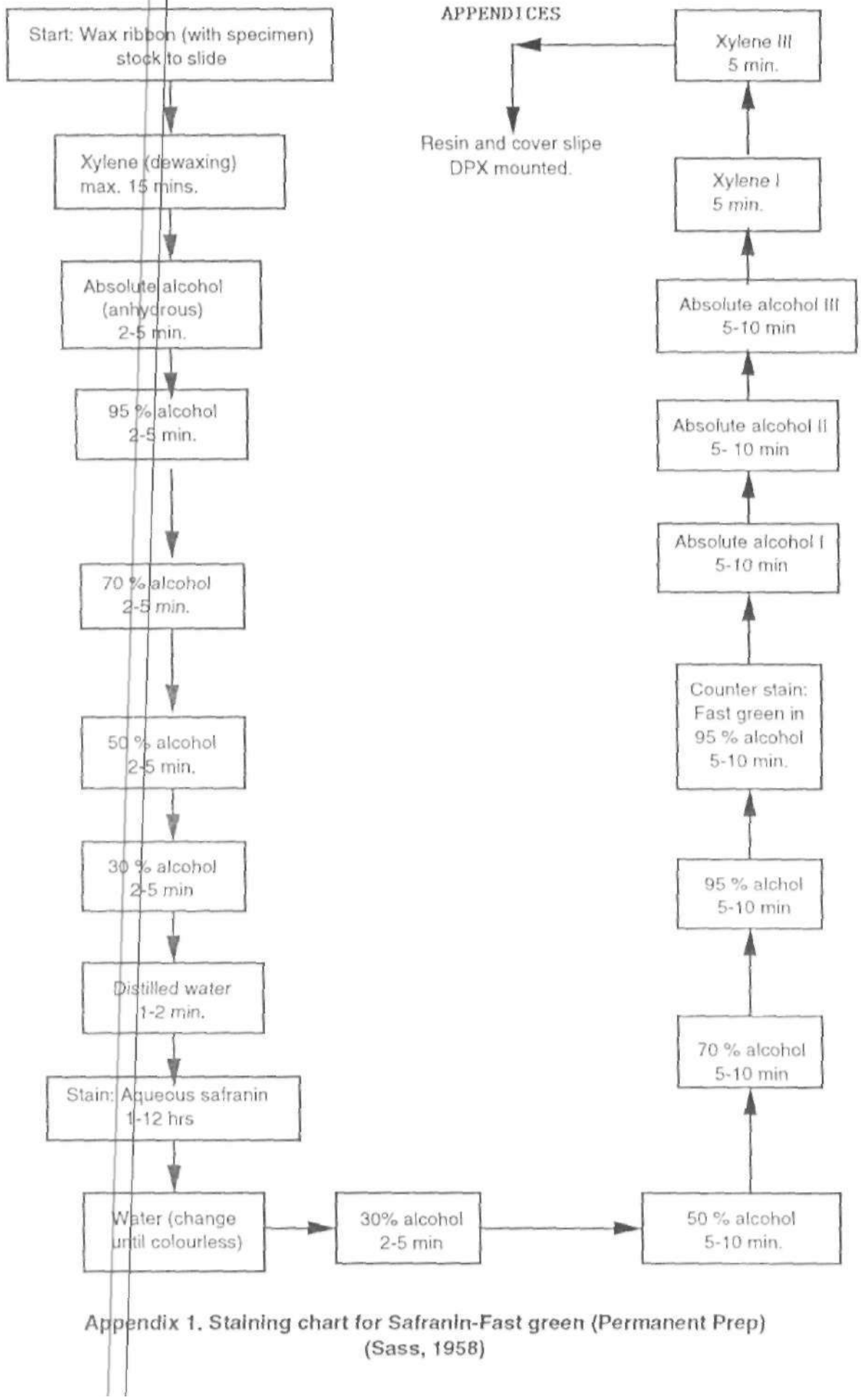
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**Appendix 1. Staining chart for Safranin-Fast green (Permanent Prep) (Sass, 1958)**

Appendix 2. Preparation of media for the isolation of *Sphaceloma* sp.

| Constituent | Quantity |
|---|---|
| Potato dextrose agar (PDA) | |
| 1 Commercial PDA | 39 grams |
| 2 Distilled water | 1 litre |
| Malt agar (MA) | |
| 1 Malt agar powder | 22 grams |
| 2 Distilled water | 1 litre |
| Cornmeal agar | |
| 1 Cornmeal agar powder | 17 grams |
| 2 Distilled water | 1 litre |
| Cowpea pod agar | |
| 1 Green cowpea pods | 250 grams |
| 2 Dextrose | 20 grams |
| 3 Agar | 15 grams |
| 4 Distilled water | 1 litre |
| Cowpea peduncle agar | |
| 1 Green cowpea peduncle | 300 grams |
| 2 Dextrose | 20 grams |
| 3 Agar | 15 grams |
| 4 Distilled water | 1 litre |
| Cowpea stem agar | |
| 1 Green cowpea stem | 300 grams |
| 2 Dextrose | 20 grams |
| 3 Agar | 15 grams |
| 4 Distilled water | 1 litre |
| Cowpea leaf agar | |
| 1 Young cowpea leaf | 300 grams |
| 2 Dextrose | 20 grams |
| 3 Agar | 15 grams |
| 4 Distilled water | 1 litre |

Appendix 3. Analysis of variance table showing the effect of 8 media on the isolation of *Sphaceloma* from different plant parts of cowpea in 1993.

| | | LEAF | | STEM | | PEDUNCLE | | POD | |
|---|---|---|---|---|---|---|---|---|---|
| Source | DF | SS | Pr > F | SS | Pr > F | SS | Pr > F | SS | Pr > F |
| REP | 5 | 1.2167 | 0.8391 | 1.1500 | 0.5787 | 4.1438 | 0.0688 | 5.1917 | 0.3375 |
| MEDIA | 7 | 87.4333 | 0.0001 | 44.8000 | 0.0001 | 74.1146 | 0.0001 | 268.9583 | 0.0001 |
| extr vs nonextr | 1 | 52.0083 | 0.0001 | 9.0750 | 0.0001 | 25.6688 | 0.0001 | 132.3000 | |
| st vs otherextr | 1 | 0.1681 | 0.5931 | 0.8681 | 0.0909 | 1.7014 | 0.0401 | 0.2347 | 0.6117 |
| ped vs pod | 1 | 1.6333 | 0.0962 | 1.6333 | 0.0206 | 1.4083 | 0.0618 | 1.6333 | 0.1809 |
| PDA vs extr | 1 | 0.0208 | 0.8508 | 6.3075 | 0.0001 | 3.9675 | 0.0018 | 2.7075 | 0.0852 |
| PDA vs media | 1 | 32.5125 | 0.0001 | 32.5125 | 0.0001 | 45.0000 | 0.0001 | 132.6125 | 0.0001 |
| Error | 467 | 274.5167 | | 141.2500 | | 187.5729 | | 424.8417 | |

Appendix 4. Analysis of variance table showing the effect of 8 media on the isolation of *sphaceloma* from different plant parts of cowpea in 1994.

| | | LEAF | | STEM | | PEDUNCLE | | POD | |
|---|---|---|---|---|---|---|---|---|---|
| Source | DF | SS | Pr > F | SS | Pr > F | SS | Pr > F | SS | Pr > F |
| REP | 5 | 5.8604 | 0.2012 | 1.5500 | 0.6402 | 0.9354 | 0.9215 | 2.3688 | 0.6261 |
| MEDIA | 7 | 121.6646 | 0.0001 | 54.1667 | 0.0001 | 73.9646 | 0.0001 | 128.9479 | 0.0001 |
| extr vs nonextr | 1 | 82.5021 | 0.0001 | 32.0333 | 0.0001 | 50.0521 | 0.0001 | 58.1021 | 0.0001 |
| st vs otherextr | 1 | 2.0056 | 0.1145 | 2.0056 | 0.0367 | 0.0125 | 0.8904 | 5.5125 | 0.0046 |
| ped vs pod | 1 | 9.0750 | 0.0008 | 2.7000 | 0.0155 | 1.8750 | 0.0919 | 22.5333 | 0.0001 |
| PDA vs extr | 1 | 2.8033 | 0.0622 | 2.8033 | 0.0136 | 1.0208 | 0.2134 | 0.0008 | 0.9721 |
| PDA vs media | 1 | 27.6125 | 0.0001 | 6.0500 | 0.0003 | 20.0000 | 0.0001 | 39.2000 | 0.0001 |
| Error | 467 | 374.6229 | | 213.4833 | | 307.0479 | | 317.5146 | |

Appendix 5. Estimates of contrasts of 8 media used in the isolation of *sphaceloma* sp. irrespective of plant parts in 1993 using Eigen vectors generated from principal component analysis.

| | 1993 | | | |
|---|---|---|---|---|
| | PRIN1 | | PRIN2 | |
| Parameter | Estimate | Pr > F | Estimate | Pr > F |
| extr vs nonextr | 6.1668 | 0.0001 | 0.7858 | 0.0180 |
| st vs otherextr | 0.2327 | 0.5799 | 0.6292 | 0.1214 |
| ped vs pod | 0.5699 | 0.0010 | 0.3580 | 0.0311 |
| PDA vs extr | -0.6350 | 0.2422 | 1.3606 | 0.0096 |
| PDA vs media | -6.8018 | 0.0001 | 0.5748 | 0.1570 |

Appendix 6. Estimates of contrasts of 8 media used in the isolation of *sphaceloma* sp. irrespective of plant parts in and 1994 using Eigen vectors generated from principal component analysis.

| | 1994 | | | |
|---|---|---|---|---|
| | PRIN1 | | PRIN2 | |
| Parameter | Estimate | Pr > F | Estimate | Pr > F |
| extr vs nonextr | 5.3375 | *0.0001* | 0.0796 | *0.8093* |
| st vs otherextr | 0.9189 | 0.0135 | -0.0918 | 0.8203 |
| ped vs pod | 1.0333 | 0.0001 | 0.1374 | 0.4050 |
| PDA vs extr | 1.0577 | 0.0275 | -0.3650 | 0.4842 |
| PDA vs media | -4.2798 | 0.0001 | -0.4446 | 0.2714 |

Appendix 7. Analysis of variance table showing the effect of 8 media on the isolation of *sphaceloma* sp. irrespective of plant parts in 1993 and 1994 using Eigen vectors generated from principal component analysis.

| | | 1993 | | | | 1994 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | PRIN1 | | PRIN2 | | PRIN1 | | PRIN2 | |
| Source | DF | SS | Pr > F | SS | Pr > F | SS | Pr > F | SS | Pr > F |
| REP | 5 | 6.9249 | 0.1671 | 4.8408 | 0.3190 | 4.1495 | 0.3035 | 3.9334 | 0.4388 |
| MEDIA | 7 | 529.1707 | 0.0001 | 12.1149 | 0.0417 | 345.5571 | 0.0001 | 8.4515 | 0.1718 |
| extr vs nonextr | 1 | 285.2218 | 0.0001 | 4.6314 | 0.0180 | 213.6632 | 0.0001 | 0.0475 | 0.8093 |
| st vs otherextr | 1 | 0.2707 | 0.5799 | 1.9795 | 0.1214 | 4.2218 | 0.0135 | 0.0421 | 0.8203 |
| ped vs pod | 1 | 9.7433 | 0.0010 | 3.8453 | 0.0311 | 32.0329 | 0.0001 | 0.5662 | 0.4050 |
| PDA vs extr | 1 | 1.2097 | 0.2422 | 5.5537 | 0.0096 | 3.3559 | 0.0275 | 0.3997 | 0.4842 |
| PDA vs media | 1 | 231.3237 | 0.0001 | 1.6518 | 0.1570 | 91.5832 | 0.0001 | 0.9883 | 0.2714 |
| Error | 467 | 412.0281 | | 383.9037 | | 320.4197 | | 380.7074 | |
| Corrected Total | 479 | 948.1238 | | 400.8595 | | 670.1263 | | 393.0923 | |

Appendix 8. Analysis of variance of the development of *Sphaceloma* sp. on cowpea after foliar spray of different plant parts with inoculum suspension at different plant ages.

| Plant part | Source | Incubation Period DF | SS | Pr > F |
|---|---|---|---|---|
| leaf | Rep | 6 | 0.79 | 0.7655 |
| | Plant ages | 3 | 4.42 | 0.0008 |
| | Error | 74 | 17.5 | |
| | Corrected Total | 83 | 22.7 | |
| Pod | Rep | 6 | 4.95 | 0.3824 |
| | Plant ages | 0 | 0 | . |
| | Error | 14 | 10 | |
| | Corrected Total | 20 | 14.95 | |
| Stem | Rep | 6 | 3.07 | 0.193 |
| | Plant ages | 3 | 132.51 | 0.0001 |
| | Error | 74 | 25.4 | |
| | Corrected Total | 83 | 160.99 | |
| Flowering cushion | Rep | 6 | 4.48 | 0.6055 |
| | Plant ages | 1 | 1.17 | 0.283 |
| | Error | 34 | 33.33 | |
| | Corrected Total | 41 | 38.98 | |
| Peduncle | Rep | 6 | 13.29 | 0.0587 |
| | Plant ages | 1 | 0 | 1 |
| | Error | 34 | 33 | |
| | Corrected Total | 41 | 46.29 | |

Appendix 9 Analysis of variance for leaf, stem, peduncle and pod by grinding lesions.

| | | Leaf | | Stem | | Peduncle | | Pod | |
|---|---|---|---|---|---|---|---|---|---|
| *Source* | DF | SS | Pr > F | SS | Pr > F | SS | Pr > F | SS | Pr > F |
| REP | 2 | 0.0262 | 0.6045 | 0.0003 | 0.9922 | 0.0349 | 0.6385 | 0.2002 | 0.0076 |
| MEDIA | 5 | 0.0664 | 0.7445 | 0.0330 | 0.9014 | 0.0931 | 0.7693 | 0.0384 | 0.6789 |
| Error | 10 | 0.2472 | | 0.2192 | | 0.3716 | | 0.1210 | |
| Corrected Total | 17 | 0.3397 | | 0.2525 | | 0.4996 | | 0.3596 | |

Appendix 10 . General linear model procedure for the effects of five temperatures and three media on the weight of *Sphaceloma* sp. assessed at five different time intervals

| Source | DF | Day 3 SS | Day 3 Pr > F | Day 6 SS | Day 6 Pr > F | Day 9 SS | Day 9 Pr > F | Day 12 SS | Day 12 Pr > F | Day 15 SS | Day 15 Pr > F |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Rep | 5 | 0.0000 | 0.7748 | 0.0001 | 0.6447 | 0.0001 | 0.6150 | 0.0016 | 0.5233 | 0.0015 | 0.4857 |
| Temp | 4 | 0.0078 | 0.0001 | 0.1225 | 0.0001 | 1.2164 | 0.0001 | 4.8230 | 0.0001 | 7.7803 | 0.0001 |
| Rep*temp | 20 | 0.0001 | 0.9934 | 0.0004 | 0.9661 | 0.0011 | 0.1154 | 0.0058 | 0.7560 | 0.0144 | 0.0176 |
| Media | 2 | 0.0001 | 0.0013 | 0.0237 | 0.0001 | 0.0985 | 0.0001 | 0.2361 | 0.0001 | 0.5988 | 0.0001 |
| Temp*Media | 8 | 0.0002 | 0.0011 | 0.0597 | 0.0001 | 0.1487 | 0.0001 | 0.3834 | 0.0001 | 0.5423 | 0.0001 |
| Error | 50 | 0.0004 | | 0.0019 | | 0.0018 | | 0.0192 | | 0.0171 | |
| Corrected total | 89 | 0.0086 | | 0.2083 | | 1.4666 | | 5.4692 | | 8.9544 | |
| CV | | 16.6031 | | 16.9639 | | 5.8539 | | 10.3530 | | 6.7330 | |
| R-Square | | 0.95624 | | 0.99082 | | 0.9988 | | 0.99649 | | 0.9981 | |

Appendix 11 . Repeated measures analysis of variance- Univariate tests of hypotheses for within time effects

| Source | DF | Type III SS | Mean Square | F Value |
|---|---|---|---|---|
| Time | 4 | 4.20276311 | 1.05069078 | 6475.97 |
| Time*Rep | 20 | 0.00303422 | 0.00015171 | 0.94 |
| Time*Temp | 16 | 5.61081244 | 0.35067578 | 2161.40 |
| Time*Rep*Temp | 80 | 0.01796356 | 0.00022454 | 1.38 |
| Time*Media | 8 | 0.36821289 | 0.04602661 | 283.69 |
| Time*Temp*Media | 32 | 0.43428489 | 0.01357140 | 83.65 |
| Error(Time) | 200 | 0.03244889 | 0.00016224 | |

Appendix 13 . Analysis of variance of rate of growth of *Sphaceloma* sp. on 3 media at 5 different temperatures

| Source | DF | Sum of Squares | F Value | Pr > F |
|---|---|---|---|---|
| Rep | 5 | 0.00057000 | 1.63 | 0.1681 |
| Temp | 4 | 0.08790193 | 179.51 | 0.0001 |
| Rep*Temp | 20 | 0.00244833 | 1.76 | 0.0549 |
| Media | 2 | 0.01470887 | 105.47 | 0.0001 |
| Temp*Media | 8 | 0.02791380 | 50.04 | 0.0001 |
| Error | 50 | 0.00348667 | | |
| Corrected Total | 89 | 0.13702960 | | |

Appendix 15. Analysis of variance of seed as a possible source of primary inoculum of *Sphaceloma* sp. in 1994

| Source | DF | SS | MS | F Value | Pr > F |
|---|---|---|---|---|---|
| Rep | 9 | 164.3625 | 18.2625 | 1.31 | 0.3474 |
| Treatment | 1 | 6937.8125 | 6937.8125 | 497.28 | 0.0001 |
| Error | 9 | 125.5625 | 13.951389 | | |
| Corrected Total | 19 | 7227.7375 | | | |

Appendix 16. Analysis of variance table showing the effect of soil as a probable source of primary inoculum for cowpea scab using polythene mulch as a control for secondary spread by soil splash in 1993.

| Source | DF | incidence * (%) SS | Pr > F | incidence on epicotyl SS | Pr > F | severity on epicotyl SS | Pr > F | incidence on hypocotyl SS | Pr > F | severity on hypocotyl SS | Pr > F | incidence on both hypcot & epicot SS | Pr > F | severity on both hypcot & epicot SS | Pr > F |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Rated August 26 | | | | | | | | | | | | | | | |
| Rep | 5 | 0.0016 | 0.6638 | 0.0013 | 0.7939 | 12.4838 | 0.9409 | 0.0007 | 0.5727 | 13.8038 | 0.5747 | 0.0009 | 0.5299 | 22.1875 | 0.4001 |
| Location | 2 | 0.0088 | 0.0046 | 0.0082 | 0.0084 | 80.7005 | 0.0541 | 0.0012 | 0.0772 | 32.3458 | 0.0320 | 0.0008 | 0.1831 | 22.4500 | 0.0980 |
| Location*Rep | 10 | 0.0059 | 0.3854 | 0.0043 | 0.6550 | 74.0727 | 0.7180 | 0.0017 | 0.5162 | 51.0181 | 0.2619 | 0.0018 | 0.5852 | 71.4167 | 0.1653 |
| Treatment | 1 | 0.0013 | 0.1354 | 0.0005 | 0.3555 | 0.4219 | 0.8462 | 0.0003 | 0.2347 | 5.0052 | 0.2536 | 0.0002 | 0.3731 | 3.0000 | 0.4011 |
| Location*Treatment | 2 | 0.0020 | 0.1832 | 0.0009 | 0.4732 | 1.0125 | 0.9541 | 0.0020 | 0.0209 | 37.9458 | 0.0207 | 0.0015 | 0.0547 | 23.9167 | 0.0866 |
| Error | 12 | 0.0060 | | 0.0067 | | 128.9275 | | 0.0022 | | 41.7708 | | 0.0025 | | 47.5000 | |
| Corrected total | | 0.0256 | | 0.0219 | | 297.6189 | | 0.0080 | | 181.8896 | | 0.0076 | | 190.4708 | |
| Rated Sept. 4 | | | | | | | | | | | | | | | |
| Rep | 5 | 0.0150 | 0.1642 | 0.0129 | 0.1147 | 5.9913 | 0.7036 | 0.0049 | 0.0624 | 12.9528 | 0.4216 | 0.0040 | 0.0382 | 6.0278 | 0.7387 |
| Location | 2 | 0.1337 | 0.0001 | 0.0909 | 0.0001 | 188.2625 | 0.0001 | 0.0344 | 0.0001 | 93.0320 | 0.0002 | 0.0349 | 0.0001 | 161.6080 | 0.0001 |
| Location*Rep | 10 | 0.0199 | 0.3410 | 0.0192 | 0.1963 | 18.2847 | 0.5529 | 0.0038 | 0.4344 | 48.8522 | 0.1232 | 0.0051 | 0.1003 | 16.3122 | 0.6799 |
| Treatment | 1 | 0.0105 | 0.0235 | 0.0070 | 0.0290 | 3.2552 | 0.2271 | 0.0052 | 0.0021 | 9.3633 | 0.0723 | 0.0081 | 0.0001 | 8.0033 | 0.0812 |
| Location*Treatment | 2 | 0.0213 | 0.0105 | 0.0149 | 0.0122 | 6.8125 | 0.2246 | 0.0041 | 0.0155 | 13.6853 | 0.0979 | 0.0047 | 0.0027 | 5.8880 | 0.3000 |
| Error | 12 | 0.0187 | | 0.0137 | | 24.1042 | | 0.0041 | | 28.9333 | | 0.0028 | | 26.5000 | |
| Corrected total | | 0.2190 | | 0.1586 | | 246.7104 | | 0.0566 | | 206.8190 | | 0.0595 | | 224.3393 | |
| Rated Sept. 11 | | | | | | | | | | | | | | | |
| Rep | 5 | 0.0596 | 0.1927 | 0.0652 | 0.1130 | 27.3286 | 0.2653 | 0.0106 | 0.5312 | 18.9028 | 0.3963 | 0.0110 | 0.5170 | 18.4911 | 0.6892 |
| Location | 2 | 0.3703 | 0.0001 | 0.2742 | 0.0001 | 73.4047 | 0.0028 | 0.0950 | 0.0002 | 23.2000 | 0.0649 | 0.1016 | 0.0001 | 45.7680 | 0.0520 |
| Location*Rep | 10 | 0.0796 | 0.3855 | 0.0762 | 0.3157 | 45.2602 | 0.3617 | 0.0408 | 0.1996 | 50.0889 | 0.2511 | 0.0439 | 0.1697 | 34.4609 | 0.8053 |
| Treatment | 1 | 0.0188 | 0.1202 | 0.0158 | 0.1222 | 2.9008 | 0.3920 | 0.0015 | 0.4442 | 3.5208 | 0.3253 | 0.0008 | 0.5852 | 0.0300 | 0.9447 |
| Location*Treatment | 2 | 0.0614 | 0.0335 | 0.0504 | 0.0370 | 7.3620 | 0.3964 | 0.0299 | 0.0149 | 6.4500 | 0.4092 | 0.0223 | 0.0341 | 2.2587 | 0.8305 |
| Error | 12 | 0.0807 | | 0.0688 | | 44.1467 | | 0.0294 | | 40.1667 | | 0.0295 | | 71.8500 | |
| Corrected total | | 0.6704 | | 0.5505 | | 200.4030 | | 0.2074 | | 142.3292 | | 0.2090 | | 172.8587 | |

*Data for incidence transformed by arcsine

Appendix 17. Analysis of variance table showing the effect of soil as a probable source of primary inoculum for cowpea scab using polythene mulch as a control for secondary spread by soil splash in 1994.

| Source | DF | Incidence* (%) SS | Pr > F | Incidence on epicotyl SS | Pr > F | severity on epicotyl SS | Pr > F | incidence on hypocotyl SS | Pr > F | severity on hypocotyl SS | Pr > F | Incidence on Hypo-Epicoty SS | Pr > F | severity on Hypo-Epicoty SS | Pr > F |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Rated August 13 | | | | | | | | | | | | | | | |
| REP | 5 | 0.0000002 | 0.1095 | 0.0000002 | 0.0691 | 17.8889 | 0.7443 | 0.0000001 | 0.1727 | 9.8681 | 0.8091 | 0.0000001 | 0.1727 | 9.8681 | 0.8091 |
| LOC | 2 | 0.0000003 | 0.0056 | 0.0000003 | 0.0022 | 27.5556 | 0.1603 | 0.0000003 | 0.2857 | 16.6806 | 0.1857 | 0.0000002 | 0.2857 | 16.6806 | 0.1857 |
| LOC*REP | 10 | 0.0000005 | 0.0606 | 0.0000004 | 0.0467 | 105.7778 | 0.2013 | 0.0000003 | 0.0281 | 49.0694 | 0.4139 | 0.0000003 | 0.0281 | 49.0694 | 0.4139 |
| TRT | 1 | 0.0000001 | 0.0150 | 0.0000002 | 0.0041 | 2.7778 | 0.5277 | 0.0000001 | 0.3231 | 3.0625 | 0.4181 | 0.0000001 | 0.3231 | 3.0625 | 0.4181 |
| LOC*TRT | 2 | 0.0000006 | 0.0003 | 0.0000005 | 0.0002 | 43.5556 | 0.0659 | 0.0000001 | 0.1046 | 21.7917 | 0.1186 | 0.0000006 | 0.1046 | 21.7917 | 0.1186 |
| Error | 15 | 0.0000003 | | 0.0000002 | | 99.6667 | | 0.0000002 | | 66.2708 | | 0.0000002 | | 66.2708 | |
| Corrected Total | 35 | 0.0000019 | | 0.0000017 | | 297.2222 | | 0.0000007 | | 166.7431 | | 0.0000007 | | 166.7431 | |
| Rated August 20 | | | | | | | | | | | | | | | |
| REP | 5 | 0.0000125 | 0.0636 | 0.0000126 | 0.0509 | 36.0000 | 0.0533 | 0.0000060 | 0.1271 | 33.8889 | 0.3772 | 0.0000060 | 0.1182 | 48.0000 | 0.2759 |
| LOC | 2 | 0.0000191 | 0.0016 | 0.0000191 | 0.0012 | 26.0000 | 0.0200 | 0.0000085 | 0.0060 | 46.8889 | 0.0410 | 0.0000084 | 0.0059 | 42.0000 | 0.0753 |
| LOC*REP | 10 | 0.0000127 | 0.2863 | 0.0000114 | 0.3148 | 34.0000 | 0.2938 | 0.0000070 | 0.3540 | 61.1111 | 0.4594 | 0.0000071 | 0.3370 | 54.0000 | 0.6364 |
| TRT | 1 | 0.0000126 | 0.0022 | 0.0000118 | 0.0023 | 11.1111 | 0.0536 | 0.0000033 | 0.0309 | 0.1111 | 0.8926 | 0.0000033 | 0.0287 | 0.0000 | 1.0000 |
| LOC*TRT | 2 | 0.0000202 | 0.0012 | 0.0000214 | 0.0007 | 10.8889 | 0.1511 | 0.0000127 | 0.0011 | 29.5556 | 0.1148 | 0.0000129 | 0.0010 | 42.0000 | 0.0753 |
| Error | 15 | 0.0000140 | | 0.0000131 | | 38.0000 | | 0.0000087 | | 88.3333 | | 0.0000085 | | 102.0000 | |
| Corrected Total | 35 | 0.0000909 | | 0.0000895 | | 156.0000 | | 0.0000461 | | 259.8889 | | 0.0000462 | | 288.0000 | |
| Rated August 27 | | | | | | | | | | | | | | | |
| REP | 5 | 0.0000061 | 0.6841 | 0.0000038 | 0.7862 | 0.0000 | 0.0000 | 0.0000055 | 0.2947 | 0.0000 | 0.0000 | 0.0000055 | 0.2947 | 1.6789 | 0.6946 |
| LOC | 2 | 0.0000655 | 0.0001 | 0.0000541 | 0.0001 | 0.0000 | 0.0000 | 0.0000342 | 0.0001 | 0.0000 | 0.0000 | 0.0000342 | 0.0001 | 1.0006 | 0.2170 |
| LOC*REP | 10 | 0.0000454 | 0.0673 | 0.0000281 | 0.1573 | 0.0000 | 0.0000 | 0.0000226 | 0.0361 | 0.0000 | 0.0000 | 0.0000226 | 0.0361 | 2.7994 | 0.8593 |
| TRT | 1 | 0.0000346 | 0.0007 | 0.0000249 | 0.0013 | 0.0000 | 0.0000 | 0.0000090 | 0.0046 | 0.0000 | 0.0000 | 0.0000090 | 0.0046 | 0.0011 | 0.9648 |
| LOC*TRT | 2 | 0.0000453 | 0.0009 | 0.0000437 | 0.0004 | 0.0000 | 0.0000 | 0.0000194 | 0.0008 | 0.0000 | 0.0000 | 0.0000194 | 0.0008 | 0.4272 | 0.6854 |
| Error | 15 | 0.0000292 | | 0.0000240 | | 0.0000 | | 0.0000122 | | 0.0000 | | 0.0000122 | | 8.2717 | |
| Corrected Total | 35 | 0.0002260 | | 0.0001785 | | 0.0000 | | 0.0001029 | | 0.0000 | | 0.0001029 | | 14.1789 | |

*Data for incidence transformed by arcsine

Appendix 18. Analysis of variance of scab incidence at 42 days after sowing (DAS) on leaf and stem of IT 81D-1137 obtained from three planting dates experiment in 1992.

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 3 | 317.51666667 | 105.83888889 | 2.39 | 0.0818 |
| INTER2 | 14 | 44931.23333333 | 3209.37380952 | 72.62 | 0.0001 |
| Error | 42 | 1856.23333333 | 44.19603175 | | |
| Corrected Total | 59 | 47104.98333333 | | | |

Appendix 19. Analysis of variance of scab incidence at 42 days after sowing (DAS) on leaf and stem of IT 85F-1517 obtained from three planting dates experiment in 1992.

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 3 | 74.58333333 | 24.86111111 | 0.60 | 0.6204 |
| INTER2 | 14 | 67034.40000000 | 4788.17142857 | 115.00 | 0.0001 |
| Error | 42 | 1748.66666667 | 41.63492063 | | |
| Corrected Total | 59 | 68857.65000000 | | | |

Appendix 20. Analysis of variance of scab incidence at 42 days after sowing (DAS) on leaf and stem of IT 88S-720-2 obtained from three planting dates experiment in 1992.

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 3 | 0 | 0 | 99999.99 | 0.0 |
| INTER2 | 14 | 0 | 0 | 99999.99 | 0.0 |
| Error | 42 | 0 | 0 | | |
| Corrected Total | 59 | 0 | | | |

Appendix 21. Analysis of variance of scab incidence at 48 days after sowing (DAS) on leaf and stem of IT 81D-1137 obtained from three planting dates experiment in 1992.

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 3 | 104.60000000 | 34.86666667 | 0.78 | 0.5094 |
| INTER2 | 14 | 66634.43333333 | 4759.60238095 | 107.08 | 0.0001 |
| Error | 42 | 1866.90000000 | 44.45000000 | | |
| Corrected Total | 59 | 68605.93333333 | | | |

Appendix 22. Analysis of variance of scab incidence at 48 days after sowing (DAS) on leaf and stem of IT 85F-1517 obtained from three planting dates experiment in 1992.

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 3 | 19.78333333 | 6.59444444 | 0.25 | 0.8584 |
| INTER2 | 14 | 66763.93333333 | 4768.85238095 | 183.34 | 0.0001 |
| Error | 42 | 1092.46666667 | 26.01111111 | | |
| Corrected Total | 59 | 67876.18333333 | | | |

Appendix 23. Analysis of variance of scab incidence at 48 days after sowing (DAS) on leaf and stem of IT 83S-720-2 obtained from three planting dates experiment in 1992.

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 3 | 4.98333333 | 1.66111111 | 1.00 | 0.4023 |
| INTER2 | 14 | 52.50000000 | 3.75000000 | 2.26 | 0.0212 |
| Error | 42 | 69.76666667 | 1.66111111 | | |
| Corrected Total | 59 | 127.25000000 | | | |

Appendix 24. Analysis of variance of scab incidence at 54 days after sowing (DAS) on peduncle and flowering cushion of IT 81D-1137 obtained from three planting dates experiment in 1992.

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 3 | 48.18333333 | 16.06111111 | 0.42 | 0.7407 |
| INTER2 | 14 | 82909.93333333 | 5922.13809524 | 154.29 | 0.0001 |
| Error | 42 | 1612.06666667 | 38.38253968 | | |
| Corrected Total | 59 | 84570.18333333 | | | |

Appendix 25. Analysis of variance of scab incidence at 54 days after sowing (DAS) on peduncle and flowering cushion of IT 85F-1517 obtained from three planting dates experiment in 1992.

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 3 | 9.60088 | 32.0029 | 0.65 | 0.5877 |
| INTER2 | 14 | 16969.28582 | 12.1209184 | 246.00 | 0.0001 |
| Error | 42 | 206.94357 | 49.2723 | | |
| Corrected Total | 59 | 17185.83028 | | | |

Appendix 26. Analysis of variance of scab incidence at 54 days after sowing (DAS) on peduncle and flowering cushion of IT 83S-720-2 obtained from three planting dates experiment in 1992.

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|

| | | | | | |
|---|---|---|---|---|---|
| REP | 3 | 6.85000000 | 2.28333333 | 1.00 | 0.4023 |
| INTER2 | 14 | 170.10000000 | 12.15000000 | 5.32 | 0.0001 |
| Error | 42 | 95.90000000 | 2.28333333 | | |
| Corrected Total | 59 | 272.85000000 | | | |

Appendix 27. Analysis of variance of scab incidence at 60 days after sowing (DAS) on peduncle and flowering cushion of IT 81D-1137 obtained from three planting dates experiment in 1992.

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 3 | 33.65000000 | 11.21666667 | 0.27 | 0.8492 |
| INTER2 | 14 | 67507.50000000 | 4821.96428571 | 114.54 | 0.0001 |
| Error | 42 | 1768.10000000 | 42.09761905 | | |
| Corrected Total | 59 | 69309.25000000 | | | |

Appendix 28. Analysis of variance of scab incidence at 60 days after sowing (DAS) on peduncle and flowering cushion of IT 85F-1517 obtained from three planting dates experiment in 1992.

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 3 | 121.65000000 | 40.55000000 | 2.28 | 0.0931 |
| INTER2 | 14 | 62912.73333333 | 4493.76666667 | 252.80 | 0.0001 |
| Error | 42 | 746.60000000 | 17.77619048 | | |
| Corrected Total | 59 | 63780.98333333 | | | |

Appendix 29. Analysis of variance of scab incidence at 60 days after sowing (DAS) on peduncle and flowering cushion of IT 83S-720-2 obtained from three planting dates experiment in 1992.

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 3 | 6.85000000 | 2.28333333 | 1.00 | 0.4023 |
| INTER2 | 14 | 170.10000000 | 12.15000000 | 5.32 | 0.0001 |
| Error | 42 | 95.90000000 | 2.28333333 | | |
| Corrected Total | 59 | 272.85000000 | | | |

Appendix 30. Analysis of variance of scab incidence at 73 days after sowing (DAS) on pod of IT 81D-1137 obtained from three planting dates experiment in 1992.

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 3 | 75.91666667 | 25.30555556 | 0.52 | 0.6735 |
| INTER2 | 14 | 63551.23333333 | 4539.37380952 | 92.56 | 0.0001 |
| Error | 42 | 2059.83333333 | 49.04365079 | | |
| Corrected Total | 59 | 65686.98333333 | | | |

Appendix 31. Analysis of variance of scab incidence at 73 days after sowing (DAS) on pod of IT 85F-1517 obtained from three planting dates experiment in 1992.

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 3 | 106.18333333 | 35.39444444 | 2.09 | 0.1155 |
| INTER2 | 14 | 65130.33333333 | 4652.16666667 | 275.17 | 0.0001 |
| Error | 42 | 710.06666667 | 16.90634921 | | |
| Corrected Total | 59 | 65946.58333333 | | | |

Appendix 32. Analysis of variance of scab incidence at 73 days after sowing (DAS) on pod of IT 83S-720-2 obtained from three planting dates experiment in 1992.

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 3 | 6.85000000 | 2.28333333 | 1.00 | 0.4023 |
| INTER2 | 14 | 170.10000000 | 12.15000000 | 5.32 | 0.0001 |
| Error | 42 | 95.90000000 | 2.28333333 | | |
| Corrected Total | 59 | 272.85000000 | | | |

Appendix 33. Analysis of variance of scab incidence at 42 days after sowing (DAS) on leaf and stem of IT 81D-1137 obtained from three planting dates experiment in 1993.

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 3 | 9.933333 | 3.311111 | 0.48 | 0.6989 |
| INTER2 | 14 | 10074.233333 | 719.588095 | 104.01 | 0.0001 |
| Error | 42 | 290.566667 | 6.918254 | | |
| Corrected Total | 59 | 10374.733333 | | | |

Appendix 34. Analysis of variance of scab incidence at 42 days after sowing (DAS) on leaf and stem of IT 85F-1517 obtained from three planting dates experiment in 1993.

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 3 | 67.250000 | 22.416667 | 1.38 | 0.2628 |
| INTER2 | 14 | 25315.833333 | 1808.273810 | 111.12 | 0.0001 |
| Error | 42 | 683.500000 | 16.273810 | | |
| Corrected Total | 59 | 26066.583333 | | | |

Appendix 35. Analysis of variance of scab incidence at 42 days after sowing (DAS) on leaf and stem of IT 83S-720-2 obtained from three planting dates experiment in 1993.

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 3 | 0 | 0 | 99999.99 | 0.0 |
| INTER2 | 14 | 0 | 0 | 99999.99 | 0.0 |
| Error | 42 | 0 | 0 | | |
| Corrected Total | 59 | 0 | | | |

Appendix 36. Analysis of variance of scab incidence at 51 days after sowing (DAS) on leaf and stem of IT 81D-1137 obtained from three planting dates experiment in 1993.

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 3 | 42.316667 | 14.105556 | 1.48 | 0.2349 |
| INTER2 | 14 | 31380.833333 | 2241.488095 | 234.52 | 0.0001 |
| Error | 42 | 401.433333 | 9.557937 | | |
| Corrected Total | 59 | 31824.583333 | | | |

Appendix 37. Analysis of variance of scab incidence at 51 days after sowing (DAS) on leaf and stem of IT 85F-1517 obtained from three planting dates experiment in 1993.

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 3 | 183.250000 | 61.083333 | 2.19 | 0.1034 |
| INTER2 | 14 | 43102.900000 | 3078.778571 | 110.38 | 0.0001 |
| Error | 42 | 1171.500000 | 27.892857 | | |
| Corrected Total | 59 | 44457.650000 | | | |

Appendix 38. Analysis of variance of scab incidence at 51 days after sowing (DAS) on leaf and stem of IT 83S-720-2 obtained from three planting dates experiment in 1993.

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 3 | 0 | 0 | 99999.99 | 0.0 |
| INTER2 | 14 | 0 | 0 | 99999.99 | 0.0 |
| Error | 42 | 0 | 0 | | |
| Corrected Total | 59 | 0 | | | |

Appendix 39. Analysis of variance of scab incidence at 57 days after sowing (DAS) on peduncle and flowering cushion of IT 81D-1137 obtained from three planting dates experiment in 1993.

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 3 | 39.333333 | 13.111111 | 0.83 | 0.4827 |
| INTER2 | 14 | 38262.500000 | 2733.035714 | 173.88 | 0.0001 |
| Error | 42 | 660.166667 | 15.718254 | | |
| Corrected Total | 59 | 38962.000000 | | | |

Appendix 40. Analysis of variance of scab incidence at 57 days after sowing (DAS) on peduncle and flowering cushion of IT 81D-1137 obtained from three planting dates experiment in 1993.

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 3 | 150.000000 | 50.000000 | 1.64 | 0.1950 |
| INTER2 | 14 | 48905.733333 | 3493.266667 | 114.44 | 0.0001 |
| Error | 42 | 1282.000000 | 30.523810 | | |
| Corrected Total | 59 | 50337.733333 | | | |

Appendix 41. Analysis of variance of scab incidence at 57 days after sowing (DAS) on peduncle and flowering cushion of IT 83S-720-2 obtained from three planting dates experiment in 1993.

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 3 | 0.18333333 | 0.06111111 | 1.00 | 0.4023 |
| INTER2 | 14 | 123.43333333 | 8.81666667 | 144.27 | 0.0001 |
| Error | 42 | 2.56666667 | 0.06111111 | | |
| Corrected Total | 59 | 126.18333333 | | | |

Appendix 42. Analysis of variance of scab incidence at 66 days after sowing (DAS) on peduncle and flowering cushion of IT 81D-1137 obtained from three planting dates experiment in 1993.

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 3 | 138.733333 | 46.244444 | 2.10 | 0.1152 |
| INTER2 | 14 | 44508.433333 | 3179.173810 | 144.08 | 0.0001 |
| Error | 42 | 926.766667 | 22.065873 | | |
| Corrected Total | 59 | 45573.933333 | | | |

Appendix 43. Analysis of variance of scab incidence at 66 days after sowing (DAS) on peduncle and flowering cushion of IT 85F-1517 obtained from three planting dates experiment in 1993.

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 3 | 227.783333 | 75.927778 | 2.16 | 0.1068 |
| INTER2 | 14 | 44472.400000 | 3176.600000 | 90.42 | 0.0001 |
| Error | 42 | 1475.466667 | 35.130159 | | |
| Corrected Total | 59 | 46175.650000 | | | |

Appendix 44. Analysis of variance of scab incidence at 66 days after sowing (DAS) on peduncle and flowering cushion of IT 83S-720-2 obtained from three planting dates experiment in 1993.

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 3 | 0.18333333 | 0.06111111 | 1.00 | 0.4023 |
| INTER2 | 14 | 123.43333333 | 8.81666667 | 144.27 | 0.0001 |
| Error | 42 | 2.56666667 | 0.06111111 | | |
| Corrected Total | 59 | 126.18333333 | | | |

Appendix 45. Analysis of variance of scab incidence at 60 days after sowing (DAS) on pod of IT 81D-1137 obtained from three planting dates experiment in 1993.

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 3 | 138.733333 | 46.244444 | 2.10 | 0.1152 |
| INTER2 | 14 | 44508.433333 | 3179.173810 | 144.08 | 0.0001 |
| Error | 42 | 926.766667 | 22.065873 | | |
| Corrected Total | 59 | 45573.933333 | | | |

Appendix 46. Analysis of variance of scab incidence at 60 days after sowing (DAS) on pod of

IT 85F-1517 obtained from three planting dates experiment in 1993.

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 3 | 227.783333 | 75.927778 | 2.16 | 0.1068 |
| INTER2 | 14 | 44472.400000 | 3176.600000 | 90.42 | 0.0001 |
| Error | 42 | 1475.466667 | 35.130159 | | |
| Corrected Total | 59 | 46175.650000 | | | |

Appendix 47. Analysis of variance of scab incidence at 60 days after sowing (DAS) on pod of IT 83S-720-2 obtained from three planting dates experiment in 1993.

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 3 | 0.18333333 | 0.06111111 | 1.00 | 0.4023 |
| INTER2 | 14 | 123.43333333 | 8.81666667 | 144.27 | 0.0001 |
| Error | 42 | 2.56666667 | 0.06111111 | | |
| Corrected Total | 59 | 126.18333333 | | | |

Appendix 48. Analysis of variance of scab incidence at 69 days after sowing (DAS) on pod of IT 81D-1137 obtained from three planting dates experiment in 1993.

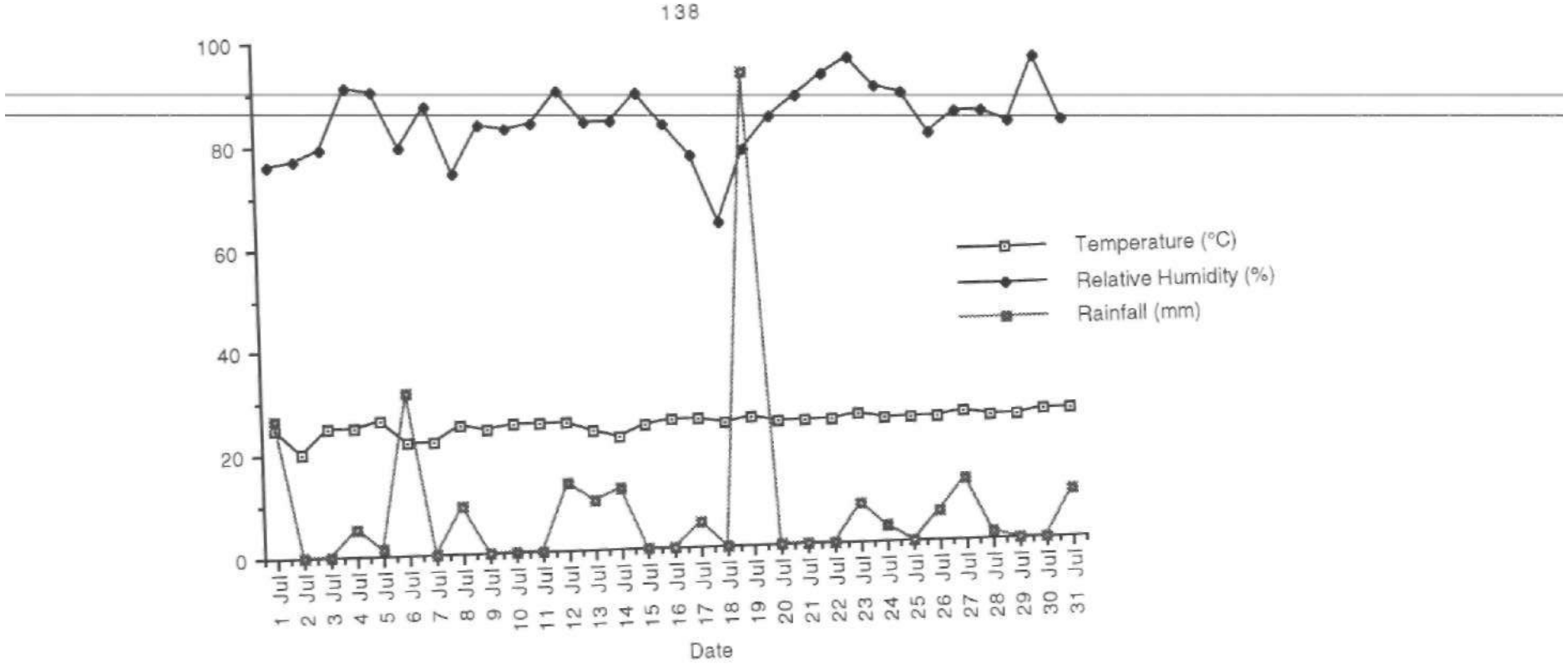
| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 3 | 138.733333 | 46.244444 | 2.10 | 0.1152 |
| INTER2 | 14 | 44508.433333 | 3179.173810 | 144.08 | 0.0001 |
| Error | 42 | 926.766667 | 22.065873 | | |
| Corrected Total | 59 | 45573.933333 | | | |

Appendix 49 Analysis of variance of scab incidence at 69 days after sowing (DAS) on pod of IT 85F-1517 obtained from three planting dates experiment in 1993.

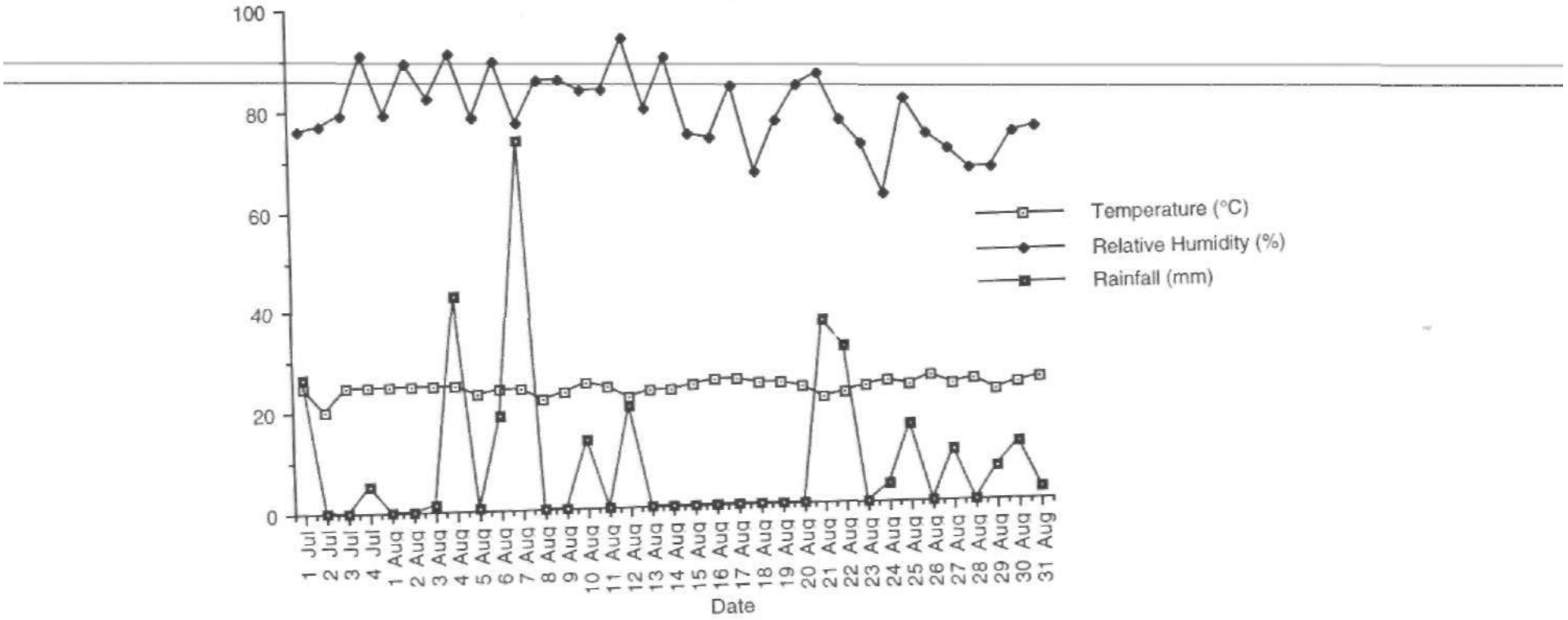
| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 3 | 227.783333 | 75.927778 | 2.16 | 0.1068 |
| INTER2 | 14 | 44472.400000 | 3176.600000 | 90.42 | 0.0001 |
| Error | 42 | 1475.466667 | 35.130159 | | |
| Corrected Total | 59 | 46175.650000 | | | |

Appendix 50. Analysis of variance of scab incidence at 69 days after sowing (DAS) on pod of IT 83S-720-2 obtained from three planting dates experiment in 1993.

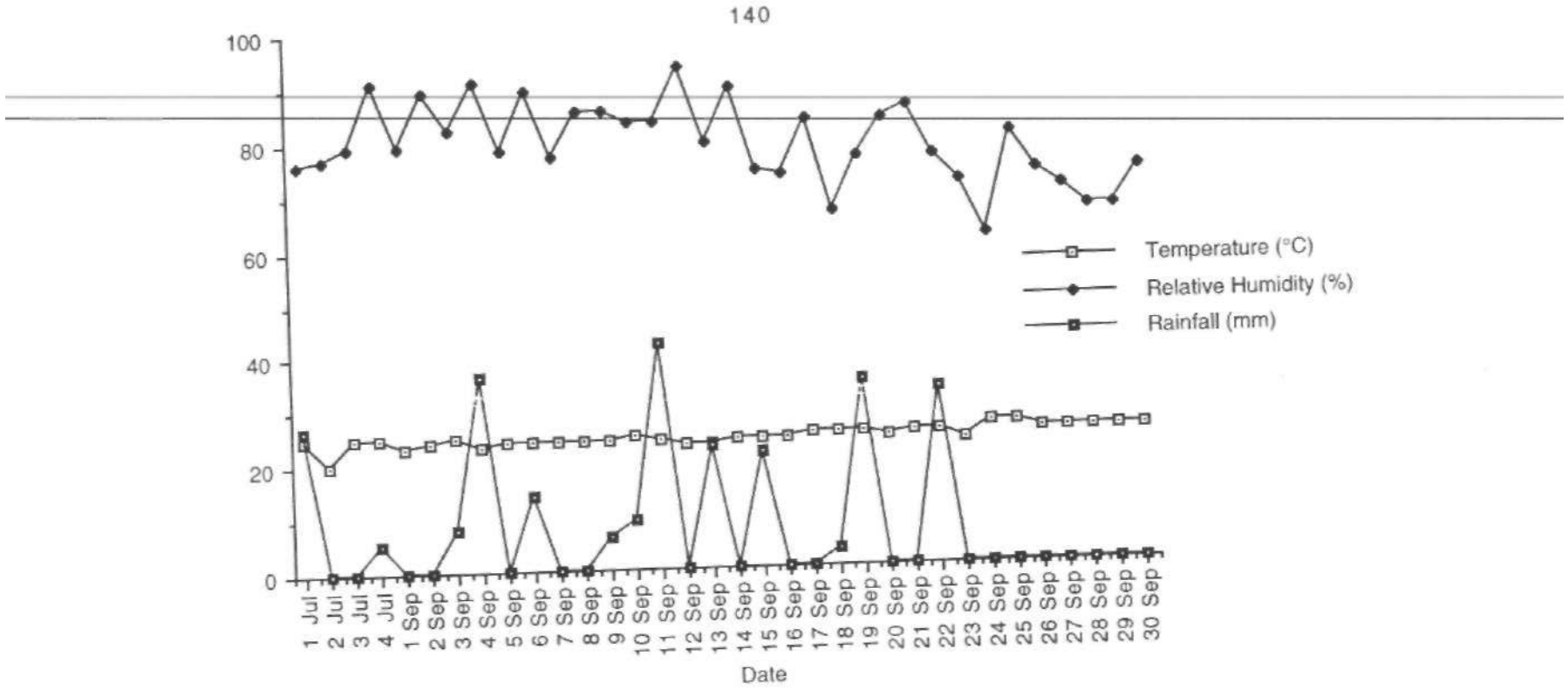
| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 3 | 0.18333333 | 0.06111111 | 1.00 | 0.4023 |
| INTER2 | 14 | 123.43333333 | 8.81666667 | 144.27 | 0.0001 |
| Error | 42 | 2.56666667 | 0.06111111 | | |
| Corrected Total | 59 | 126.18333333 | | | |



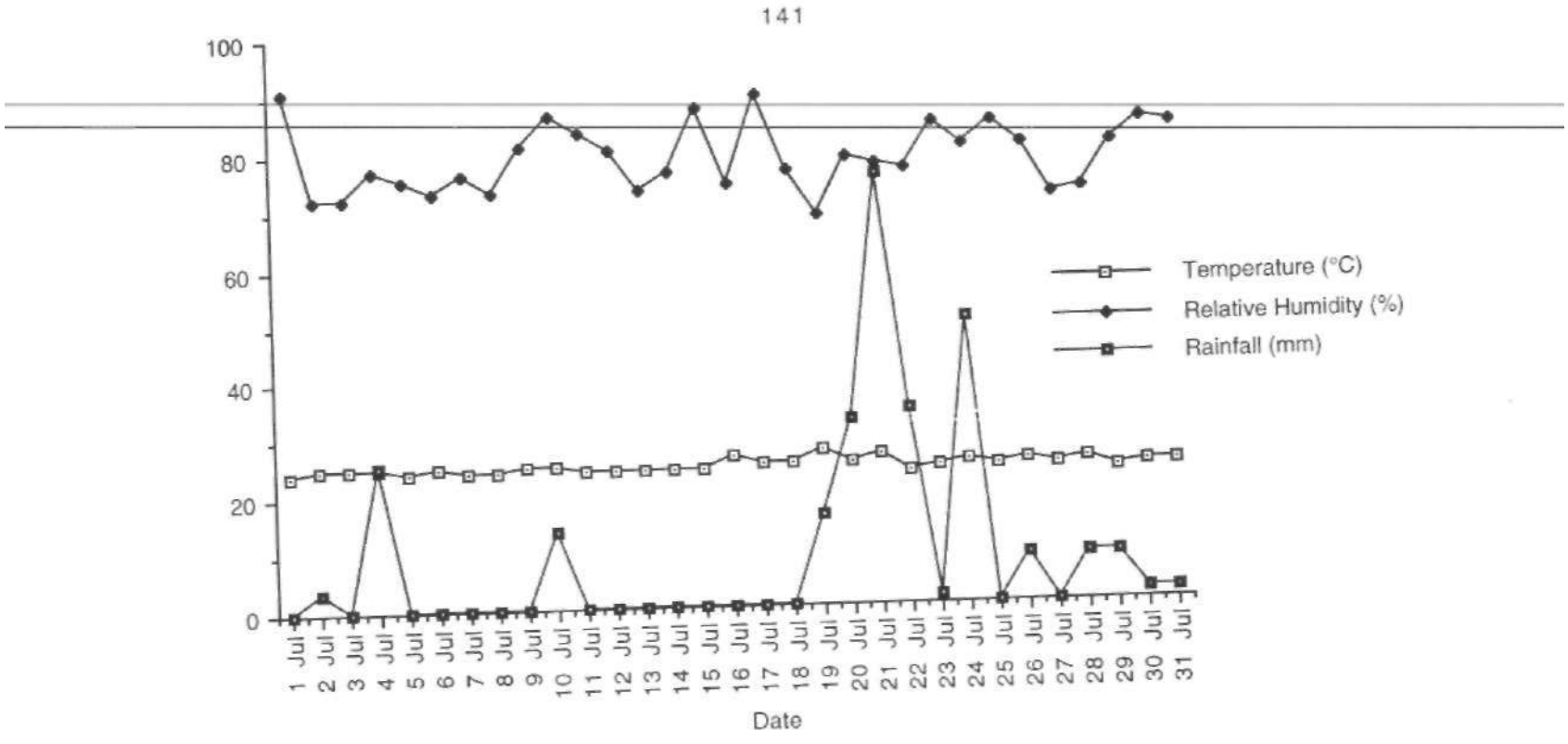

Appendix 51. Pattern of daily rainfall, temperature and relative humidity in Samaru of July 1992



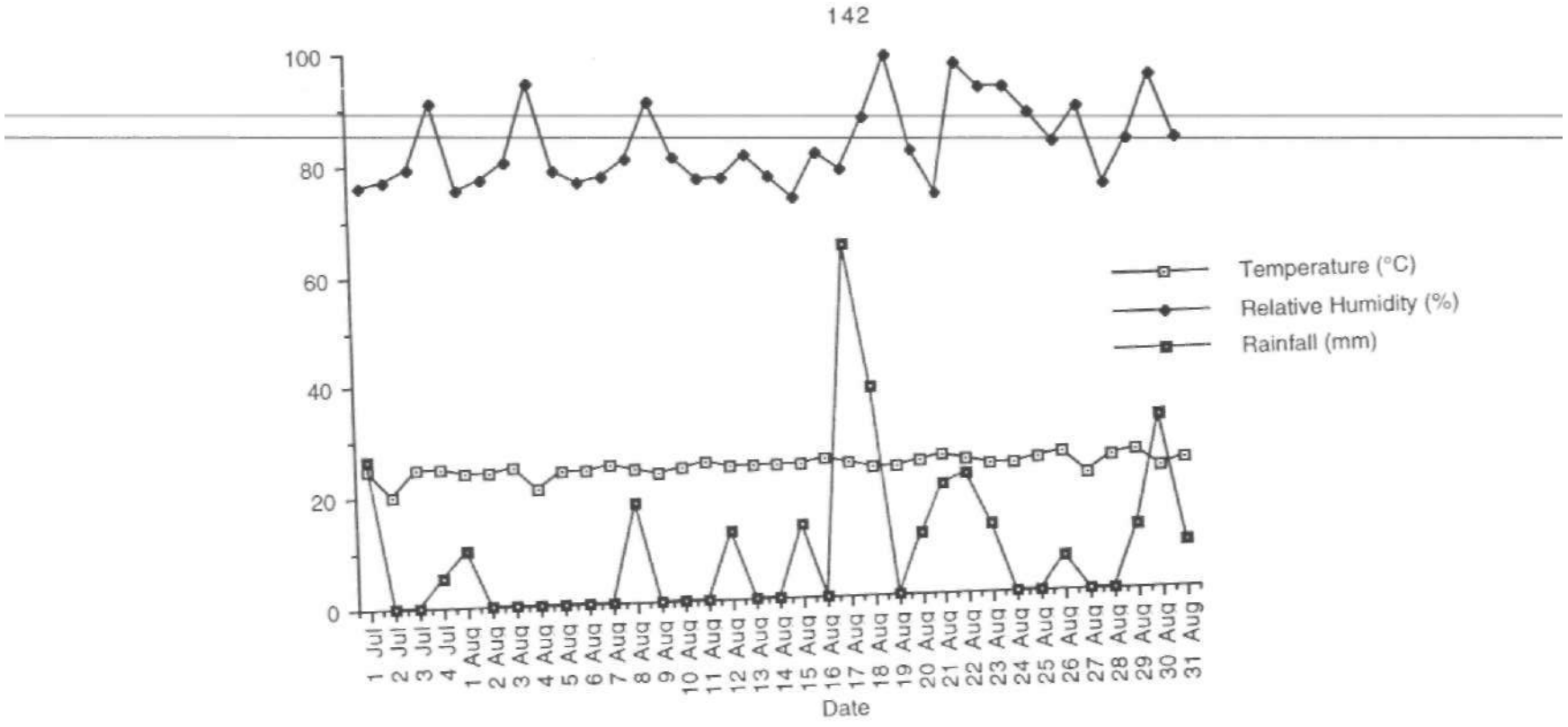

Appendix 52. Pattern of daily rainfall, temperature and relative humidity in Samaru of August 1992




Appendix 53. Pattern of daily rainfall, temperature and relative humidity in Samaru of September 1992




Appendix 54. Pattern of daily rainfall, temperature and relative humidity in Samaru of July 1993




Appendix 55. Pattern of daily rainfall, temperature and relative humidity in Samaru of August 1993

Appendix 57. Analysis of variance table of the areas under disease progress curves (AUDPC) for fungicide applied weekly to control scab of cowpea in 1992 at Samaru.

| Source | DF | IT81D - 1137 SS | Pr > F | IT85F - 1517 SS | Pr > F | IT83S-720-2 SS | Pr > F |
|---|---|---|---|---|---|---|---|
| Leaf | | | | | | | |
| Replication | 3 | 10531.5469 | 0.2541 | 11233.6875 | 0.1155 | | |
| Fungicide | 3 | 52705.5469 | 0.0064 | 9024.1875 | 0.1706 | | |
| untreated control vs others | 1 | 12529.1719 | 0.0400 | 67.6875 | 0.8328 | | |
| benomyl vs del. + man. | 1 | 35921.3438 | 0.0028 | 8550.3750 | 0.0372 | | |
| Delsene M vs mancozeb | 1 | 4255.0313 | 0.1956 | 406.1250 | 0.6074 | | |
| Error | 9 | 19595.3906 | | 12897.5625 | | | |
| Corrected Total | 15 | 82832.4844 | | 33155.4375 | | | |
| Stem | | | | | | | |
| Replication | 3 | 276.1875 | 0.9394 | 12217.5000 | 0.0094 | | |
| Fungicide | 3 | 103056.1875 | 0.0001 | 14836.5000 | 0.0051 | | |
| untreated control vs others | 1 | 11254.6875 | 0.0031 | 3888.0000 | 0.0283 | | |
| benomyl vs del. + man. | 1 | 91513.5000 | 0.0001 | 10584.0000 | 0.0020 | | |
| Delsene M vs mancozeb | 1 | 288.0000 | 0.5384 | 364.5000 | 0.4449 | | |
| Error | 9 | 6336.5625 | | 5139.0000 | | | |
| Corrected Total | 15 | 109668.9375 | | 32193.0000 | | | |
| Peduncle | | | | | | | |
| Replication | 3 | 7737.1875 | 0.1764 | 112.5000 | 0.8515 | | |
| Fungicide | 3 | 62953.3125 | 0.0005 | 27666.0000 | 0.0001 | | |
| untreated control vs others | 1 | 8667.1875 | 0.0274 | 6912.0000 | 0.0001 | | |
| benomyl vs del. + man. | 1 | 54150.0000 | 0.0001 | 20533.5000 | 0.0001 | | |
| delsene M vs mancozeb | 1 | 136.1250 | 0.7493 | 220.5000 | 0.2465 | | |
| Error | 9 | 11285.4375 | | 1291.5000 | | | |
| Corrected Total | 15 | 81975.9375 | | 29070.0000 | | | |
| Flowering cushion | | | | | | | |
| Replication | 3 | 258.1875 | 0.7500 | 82.6875 | 0.9362 | | |
| Fungicide | 3 | 26979.1875 | 0.0001 | 76015.6875 | 0.0001 | | |
| untreated control vs others | 1 | 9157.6875 | 0.0001 | 8190.1875 | 0.0001 | | |
| benomyl vs del. + man. | 1 | 17821.5000 | 0.0001 | 58509.3750 | 0.0001 | | |
| delsene M vs mancozeb | 1 | 0.0000 | 1.0000 | 9316.1250 | 0.0001 | | |
| Error | 9 | 1890.5625 | | 1827.5625 | | | |
| Corrected Total | 15 | 29127.9375 | | 77925.9375 | | | |
| Pod | | | | | | | |
| Replication | 3 | 262.1250 | 0.9615 | 1259.2969 | 0.5561 | 9317.0625 | 0.2022 |
| Fungicide | 3 | 34555.5000 | 0.0015 | 77683.9219 | 0.0001 | 5852.6250 | 0.2193 |
| untreated control vs others | 1 | 75.0000 | 0.7828 | 25877.2969 | 0.0001 | | |
| benomyl vs del. + man. | 1 | 32856.0000 | 0.0002 | 48465.0938 | 0.0001 | | |
| delsene M vs mancozeb | 1 | 1624.5000 | 0.2189 | 3341.5313 | 0.0385 | | |
| Error | 9 | 8368.8750 | | 5127.8906 | | 8889.3750 | |
| Corrected Total | 15 | 43186.5000 | | 84071.1094 | | 24059.0625 | |

del: delsene M
man: mancozeb

Appendix 58. Analysis of variance table of the areas under disease progress curves (AUDPC) for fungicide applied weekly to control scab of cowpea in 1993 at Samaru.

| Source | DF | IT81D - 1137 SS | Pr > F | IT85F - 1517 SS | Pr > F | IT83S-720-2 SS | Pr > F |
|---|---|---|---|---|---|---|---|
| Leaf | | | | | | | |
| Replication | 3 | 4902.7500 | 0.0751 | 1809.0000 | 0.5074 | | |
| Fungicide | 3 | 25620.7500 | 0.0005 | 21935.2500 | 0.0030 | | |
| untreated control vs others | 1 | 126.7500 | 0.6288 | 168.7500 | 0.6402 | | |
| benomyl vs del. + man. | 1 | 11881.5000 | 0.0009 | 30.3750 | 0.8420 | | |
| Delsene M vs mancozeb | 1 | 13612.5000 | 0.0006 | 21736.1250 | 0.0004 | | |
| Error | 9 | 4556.2500 | | 6493.5000 | | | |
| Corrected Total | 15 | 35079.7500 | | 30237.7500 | | | |
| Stem | | | | | | | |
| Replication | 3 | 1874.2500 | 0.5406 | 303.1875 | 0.8503 | | |
| Fungicide | 3 | 113681.2500 | 0.0001 | 6373.6875 | 0.0198 | | |
| untreated control vs others | 1 | 37968.7500 | 0.0001 | 31.6875 | 0.7804 | | |
| benomyl vs del. + man. | 1 | 37209.3750 | 0.0001 | 181.5000 | 0.5091 | | |
| Delsene M vs mancozeb | 1 | 38503.1250 | 0.0001 | 6160.5000 | 0.0031 | | |
| Error | 9 | 7328.2500 | | 3456.5625 | | | |
| Corrected Total | 15 | 122883.7500 | | 10133.4375 | | | |
| Peduncle | | | | | | | |
| Replication | 3 | 668.2500 | 0.6042 | 573.1875 | 0.8235 | | |
| Fungicide | 3 | 6617.2500 | 0.0130 | 19455.1875 | 0.0029 | | |
| untreated control vs others | 1 | 2790.7500 | 0.0192 | 229.6875 | 0.5618 | | |
| benomyl vs del. + man. | 1 | 1014.0000 | 0.1202 | 10710.3750 | 0.0026 | | |
| Delsene M vs mancozeb | 1 | 2812.5000 | 0.0188 | 8515.1250 | 0.0052 | | |
| Error | 9 | 3098.2500 | | 5697.5625 | | | |
| Corrected Total | 15 | 10383.7500 | | 25725.9375 | | | |
| Flowering cushion | | | | | | | |
| Replication | 3 | 3870.5625 | 0.4900 | 420.1875 | 0.1678 | | |
| Fungicide | 3 | 35021.8125 | 0.0068 | 52989.1875 | 0.0001 | | |
| untreated control vs others | | 129551.6875 | 0.0001 | 10005.1875 | 0.0286 | | |
| benomyl vs del. + man. | 1 | 5859.3750 | 0.0001 | 7297.5938 | 0.0533 | | |
| Delsene M vs mancozeb | 1 | 17578.1250 | 0.0001 | 17719.0313 | 0.0071 | | |
| Error | 9 | 13293.5625 | | 594.5625 | | | |
| Corrected Total | 15 | 52185.9375 | | 54003.9375 | | | |
| Pod | | | | | | | |
| Replication | 3 | 328.5000 | 0.7138 | 8438.0625 | 0.0850 | 2164.9219 | 0.4716 |
| Fungicide | 3 | 49734.0000 | 0.0001 | 84075.1875 | 0.0001 | 28070.2969 | 0.0018 |
| untreated control vs others | 1 | 12288.0000 | 0.0001 | 15016.6875 | 0.0030 | 2632.9219 | 0.1009 |
| benomyl vs del. + man. | 1 | 9361.5000 | 0.0001 | 37683.3750 | 0.0001 | 24098.3438 | 0.0004 |
| Delsene M vs mancozeb | 1 | 28084.5000 | 0.0001 | 31375.1250 | 0.0003 | 1339.0313 | 0.2249 |
| Error | 9 | 2119.5000 | | 8313.1875 | | 7097.7656 | |
| Corrected Total | 15 | 52182.0000 | | 100826.4375 | | 37332.9844 | |

del: delsene M
man: mancozeb

# BIOGRAPHY

Name: Mrs Catherine M. Mungo-Egbe

Date of Birth: August 26th 1964

Nationality: Cameroonian

Home address: Ojoo Ibadan

Previous Qualification:

| Institutions attended | From | To | Certificate obtained |
|---|---|---|---|
| A.B.U. Zaria | 1989 | 1992 | M.Sc. (Crop Protection) |
| A.B.U. Zaria | 1986 | 1989 | B.Sc. (Hons) Botany |
| College of Arts and Science Bambili | 1983 | 1985 | "A" level passed |
| Queen of the Holy Rosary Secondary School Okoyong | 1978 | 1982 | "O" level passed |
| St. Pius Primary School Buea | 1971 | 1978 | Primary School Certificate |

Fellowship Awards since the Award of first Degree

| Organisation | From | To | Research Degrees |
|---|---|---|---|
| IITA Ibadan Nigeria | 1990 | 1991 | M.Sc. (Crop Protection) |
| IITA Ibadan Nigeria | 1992 | 1995 | Ph.D. Plant Pathology |

Year of Ph.D. admission for Postgraduate studies: 1992

Admission number: Ph.D./Agric/1458/92