EPIDEMIOLOGY OF COWPEA BROWN BLOTCH INDUCED BY *COLLETOTRICHUM CAPSICI* AND ASSESSMENT OF CROP LOSSES DUE TO THE DISEASE

BY

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A thesis submitted to the Postgraduate School, Ahmadu Bello University in partial fulfilment of the requirements for the degree of Doctor of Philosophy

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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.

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CERTIFICATION

This thesis entitled "EPIDEMIOLOGY OF COWPEA BROWN BLOTCH INDUCED BY COLLETOTRICHUM CAPSICI AND ASSESSMENT OF CROP LOSSES DUE TO THE DISEASE" by Olufunmilola Alabi 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.

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DEDICATION

This thesis is dedicated to: my darling husband, Segun and our lovely children, Seyi, Bukky, Folake and Fisayo for their loving care and support throughout the time of this study.

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ABSTRACT

Colletotrichum capsici (Syd.) Butler and Bisby which induces brown blotch in cowpea was found to grow and sporulate between 20 and 3 5°C on potato dextrose agar incorporated with streptomycin, the optimum temperature being 25°C. When plants aged 1-6 weeks were inoculated with the conidial suspension of the fungus containing 4.16 x $1Q^5$ to 3.7 5 x 10^6 conidia/ml, plants aged 1 or 2 weeks at inoculation were more severely affected than older plants aged 3 to 6 weeks at the time of inoculation. There was a highly significant (P=0.01) interaction between plant age at inoculation and inoculum concentration. The incubation period was 2-3 days for all the aerial parts of the plants inoculated irrespective of age.

C. capsici sporulated faster on relatively tender petiole tissues than on less succulent stem tissues. The latent period varied from 5 to 16 days, depending on the plant part.

Cowpea sown early at the third week of July or first week of August (when there was high and frequent rainfall) had higher incidence of brown blotch than those sown late August. In addition the quality of the grain produced in the early sown crop was poor (being mostly wrinkled) while that of the late sown crop, was relatively high, being mostly wholesome and non-discoloured.

It appears that both rainfall and relative humidity enhance the development of brown blotch in cowpea provided that there is enough rainfall and consequently, sufficiently

high relative humidity for disease establishment, although among the two factors, the progress of the disease appears to depend more on relative humidity.

Loss in grain yield of cowpea due to brown blotch ranged from 10% for variety IT83S-742-11 to 44% for Ife Brown.

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CHAPTER ONE

INTRODUCTION

The cowpea (Vigna unguiculata) (L.) Walp.), is a widely cultivated grain legume in the tropics and subtropics. On a worldwide basis it is grown on about 7.7 million hectares, of which over 80% are located in sub-Saharan Africa, where it is usually grown in mixtures with sorghum, millet, maize or cassava (Singh and Rachie, 1985). The crop is highly valued both for its grain and forage.

The annual production of cowpea in Nigeria has been estimated to be between 700,000 and 800,000 tons (Raheja and Apeji, 1979). As much as 80% of this is produced in the northern parts of Nigeria (Emechebe and McDonald, 1979).

Cowpea suffers from many diseases wherever it is grown in this country. Williams (1975) and Singh and Allen (1979) listed over 35 diseases of cowpea mostly induced by fungi, bacteria and viruses. Emechebe (1980) considered cowpea scab induced by Sphaceloma sp. as the most important disease of cowpea in the northern Guinea savanna zone of Nigeria while brown blotch, induced by Colletotrichum capsici, was considered as the second most important disease of the crop.

Brown blotch of cowpea, which occurs in all cowpea-growing areas of Nigeria, is internally and externally seed-borne as well as being seed transmitted (Emechebe, 1981a). A full account of the symptoms of this disease has been given by Emechebe (1981a), who first described the disease.

Earlier workers (Emechebe, 1981b; Emechebe and Shoyinka, 1985) have concentrated their efforts on the control of the disease by the use of fungicides and hosts plant resistance. The incorporation of resistance to brown blotch and other important diseases into cowpea germplasm is one of the main objectives in the cowpea improvement programmes of the International Institute of Tropical Agriculture (IITA) Ibadan and the Institute for Agricultural Research (IAR) Samaru, in Nigeria.

Epidemiology has been conceived in various ways by plant pathologists. Van der Plank (1963) defined epidemiology as the science of disease in populations while Kranz (1974) conceived epidemics as open, coupled and dynamic systems of pathogen and host populations interacting under the influence of environment and human interference. In general, study of the epidemiology of a disease should normally result in the development of tactical and strategic concepts for the control of the disease.

Most homes in Nigeria cannot afford the high price of animal proteins and so they depend on the cheaper plant proteins, especially those from leguminous crops notably cowpea. A fall in the production of cowpea as a result of brown blotch would mean that many people could suffer from protein-deficiency diseases such as kwasiokor. Fungicides have been recommended for optimum cowpea production but this has not been widely adopted due to high costs of knapsack sprayers and fungicides. However, apart from the work of Emechebe (1981a),

and Okpala (1981) not much work has been done on the epidemiology of cowpea brown blotch. A good knowledge of the epidemiology of brown blotch is essential for devising effective control measures including the optimum time to apply fungicides for effective disease control, thereby minimizing the number of sprays required.

The specific objectives of the present study are:

1. To determine the effect of temperature on the growth and sporulation of C. capsici in artificial culture. This information will help one to know the minimum, maximum and optimum temperatures for growth and sporulation of the pathogen.
2. To determine the latent period of the pathogen and how it is affected by temperature and relative humidity. This information will be useful in working out a schedule for timely application of fungicides for brown blotch control.
3. To determine the effect of plant age and concentration of inoculum on brown blotch.
4. To study the effect of temperature, relative humidity, and rainfall on the development of the disease from time of planting to harvest. The results of these experiments will help in forecasting outbreaks of the disease.
5. To study the effect of sowing dates and fungicide application on some of the epidemiological

characteristics of the disease. The result of these experiments will not only help in deciding when and how to control the disease effectively without wasting fungicides, it will also help in deciding whether adjustment of sowing date can facilitate disease escape by cowpea crop.

6. To assess the crop losses due to the disease. This is necessary for estimation of the economic importance of the disease, for evaluating control strategies, for making yield predictions and for evaluating the need for research on the disease in relation to cowpea production.

CHAPTER 2
2.0 LITERATURE REVIEW

Cowpea brown blotch is induced by Colletotrichum capsici (Syd.) Butler & Bisby. The pathogen was first recorded on stem base of cowpea in Bailey's (1966) check list of plant diseases in Nigeria; it was later recorded by Williams (1975) on cowpea stems, primarily killed by Pythium stem rot in southern Nigeria.

Brown blotch occurs in all cowpea growing areas and is one of the major diseases that reduces productivity of cowpea in northern Nigeria, especially in years when the environmental conditions are favourable for high severity of the disease (Emechebe, 1981a).

2.1. The Pathogen

Colletotrichum capsici belongs to the class Deuteromycetes, and order Melaconiales which are characterized by the production of acervuli with setae. The structure of the fungus and conidia have been described by the Commonwealth Agricultural Bureau International Mycological Institute (CMI). Acervuli (110 to 34 μm) are formed within 2 days. The conidial mass is initially creamish but later becomes pinkish. The setae (76 to 196 x 4.5 to 7.0 μm) are dark and brown and two to five-septate and have an apical lanceolate cell (apex width, 2.5 x 3.3μm) and a basal cell that is either slightly swollen (width, 6.9 to 10.3μm) or has the same diameter as central cells. The conidiophores (8.5

and has been found to continue for many days after that.

Seedling death is caused by a purplish-brown lesion that girdles the stem at soil level or at cotyledonary node. Occasionally purplish-brown spots occur on the unifoliate leaves (Emechebe, 1981a). Stem lesions that do not cause seedling collapse persist on the growing and mature plants. Those at the soil level may eventually girdle stem base, causing wilting and death of the plant.

One of the most destructive phases of the disease is the blotch of the peduncle and the flowering axis. Early infection of the axis results in a brown necrosis that causes either complete floral abortion or distortion, shrivelling and mummification of immature pods. Pods may be infected at all stages of maturity. Under humid field conditions the fungus sporulates on the dry pod to produce a lesion with alternating brown and black bands, the latter being due to acervuli of the fungus. This is considered to be the most diagnostic phase of the disease (Emechebe, 1981a).

2.3 Host Range

Okpala (1981) studied the host range of the cowpea isolate of *C. capsici* and found that apart from causing brown blotch in cowpea varieties it also induced similar symptoms in yard long bean (*Vigna sesquipedalis*), cotton (*Gossypium hirsutum*), soybean (*Glycine max*), hyacinth bean (*Lablab niger*), Lima bean (*Phaseolus lunatus*), broad bean (*Vicia faba*), Bambara groundnut

(Vigna subterranea) and maize (Zea mays). Ling and Lin (1944) reported that pepper isolate of C.capsici was pathogenic to detached, artificially inoculated soybean pods. Several workers have reported that the Capsicum isolate of C.capsici causes ripe fruit rot of Chilli (Ling and Lin 1944; Chowdhury 1957; Smith and Crossan, 1958). Earlier, Thompson (1931) had reported that the Capsicum isolate of C.capsici caused ripe fruit rots of tomato and egg plant, while Deighton (1936) observed that it caused ripe fruit rot of pawpaw in Sierra Leone.

2.4 Survival of C.capsici and some other pathogenic species of Colletotrichum

Earlier workers have investigated the survival of C.capsici and some other species of Colletotrichum. For example, 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 (1968) who found that conidia of C.piperatum survived for up to 10 months in dry chilli fruits 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 - 15cm.

Warren (1977) 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° 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 source 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).

2.5 Epidemiology of Some Diseases of Cowpea and French Beans

Epidemiology is the science of disease in populations; it attempts to optimize the use of chemicals and various measures in plant disease control (Van der Plank, 1963).

An epidemic results from a combination of many factors interacting with each other, compensating for limitations in different locations, seasons and climatic zones (Rotem, 1978). Atmospheric factors affect an epidemic through their effects on the various phases of the pathogen's life cycle as they interact with the specific responses of individual plants. Amin et al. (1976) studied the epidemiology of Cercospora cruenta in cowpea and found that a dense suscept population

coupled with continued high relative humidity for 3 to 4 days (provided by sprinkler irrigation) and moderately warm temperatures were conducive for epidemic development.

Sindhan and Bose (1981) studied the epidemiology of anthracnose of French bean (Phaseolus vulgaris) induced by Colletotrichum lindemuthianum in India. They reported that the disease appeared in the second or third week of June with a maximum damage from first week of August, which continued until the middle of September. French bean plants of all ages were susceptible but infection was less on two weeks than six weeks old plants; in addition, disease intensity increased with the age of the plant. The best sowing time for French bean for maximizing yield and minimizing the disease incidence was between mid April to mid May.

2.5.1. Effect of temperature on growth and sporulation of some Colletotrichum species.

Temperature is one of the environmental parameters frequently reported as being of great importance in the alteration of disease epidemics in crop plants (Rache and Kuc, 1970). 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). 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. Solanki et al. (1974) reported an optimum temperature of 25°C for germination

of the conidia of the Capsicum isolate of C.capsici. Fakorede (1976) provided evidence that 25 to 26°C was the optimum temperature for growth, sporulation and infection of cowpea by C.lindemuthianum. 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 IT82E-16 which are less susceptible.

The study by Mazlan and Sariah (1980) on anthracnose of chilli caused 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, caused by 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.

Similarly, Ghogre and Kodmelwar (1986) reported that maximum growth of C.curcumae, causal agent of leaf blotch of turmeric, occurred at 27°C. In storage, temperature of 30°C favoured development of fruit anthracnose of chilli caused by C.capsici and Gloeosporium piperatum.

In contrast to the above reports, Sartorato (1988) reported from Brazil that French bean anthracnose (C. lindemuthianum) developed between 13 and 17°C, the optimum temperature being 17°C; and high relative humidity favoured disease development.

2.5.2. Effect of age of the suscepts on disease development

In most cases, the age of a plant influences the susceptibility of the plant to diseases. Verma and Patel (1969) found that 7- and 15-day old cowpea seedlings were less susceptible to Cercospora leaf spot than 21- and 27-day old plants and that susceptibility decreased with age of plants after 32 days. Similarly, Sindhan and Bose (1981) studied anthracnose of French bean and found that infection was less on two weeks than on 6 weeks old plants, that is, disease intensity increased with age from 2 to 6 weeks.

The above are similar to earlier report by Griffey and Leach (1965) that "old" tissues (hypocotyl of 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 when inoculated with C.lindemuthianum compared to larger lesions on "young" (plants are said to be young from emergence of the cotyledon until the leaves of the first node above the cotyledons were approximately half their mature size) plants. This "mature plant" resistance in French bean to C.lindemuthianum has been subsequently coroborated by

other workers. For example, Coronel and Chaves (1975) studied the effect of the interaction of plant age and inoculum concentration on the susceptibility of bean plants to races of C.lindemuthianum and found that the reactions of 5 -15 days old plants were similar in all the five cultivars inoculated.

By contrast, 35-day old plants of all cultivars, 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 International de Agricultura Tropical (CIAT) also reported that when different ages of a susceptible bean variety (Ecuador1056) 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).

In contrast, Leonard and Thompson (1976) studied the effect of host maturity on lesion development of corn anthracnose (Colletotrichum graminicola). They found that plants inoculated at 5 and 6 weeks after planting developed fewer and smaller lesions per square centimeter of leaf area than those inoculated at 7 or 8 weeks of age. However, studies on the

effect of fruit maturity on the incidence of anthracnose of chilli pepper, induced by C. capsici and Gloeosporium piperatum, revealed that green or young chillies were more susceptible to the disease than half mature fruits (Asuti and Suhardi, 1986).

2.5.3. Effect of planting date on disease development

The causal agents of plant disease may be avoided by selecting the best dates for planting (Pennypacker and Stevenson, 1982). Thus, the choice of an appropriate planting date may allow for selection of a period when environmental conditions are favourable for the growth and development of the crop while being unfavourable for the pathogen (Pennypacker and Stevenson, 1982).

Sindhan and Bose (1981) in their studies of the epidemiology of anthracnose of French bean (C.lindemuthianum) found that, to maximize yield and minimize disease incidence, the best sowing time of French bean in India was between mid April to mid May.

Studies on Cercospora leaf spot of cowpea caused by C. cruenta in India showed that the disease appears very late in the season but progresses very rapidly under conditions of moderate mean temperature (26°C), 61-79% relative humidity and scanty rainfall (Verma and Patel, 1969).

Emechebe (1984) studied the effect of planting date on cowpea brown blotch in Samaru, Zaria and found that the earlier

the planting date the higher the severity of the disease on cowpea. He suggested that planting cowpea in the third week of July reduces the disease, even on unsprayed plots.

CIAT (1976) reported that even under protected conditions in Colombia, yields of Phaseolus bean decreased from 2.7t/ha in June planting to 61 kg/ha in October planting because of the excessive rainfall during October and November which increased the incidence and severity of anthracnose on the crop.

2.5.4. Effect of rainfall and relative humidity on disease development

Atmospheric factors affect an epidemic through their effects on the various phases of the pathogens's life cycle as they interact with specific responses of individual plants (Rotem, 1978). Accurate knowledge of the effects of environmental factors on disease development is essential for devising effective control measures (Castor et al., 1977). Spraying only when environmental conditions are favourable for disease increase in the field is the key to effective and efficient control (Madden et al., 1978). Models for forecasting systems are based primarily on the major environmental factors required to predict the occurrence of an expected plant disease epidemic (Pennypacker and Stevenson, 1980). For example, the modelling of Alternaria solani on potato and tomato as a function of the environment resulted in the development of EPIDEM in Connecticut, United States of America which is the first

computer simulator of a plant disease (Waggoner and Horsfall, 1969). Similarly EPIMAY was developed for southern corn leaf blight on maize (Waggoner et al., 1972). These and other models have been found to be effective in the places where they were developed and used.

The three major sources of moisture for plant disease epidemics are rain, dew, and overhead irrigation. The apparent preference of plant pathogens for different patterns of rainfall seems to be connected with the specific characteristics of a given pathogen and climatic region (Rotem, 1978).

Fernadez et al. (1987) carried out studies on anthracnose of Phaseolus vulgaris at two locations during rainy and dry seasons. They found that the influence of inoculum on leaf anthracnose was much higher during the rainy season than during the dry season, whereas its influence on the pod anthracnose was higher in the dry season. Excessive rainfall reduced the yield of beans planted in October or November in Colombia due to high anthracnose incidence (CIAT, 1976). Rain splash and wind-driven rain are important in the spread of brown blotch from one point to the other or from plant to plant (Emechebe, 1981a).

Sindhan (1983) reported that the optimum relative humidity (RH) for the development of anthracnose of French bean was 91-100% and the infection was completely inhibited below 80.5% RH Chung and Lee (1986) also found that RH of at least 90% was

optimum for conidial germination of C.dematium f.sp. capsicum, the pathogen of red pepper anthracnose. In most cases, high relative humidity favours disease development. However, a RH of 80.5% was found to be optimum for mycelial growth and sporulation of C.curcumae, causal agent of leaf blotch of turmeric (Ghogre and Kodmelwar, 1986).

A pre-inoculation moist period increased infection but was not as effective as a post-inoculation moist period for initiation and development of anthracnose of French bean induced by C. lindemuthianum in India (Sindhan, 1983). The loss due to anthracnose on bean was doubled during the second half of the year compared to the first half in Colombia, because there were more frequent and heavy rains favouring the development and spread of the fungus (Guzman et al., 1979).

2.5.5. Dispersal of some diseases caused by species of *Colletotrichum*

Many species of Colletotrichum have been reported to be seed-borne; among them are C.lindemuthianum, C.capsici, C. cajani, C.truncatum and C.curvatum (Noble and Richardson, 1968; Neergaard, 1977). Rajkumar et al., 1989 found that C. capsici on urd bean (Phaseolus mungo L.) perpetuates through infected seeds. Prasanna (1985), in his transmission studies, showed that for C.lindemuthianum (cowpea anthracnose pathogen) infected seeds serve as primary source of inoculum of the disease.

Emechebe (1981a) reported that C.capsici is externally and internally seed-borne and that seed-borne infections produce the primary lesions from where secondary spread mostly by rain splash occurs in the field. C.capsici has been reported to be seed-borne on other plants such as chilli, jute and cotton (Voelcker, 1953; Grover and Bansal, 1970; Chopra et al., 1975).

Tu (1981) studied the spread of anthracnose (C. lindemuthianum delta race) from an initial infection focus in field plots planted with a susceptible white French bean cultivar (Fleewood). He found that splashing rain drops blown by gusting winds spread the disease. The disease spread from an infection focus and decreased gradually towards the periphery of the field.

Secondary infection of sorghum stalks by red rot induced by C.graminicola plays a very important role in the spread of the disease (Neya and Kaboré, 1987).

2.6. Crop Loss Assessment

Crop loss is defined as the measurable reduction in quantity and, or quality of yield (Madden et al., 1981). Crop loss estimates provide evidence that will justify or condemn the use of fungicides to control epidemics (James, 1974).

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 acceptable under biological, economic, and physical constraints.

In some experiments there are both sprayed and unsprayed plots and crop loss has been calculated as the difference in yield between the two treatments. However, this method of assessment is inferior to multiple-treatment experiment, with several amounts of disease and one healthy treatment, which allows comparison of the effect of different types of epidemics at one location (James, 1974).

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; James and Teng, 1979, Main, 1977).

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 temperature and high humidity or free moisture (Schwartz and Sanders, 1979). Yield losses of up to 100% have been reported

in many countries (Zaumeyer 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. In Brazil, Sartorato (1988) found that anthracnose may account for 100% yield loss.

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).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Isolation of the Pathogen

C.capsici was isolated from brown blotch lesions on stems, pods, and seeds. The diseased tissues were surface-sterilized with 0.5% solution of sodium hypochlorite for 5 minutes, rinsed in three changes of sterile distilled water, and plated on potato dextrose agar containing 100 units/ml of streptomycin (PDAS) in 9-cm (diameter) Petri dishes. The plates were incubated at room temperatures (25-30 °C) for 5 days.

The fungus readily sporulated on the lesions and on the agar. It was then subcultured onto fresh PDAS plates in order to get a pure culture. Cultures to be used at later dates were maintained on PDAS slants in McCartney bottles.

3.2. Effect of Temperature on Growth and Sporulation of C.capsici

Petri dishes containing PDAS were each inoculated centrally with 2-mm (diameter) disc taken with sterilized cork borer from the periphery of a 4-day old culture of C.capsici. Three Petri dishes each were incubated at 15, 20, 25, 30, 35 or 40°C for 10 days. The dishes were arranged randomly in six Gallenkamp incubators which had been preset at the above six temperatures. During the experimental period, the colony radial growth was measured at 2-day interval with a ruler. The

quantity of conidia produced was determined 10 days after inoculation. This was done by flooding the agar plate with 10 ml of sterile distilled water, scraping the agar surface with one end of a clean, surface-sterilized microscope slide, and filtering the resulting suspension through two layers of cheese cloth into 80 ml of sterile distilled water in a 600 ml beaker. The plate was rinsed with 10 ml of sterile distilled water before the suspension was transferred into a 200 ml measuring cylinder and made up to 100 ml with sterile distilled water. The number of conidia per ml was determined with the aid of a haemocytometer.

3.3. Effect of Plant Age and Inoculum Density on the Disease

3.3.1 Glasshouse experiment

A cowpea variety (Ife Brown) which is very susceptible to brown blotch was used for the study in 1986. Seeds treated with benomyl (Benlate, 50% a.i. wp., Du Pont) were sown at weekly intervals to raise healthy seedlings aged 1, 2, 3, 4, 5, or 6 weeks at the time of inoculation. The seedlings were inoculated with the conidial suspension of six different concentrations, viz 4.16×10^5, 6.7×10^5 1.25×10^6, 1.31×10^6, 2.68×10^6, or 3.75×10^6 conidia/ml. The check consisted of healthy seedlings aged 1 to 6 weeks sprayed with water. There were two replicates, each replicate consisting of three seedlings. The experiment was carried out in the glasshouse, in which temperature fluctuated between 24 and 50°C and relative humidity

between 43 and 62%.

After inoculation, the plants were enclosed in wet, clear polyethylene bags for 48 hrs but aerated after 24 hrs by removal of the bags then re-wetted before they were replaced to provide a humid atmosphere conducive for infection. The plants were subsequently observed for disease symptoms. A visual assessment scale of 1 - 10 in which 1 means no disease and 10 is the maximum score or death of plant (Alabi and Emechebe 1981), was used to determine the severity of disease, 10 and 16 days after inoculation.

3.3.2. Field experiment

A modified form of the experiment in 3.3.1 above was conducted in the field in 1986 cropping season. Dressed seeds of Ife brown were planted at weekly interval to raise seedlings aged 2 to 6 weeks at the time of inoculation. The seedlings were then inoculated with conidial suspension of three different concentrations, viz 4.38×10^5, 1×10^6, or 2.25×10^6 conidia/ml. Disease severity assessment was recorded within one week after inoculation using the same scale as for the glasshouse experiment.

3.4 Effect of Temperature and Relative Humidity on the Latent Period of C.capsici

Seeds of Ife brown, dressed with benomyl, were sown in 18-cm (diameter) pots. At about 8 weeks of age (i.e., at

flowering/young, green pod stage of growth), the plants were spray-inoculated to run-off with the conidial suspension of the fungus. The check plants were sprayed with water. The plants were covered with wet polyethylene bags for 48 hrs but they were aerated after 24 hrs by removal of the bags which were then re-wetted before they were replaced. The plants were then placed under three different conditions, viz : glasshouse, a polyethylene chamber in the laboratory and in a wire net cage outside the glasshouse.

The different parts of the plants were observed for acervulus formation daily until all the diseased parts had sporulated. This facilitated the determination of the latent period for the different plant parts under the three conditions above. Records of temperature and relative humidity were taken with a thermohydrograph.

3.5. Effect of Sowing Date on Some Epidemiological Characteristics of C.capsici

Seeds of susceptible Ife Brown variety were sown at four different dates (D_1, D_2, D_3, D_4) at one week interval. A split plot design was used for the experiment. The main plots consisted of plants sown at four different dates while the sub-plots comprised fungicide-sprayed or unsprayed plots. The sprayed plots received a tank mixture of benomyl and mancozeb at the rates of 0.6 kg and 2.5 kg of commercial product, respectively, both in 400 l of water per ha. Spraying commenced

6 weeks after sowing (WAS) and continued at 14-day interval until pod maturity. All plots received blanket, forthnightly insecticidal sprays of a tank mixture of cypermethrin and dimethoate at (1 L/ha each) starting at 6 weeks after sowing until pod filling stage. The incidence and severity of the disease in various plots were taken every 7 days, starting at 8 weeks after sowing. The experiment was carried out for three years (1986, 1987, and 1989). The apparent infection rate was determined using Van der Plank's (1963) formula :

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

Where X_1 and X_2 are the proportions of disease on dates t_1 and t_2, respectively.

3.6. Disease Progress

Healthy seeds of Ife Brown variety were sown in the field. The plants were spray-inoculated with the conidial suspension of the fungus at 4 weeks after sowing. The development of the disease was recorded at weekly interval until harvest. There were two plots, each 5 x 5 m. The amount of disease, $\log_{10}$ X/1-X, was plotted against time, where X is the proportion of diseased plants. This was done for three years (1984, 1985 and 1989). The rainfall, and relative humidity were recorded weekly during the cropping season of each year and related with those of disease incidence recorded.

3.7 Reactions of Cowpea Cultivars of Different Susceptibilities to Brown Blotch

Healthy seeds of Ife Brown (susceptible), SAMPEA-2, IAR 335 (moderately resistant) and TVx 3236 (resistant) were planted on the field. The design was a randomized complete block with three varieties, replicated three times. Each plot consisted of five, 75-cm ridges, 5 m long. The disease incidence scores were taken 6, 8, and 10 weeks after planting and apparent infection rates were calculated.

3.8. Crop Loss Assessment

Ife brown was used for this study in 1986. The field layout was a randomized complete block design with five treatments and one unsprayed check. Each experimental plot consisted of five, 75-cm ridges, 5 m long. Each plot was surrounded with 2 m planted with variety SAMPEA-3 (IAR 341) which was sprayed twice a week in order to reduce the spread of secondary inoculum from one plot to another.

Seeds were sown 20 cm apart (two seeds/hole). At 4 weeks after sowing (WAS), five plants chosen randomly were inoculated with conidial suspension of the pathogen. At 5 WAS fungicidal spraying started, the fungicidal spray comprised of a tank mixture of mancozeb (2.5 kg of Dithane M 45 per 400 l of water/ha) and benomyl (0.6 kg of Benlate per 400 l of water/ha) at different intervals. The spraying regimes were:

T_0 - no fungicide spray applied,

T_1 - fungicide spray applied at 3-4 days interval,

T_2 - fungicide spray applied at 7-day interval,

T_3 - fungicide spray applied at 14-day interval,

T_4 - fungicide spray applied every three weeks, and

T_5 - fungicide spray applied every four weeks.

Disease incidence in percentage was recorded and the three middle ridges were harvested for determining grain yield. Grain yield loss was then calculated.

In 1987 the experiment was repeated using two IITA varieties namely IT 83S - 742 - 11 and IT 83S - 728 - 13 which were earlier observed to be highly susceptible to brown blotch. The design was the same as for 1986, except that the plots were not surrounded with SAMPEA 3 (IAR 341). The experiment was repeated in 1988 using the above two varieties, but the plants escaped from infection and so no records of disease incidence were taken.

In 1989, two varieties, namely IT86D-1056 and IT86D-1057, which are resistant to other cowpea fungal diseases but susceptible to brown blotch were used for this study. The field layout was a randomized complete block design with six treatments (five different fungicidal spray treatments, one unsprayed check). Each experimental plot consisted of five, 75-cm ridges, 5 m long; plots were separated from each other at two of the four sides by 1.5 m of bare ground. Each block was separated from the next block by 2 m planted with SAMPEA-3

(IAR 341). Seeds were sown 20 cm apart (two seeds/hole), on 25 July 1989. At 4 weeks after sowing (WAS) and at flowering (7 WAS) the plants were spray-inoculated with conidial suspension of the fungus. The inoculation was done in the evening between 1900 hours and 2030 hours when the relative humidity was higher than during the day time. The fungicide spray regime was as described above.

Disease severity was estimated using a scale of 1 - 10 described by Alabi and Emechebe (1981). The three middle ridges were harvested at maturity and weighed.

CHAPTER FOUR

4.0 RESULTS

4.1 Effect of Temperature on Radial Mycelial Growth and Sporulation of *Colletotrichum capsici*

The fungus grew rapidly on PDAS at 20, 25, 30, and 35^{0}C from the second to the tenth day after inoculation (Fig.1). There was no growth at 15 and 40^{0}C. The maximum growth occurred at 25^{0}C. There was a rapid growth of the fungus at 25^{0}C from the second to the tenth day after inoculation when the fungus had grown to cover the whole surface of medium in the plate (Fig.2). There were highly significant differences (P=0.01) among radial growths at different temperatures (Fig.1 and Appendix 1). Temperatures below 20^{0}C and above 35^{0}C apparently suppress radial mycelial growth.

The radial growth rates between 48 and 72 hrs after inoculation were calculated for each temperature using a modified formula of Zadoks and Schein (1979). The results (Table 1) show that growth was relatively fast at 20^{0}C (0.35mm/hr) and it continued to increase with increase in temperature up to a maximum radial growth rate (0.44mm/hr) recorded in the present study at 25^{0}C. The data also show that the radial growth rate at 30^{0}C (0.40mm/hr) was lower than that of 25^{0}C.

The data presented in Fig.3 show that the fungus produced spores between 20 and 35^{0}C and that no sporulation occurred at 15 and 40^{0}C. The maximum

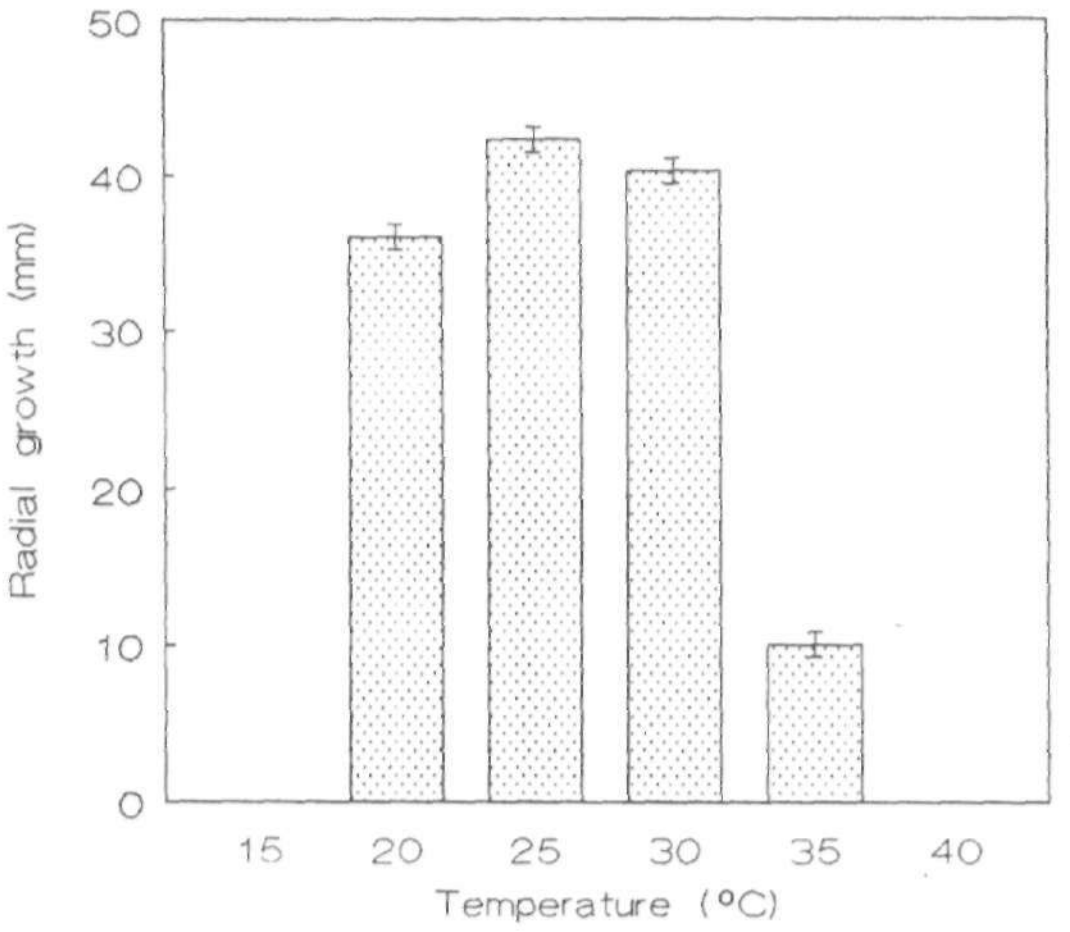


Fig. 1. Effect of temperature on radial growth of C. capsici on potato dextrose agar at 10 days after inoculation

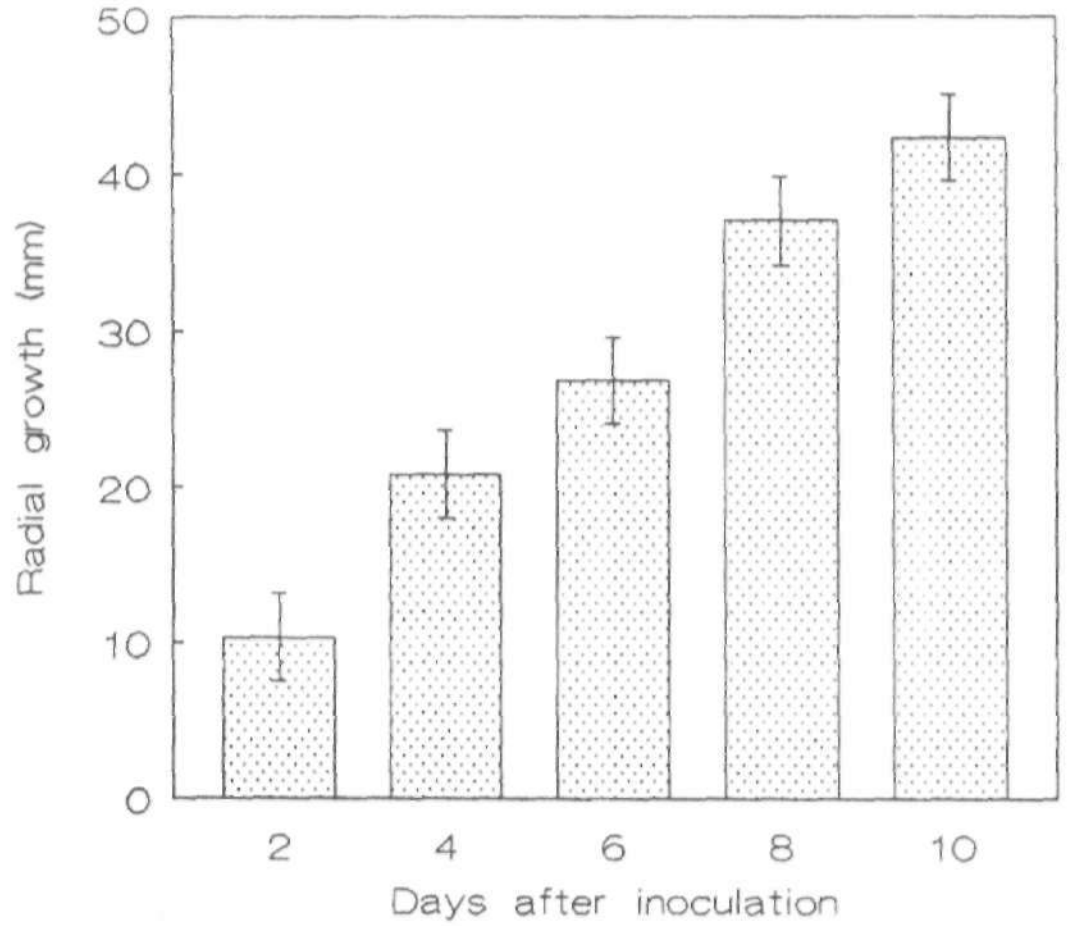


Fig. 2. Radial growth of Colletotrichum capsici on potato dextrose agar from 2nd to 10th day after inoculation at 25°C

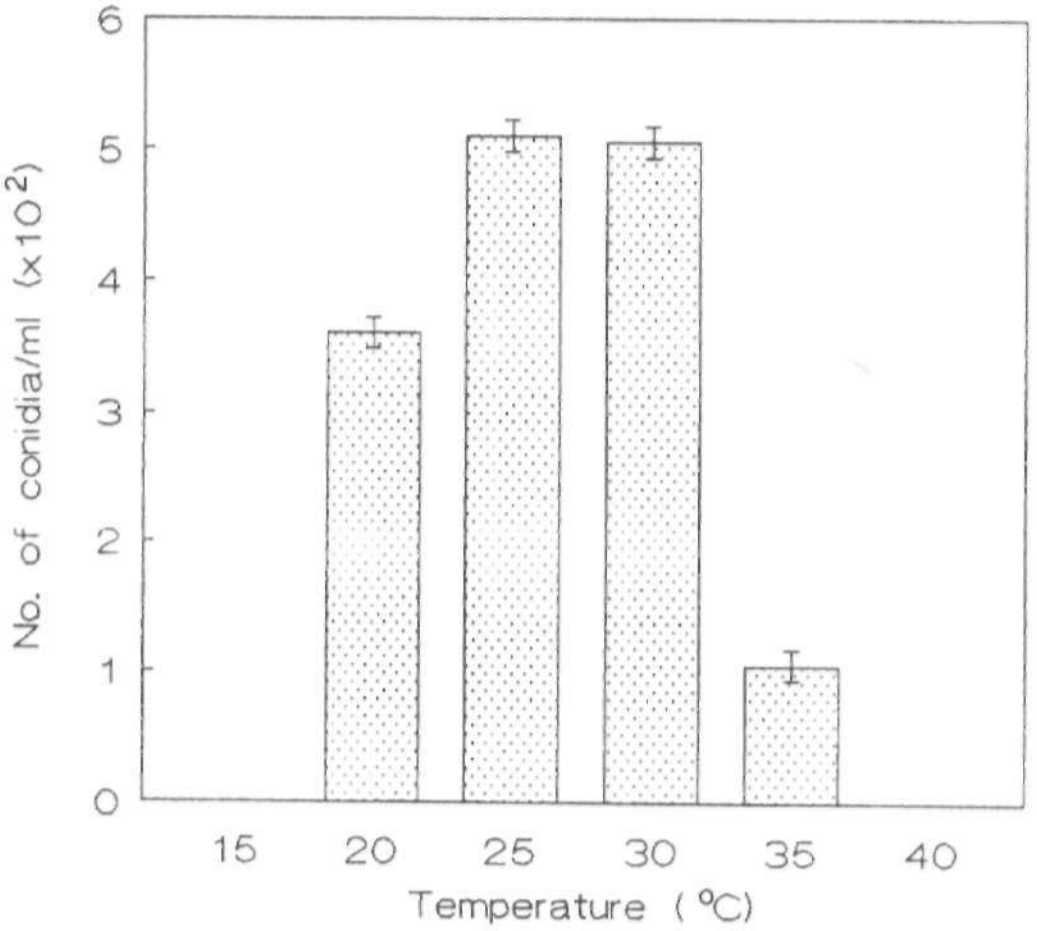


Fig. 3. Effect of temperature on sporulation of Colletotrichum capsici on potato dextrose agar at 10 days after inoculation

Table 1. Rate of growth of *Colletotrichum capsici* at different temperatures

Temperature (o_C)	Time after inoculation (h)	Radial* growth (mm)	Rate of growth (mm/h)
20	48	6.7	
	72	15.0	0.345
25	48	10.3	
	72	20.8	0.4375
30	48	10.8	
	72	20.5	0.4042
35	48	5.5	
	72	8.2	0.1125

* Mean of three replicates

The rate of growht v was calculated using the formula

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

X_1 = average radial growth at time t_1

X = average radial growth at time t_2

sporulation took place at 25°C. These results follow the same pattern with those obtained for radial mycelial growth.

4.2 Effects of Plant Age and Inoculum Density on Severity of Brown Blotch in the Glasshouse and in the Field

4.2.1 Glasshouse studies

The inoculated plants started showing symptoms of the disease 2 days after inoculation. By the third day, symptoms had appeared on all inoculated plants. This indicates that the incubation period for the fungus is 2 - 3 days, irrespective of the aerial part of plant inoculated and regardless of the plant age or inoculum density.

The disease severity scores obtained 10 days after inoculation are presented in Table 2. At low inoculum density, severity scores of disease in young plants aged 1 to 2 weeks at the time of inoculation were significantly higher ($P = 0.01$) than those in plants aged 4 to 6 weeks at the time of inoculation. With higher inoculum concentrations (1.25×10^6 conidia/ml and above) the severities were almost the same on plants inoculated at all ages studied, except that most of the plants inoculated when they were one week old died within 10 days of inoculation.

By 16 days of inoculation (Table 3) the disease severity in almost all the plants inoculated with even

Table 2. Effects of plant age at inoculation and inoculum density on severity of cowpea brown blotch at 10 days after inoculation in the glasshouse

Plant age at inoculation weeks	Disease severity in plants inoculated with inoculum containing :[a] X_0	X_1	X_2	X_3	X_4	X_5	X_6
1	1.0	8.0	8.7	10.0	10.0	10.0	10.0
2	1.0	7.3	8.0	8.0	8.0	8.0	8.0
3	1.0	4.7	8.0	8.0	8.0	8.0	8.0
4	1.0	6.0	8.0	8.0	8.0	8.0	8.0
5	1.0	4.0	6.0	8.0	8.0	8.0	8.0
6	1.0	2.0	4.0	6.0	8.0	8.0	8.0

L.S.D. (P = 0.01) 0.5292

a: X_0 - check, water spray; X_1 - 4.16×10^5 conidial/ml;

X_2 - 6.7×10^5 conidial/ml X_3 - 1.25×10^6 conidial/ml;

X_4 - 1.31×10^6 conidial/ml; X_5 - 2.68×10^6 conidial/ml and

X_6 - 3.75×10^6 conidial/ml.

Table 3. Effect of plant age at inoculation and inoculum density on severity of cowpea brown blotch at 16 days after inoculation in the glasshouse

Plant age at inoculation (weeks)	Disease severity in plant inoculated with inoculum containing :[a]						
	X_0	X_1	X_2	X_3	X_4	X_5	X_6
1	1.0	8.0	10.0	10.0	10.0	10.0	10.0
2	1.0	8.0	10.0	8.0	9.0	9.0	10.0
3	1.0	5.0	8.0	8.0	8.0	8.0	8.0
4	1.0	6.0	8.0	8.0	8.0	8.0	8.0
5	1.0	6.0	7.0	8.0	8.0	8.0	8.0
6	1.0	5.0	8.0	8.0	8.0	8.0	8.0

L.S.D. (P = 0.01) 0.6338

a: X_0 - check, water spray; X_1 - 4.16×10^5 conidial/ml;

X_2 - 6.7×10^5 conidial/ml X_3 - 1.25×10^6 conidial/ml;

X_4 - 1.31×10^6 conidial/ml; X_5 - 2.68×10^6 conidial/ml

and X_6 - 3.75×10^6 conidial/ml.

the suspension containing the lowest conidial concentration (4.16 x 10^5conidia/ml) was at least 8 (on a scale of 1-10); some plants had actually died. The check seedlings remained healthy during the experimental period.

Analysis of variance for the data in Table 3 shows that there were highly significant differences (P=0.01) in the severities of the disease in plants inoculated at different ages with suspensions having different inoculum concentrations. There was a highly significant (P=0.01) interaction between plant age at inoculation and inoculum concentration (Appendix 3).

4.2.2. Studies under natural field conditions

The results obtained under natural field conditions (Fig.4), were similar to those obtained in the glasshouse. There was a significant (P=0.05) interaction between plant age at inoculation and inoculum concentration (Appendix 4). Thus, regardless of the inoculum concentration, cowpea plants aged 2 to 3 weeks at the time of inoculation were more severely affected than older plants aged 4 to 6 weeks at the time of inoculation.

4.3 Latent Period of C. capsici on Different Parts of Cowpea Under Three Environmental Conditions

The three environmental conditions at which the latent period of C. capsici was determined were:

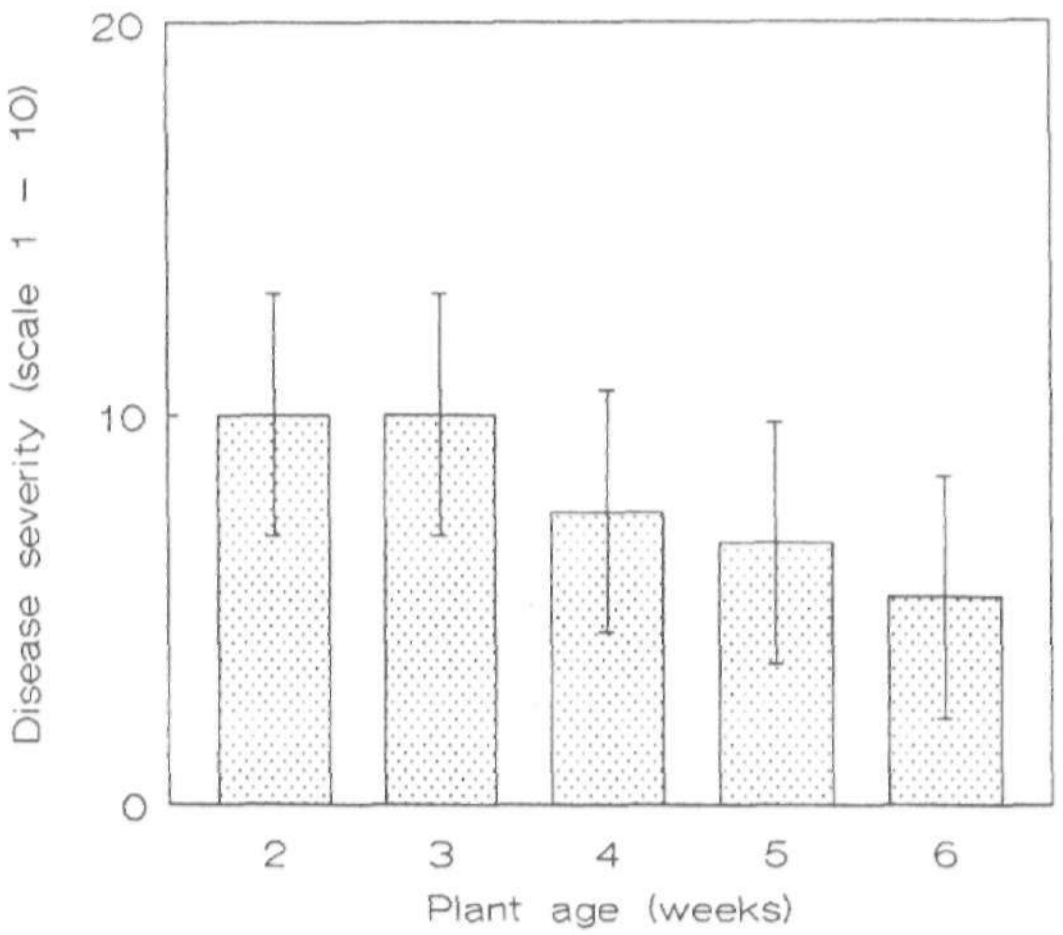


Fig. 4. Severity of brown blotch on cowpea of different ages at 3 weeks after inoculation

Table 4. The latent period of *Colletotrichum capsici* on different parts of cowpea under three environmental conditions

Plant part	Latent period (days) in the:		
	Glasshouse	Wire mesh cage	Polyethylene chamber
Stem	16	13	12
Petiole	6	5	5
Peduncle	8	8	7
Flower cushion	7	7	6
Pod	8	8	8
L.S.D. (P = 0.05)	2.0	1.8	1.7

(1) a glasshouse, where temperatures ranged from 24 to 50^{0}C and relative humidity values ranged from 43-62%;
(2) a wire mesh cage outside the glasshouse where temperatures ranged from 21 to 38^{0}C and relative humidity values from 46 - 90%
(3) A polyethylene chamber in the laboratory where temperatures ranged from 21 to 39^{0}C and relative humidity values ranged from 62 - 72%.

The results of the study of latent period of C. capsici on differnet parts of the plant under the above three environments are presented in Table 4. The latent period was shortest in the petiole lesions, being 6 days in the glasshouse and 5 days in the other two environmental conditions. The latent periods of lesions on the peduncle, flower cushion and pod were not significantly different (P=0.05) under the three conditions studied. However, there was a highly significant (P=0.05) difference between the latent period of stem and other plant parts. Thus the stem lesions had the longest latent period under the three conditions being as long as 16 days in the glasshouse; this was at least twice as long as the latent period of lesions in other plant parts at the same environment.

4.4 Effect of Sowing Date on the Incidence of Brown Blotch, Apparent Infection Rate and Cowpea Yield in the Field

The effect of date of sowing on the incidence of

brown blotch, apparent infection rate and grain yield of Ife Brown in 1986, 1987 and 1989 are presented in Tables 5, 6 and 7, respectively. In general, the incidence of brown blotch was higher in early-sown (third week of July or first week of August) cowpea plants than in those sown in the second and third week of August. The apparent infection rates of brown blotch were also higher in the early-sown than in the late-sown crop. The grain yield obtained in early-sown plots were higher than those of the late-sown plots, but the latter produced grains of higher quality than the early-sown crop.

The data obtained in 1986 (Table 5) showed that the disease incidence was generally low. However the incidence of infection on 6 october, 1986 in unsprayed plots sown on 22 July, 1986 was significantly (P=0.05) higher than the incidences of infection in crops sown on later dates. The apparent infection rates in unsprayed plots sown at different dates were significantly different (P=0.05) from each other. Specifically, the apparent infection rate in earliest-sown plots was significantly higher than that in later-sown plots. Although early-sown, unsprayed plots yielded higher than late-sown plots, the latter produced seeds of higher quality than early-sown plots.

Table 5. Effect of sowing dates on the incidence and apparent infection rate of brown blotch on cowpea yield in the field in 1986

Sowing date	Disease incidence on: 22/9/86 (t_1)*		6/10/86 (t_2)*		Calculated apparent infection rate (per unit/day) between t_2 and t_1		Grain yield (kg/plot	
	Sprayed	Unsprayed	Sprayed	Unsprayed	Sprayed	Unsprayed	Sprayed	Unsprayed
22 July	0.0	2.5	2.0	13.3	0.0	0.217	2.067	2.000
29 July	0.0	1.43	0.0	3.3	0.0	0.0213	2.43	2.033
5 August	0.8	1.43	1.67	3.3	0.107	0.0213	2.55	1.333
11 August	0.0	0.47	0.0	1.67	0.0	0.0310	2.33	1.733
L.S.D. (P = 0.05)	2.36		7.42		0.05		0.55	

* t_1 t_2 = days on which disease incidence scores were taken.

Table 6. Effect of sowing dates on the incidence and apparent infection rate of brown blotch and on cowpea yield in the field in 1987

Sowing date	Disease incidence on: 30/9/87 (t_1)*		8/10/87 (t_2)*		Calculated apparent infection rate (per unit/day) between t_2 and t_1		Grain yield (kg/plot	
	Sprayed	Unsprayed	Sprayed	Unsprayed	Sprayed	Unsprayed	Sprayed	Unsprayed
27 July	0.7	2.7	1.3	7.7	0.0	0.14	1.67	1.3
3 August	3.8	23.3	10.4	30.3	0.03	0.065	1.87	1.17
10 August	13.5	25.3	14.9	28.1	0.014	0.018	1.87	1.97
17 August	16.4	25.8	16.4	27.7	0.0	0.012	1.4	1.37
L.S.D. (P = 0.05)	15.58		16.98		0.09		0.81	

* t_1 , t_2 = days on which disease incidence scores were taken.

Table 7. Effect of sowing dates on the incidence and apparent infection rate of brown blotch and on cowpea yield in the field in 1989

Sowing date	Disease incidence on: 14/9/89 (t_1)*		25/9/89 (t_2)*		Calculated apparent infection rate (per unity/day) between t_2 and t_1		Grain yield (kg/plot	
	Sprayed	Unsprayed	Sprayed	Unsprayed	Sprayed	Unsprayed	Sprayed	Unsprayed
25 July	35.8	55.3	47.2	65.2	0.045	0.038	0.24	0.89
1 August	34.5	41.8	50.6	67.5	0.073	0.096	1.27	0.30
8 August	28.4	37.4	35.6	50.3	0.0339	0.051	1.45	0.65
15 August	14.9	27.91	16.2	37.0	0.0142	0.0244	0.75	0.37
L.S.D. (P = 0.05)	23.78		17.48		0.06		0.54	

* t_1 , t_2 = days on which disease incidence scores were taken.

The disease incidences in 1987 (Table 6) were higher than those of 1986. In 1987 there were significant differences (P=0.05) in the disease incidences in unsprayed plots sown on different dates (Table 6). The disease incidence in the earliest-sown plants (planted on 27 July 1987) was significantly (P=0.05) lower than the incidences in later sown crops; this contrasts with the data obtained in 1986.

The apparent infection rate in the unsprayed, earliest sown plot was significantly higher than that of the two later-sown plots. The grain yield obtained from the sprayed plot sown on 17 August, 1987 (1.4kg/plot) was 25% lower than that obtained from the plot sown on 3 August, 1987 (1.87 kg/plot).

The disease incidences in 1989 (Table 7) were higher than those of 1986 and 1987. Generally, plants sown early and unsprayed 46 had higher disease incidences than those sown late. For example, the incidence of infection in plots sown 01 August 1989 was 67% (Plate 1) while that in plots planted two weeks later (15 August 1989) was only 37% (Plate II). Similarly, the apparent infection rate (0.096 unit/day) in plots sown early, 01 August, 1989, was significantly higher than that in plots sown on 15 August, 1989, (0.024 unit/day)

In general, early-sown plots that received no fungicidal treatment had higher grain yield than the



Plate I. Effect of planting date on disease development: plot planted on 1 August, 1989 which received no fungicidal spray, note the shrivelled pods.



Plate II: Effect of planting date on disease development: plot planted on 15 August, 1989 which received no fungicidal spray, note the healthy green pods.

late-sown plots. Plots sown 25 July, 1989 gave a yield of 0.89 kg per plot which was 58% higher than the yield of 0.37 kg per plot obtained from plots sown three weeks later on 15 August, 1989; the differences being statistically significant. However, seeds from the early-sown plots were generally wrinkled, and hence were of lower quality than the seeds from late-sown plots.

4.5 Disease Progress

4.5.1 The incidence of brown blotch with time

The incidence of brown blotch on Ife Brown variety recorded at weekly interval starting from 4 weeks after planting are presented in Table 8, in respect of the data obtained in 1984, 1985, 1989. In each of the 3 years there was a significant (P=0.05) increase in the amount of disease with time. The disease progress graphs were obtained by plotting Log_{10} X/1-X against time, where X is the proportion of diseased plant, are presented in Figures 5,6 and 7 in respect of the data obtained in 1984, 1985 and 1989 respectively. The gradient of the best straight line obtained for each graph was calculated. In 1984, the gradient, which is an estimate of apparent infection rate, was 0.38 per unit per week or 0.05 per unit per day which was comparable to 0.35 per unit per week or 0.05 per unit per day obtained in 1985. However, in 1989 the apparent infection rate was 0.16 per unit per week or 0.021 per unit per day.

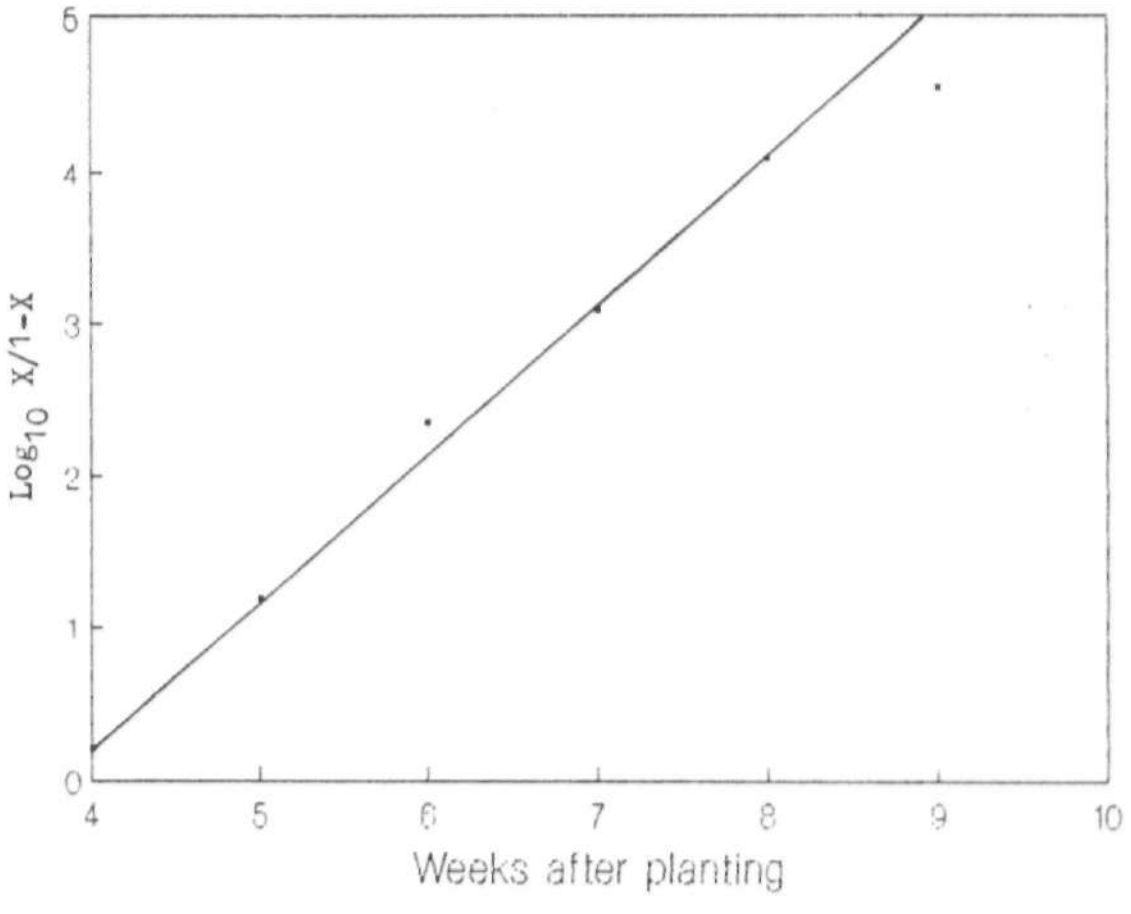


Fig. 5. Progress of brown blotch in 1984

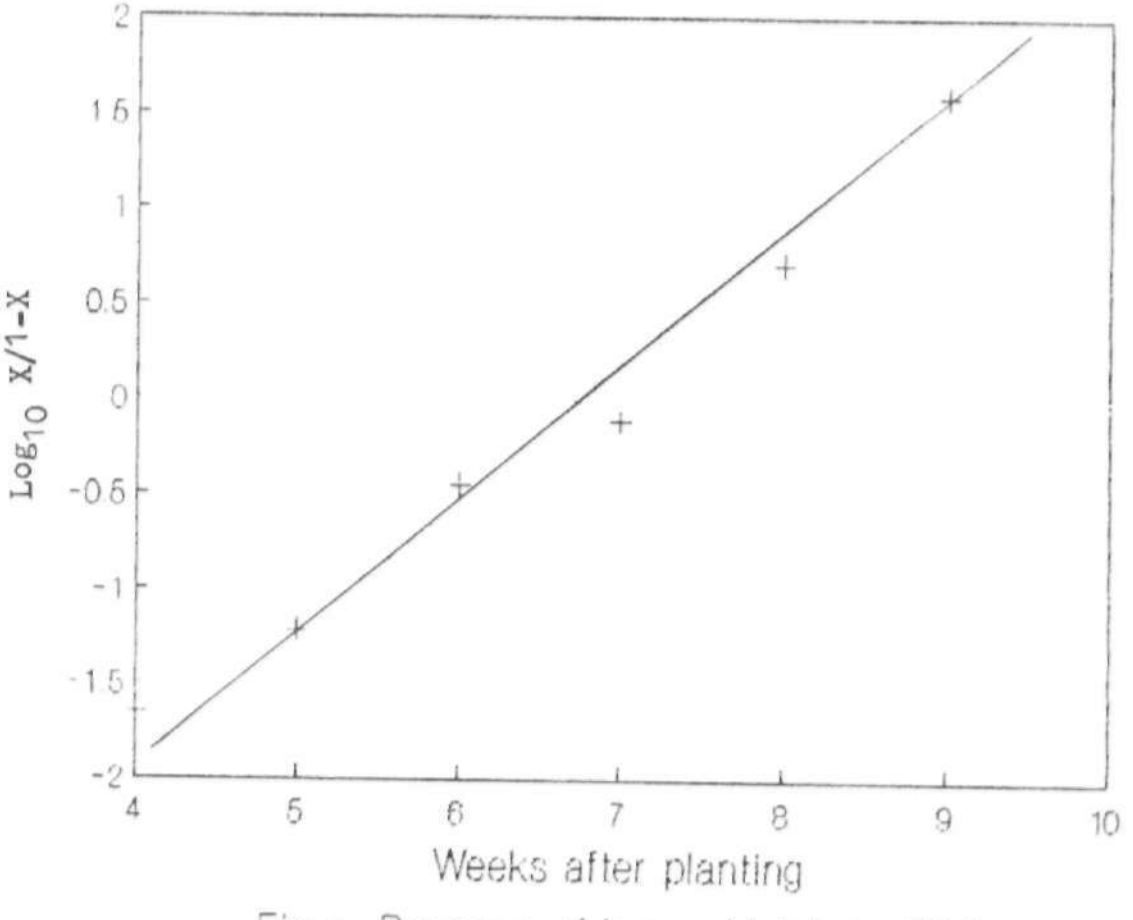


Fig. 6. Progress of brown blotch in 1985

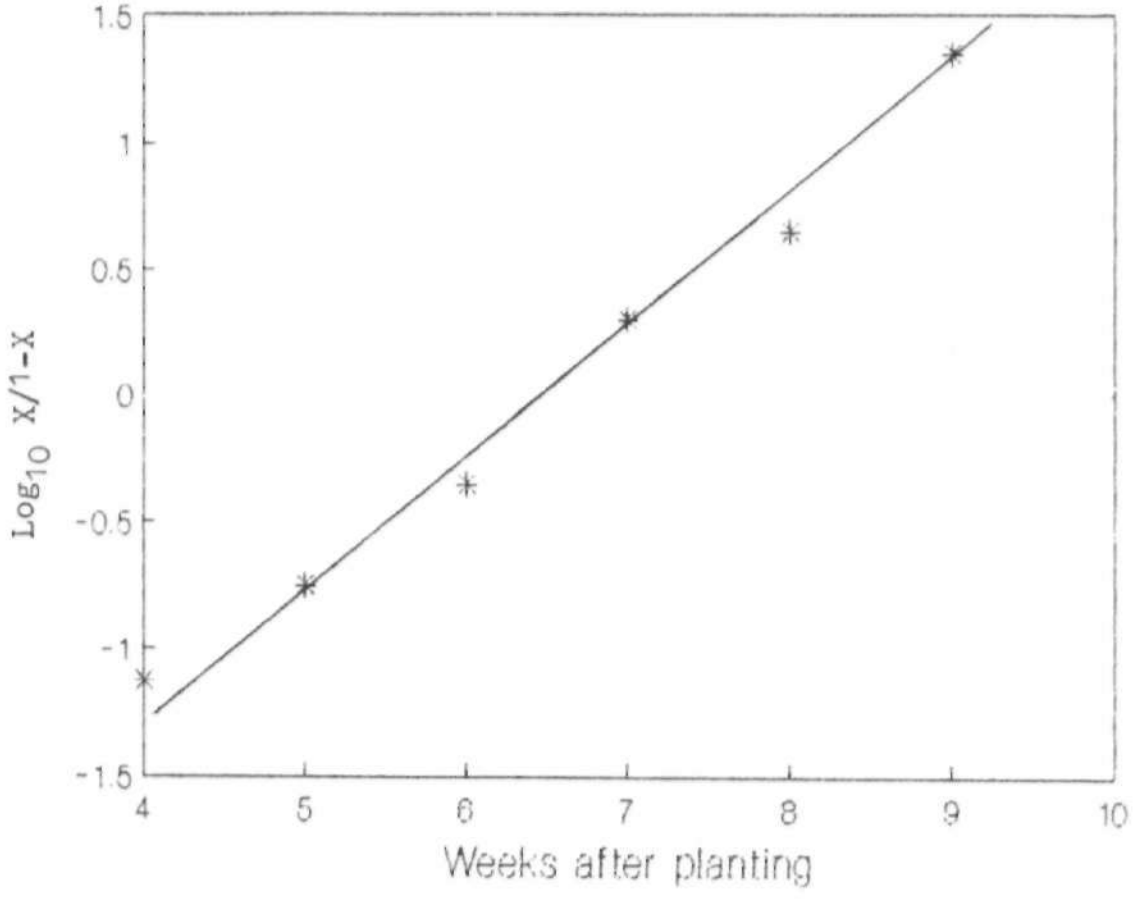


Fig. 7 Progress of brown blotch in 1989

Table 8: Incidence of brown blotch on Ife brown in 1984, 1985 and 1989 in the field at Samaru

Weeks after planting	Disease incidence (%) in: 1984	1985	1989
4	54.9	16.0	24.4
5	76.8	22.9	32.0
6	91.3	38.9	42.3
7	95.6	47.1	57.4
8	98.2	67.1	65.7
9	98.8	83.1	79.2
L.S.D. (P=0.05)	5.8	3.0	4.7

4.5.2 The relationship between weekly increase in disease incidence, weekly rainfall and weekly relative humidity in the field

The amount of weekly rainfall and average weekly relative humidity recorded during the planting season in 1985 and 1989 are presented in Appendices 5 and 6 respectively. Figure 8 presents the weekly increases in disease incidence, weekly relative humidity values and the corresponding weekly rainfall for 1985. The highest amount of rainfall per week (70.4mm) and the corresponding high relative humidity (77%) were recorded at 4 WAS, the very week that inoculation was done and at which scoring of the disease started.

Although the amount of rainfall during the sixth week of planting was 51.8 mm, the relative humidity was high especially at morning hours (82%); these conditions were associated with 16% increase in disease incidence during that week. The amount of rainfall was 50.6 mm during the eighth WAS, almost the same as the 51.4 mm of the seventh week, the corresponding high average relative humidity (78%) was associated with the highest (20%) weekly increase in disease incidence. The decrease in the amount of rainfall (25.7mm) during the nineth WAS with a corresponding average relative humidity of 67% was still associated with 16% increase in disease incidence that week.

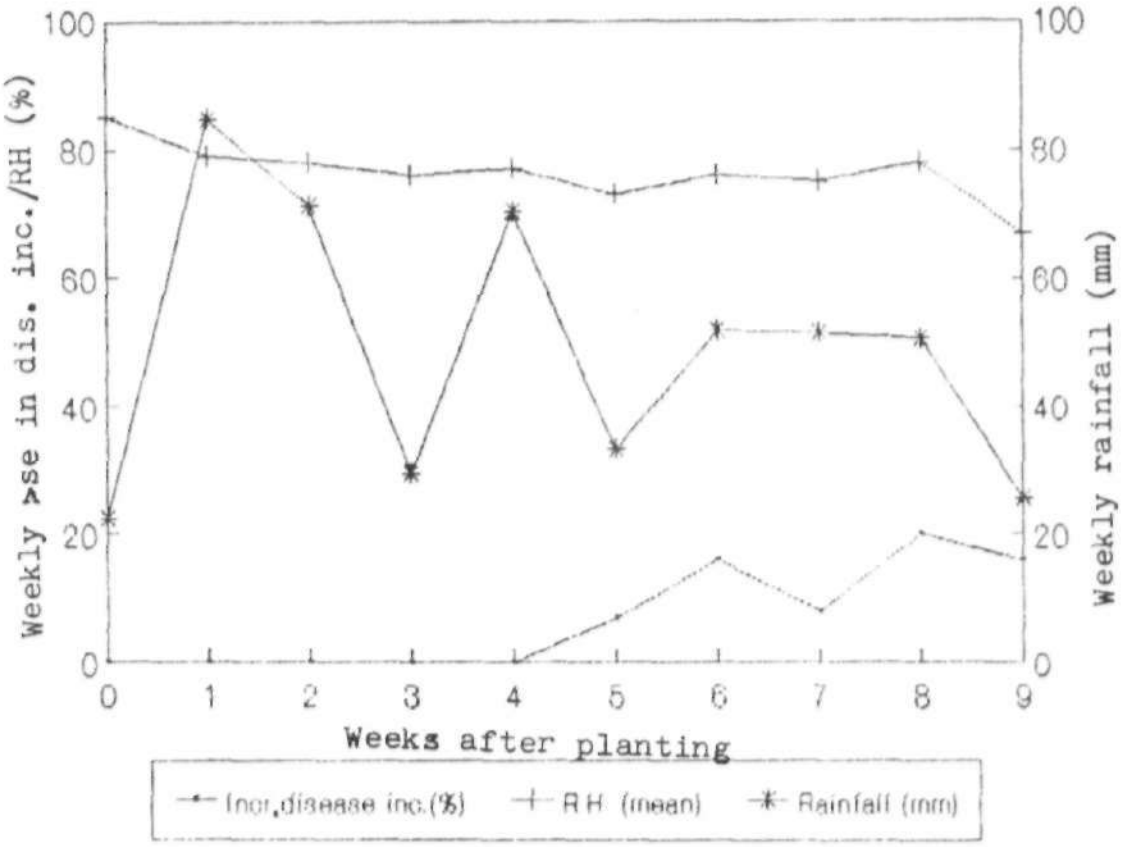


Fig. 8. Pattern of weekly increase in disease incidence, relative humidity, and weekly rainfall in 1985

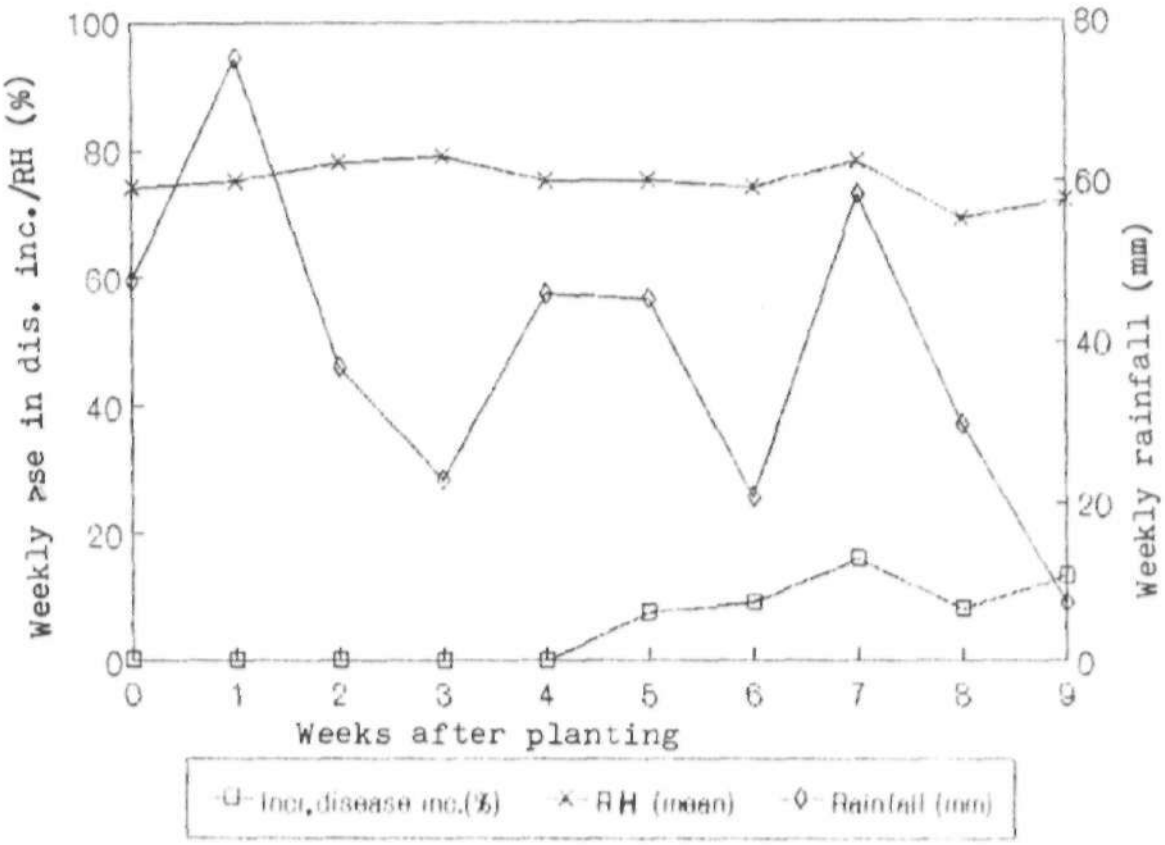


Fig. 9. Pattern of weekly increase in disease incidence, relative humidity and weekly rainfall in 1989

The results obtained in 1989 are presented in Figure 9. The amounts of rainfall during the fourth and fifth WAS were 46 and 45.3 mm respectively and this reduced to 20.7 mm during the sixth week after planting; however, the corresponding relative humidity for the week remained high (74%). These conditions were associated with an increase in the weekly disease incidence from 0% at 4 WAS and 7.6% at 5 WAS to 9% at 6 WAS. At 7 WAS the amount of rainfall increased to 58.3 mm with a corresponding increase in relative humidity (78%) which was associated with 16% increase in weekly disease incidence which was the highest for the year. At 8 WAS there was a decline in the amount of rainfall with a corresponding slight reduction in average relative humidity which was associated with 8.3% increase in weekly disease incidence. Although the amount of rainfall declined continuously after the eighth week, the relative humidity remained high being 72% in the nineth week during which weekly increase in disease incidence was 13%.

4.6 Response of Cowpea Cultivars to Infection by *Colletotrichum capsici* in the Field at Samaru in 1988 and 1989

The results obtained for brown blotch incidence and calculated apparent infection rate on three cowpea cultivars which differ in their susceptibilities to the disease are presented in Tables 9 and 10 for 1988 and

Table 9: Response of cowpea cultivars to infection by *C. capsici* in 1988 in the field at Samaru

Cowpea variety	Stem blotch incidence on: 20/9/88 (t_1)	30/9/88 (t_2)	Apparent infection rate (r) between t_1 and t_2 per unit/day
Ife Brown	31.1	48.8	0.08880
SAMPEA 2	16.4	17.7	0.0065
Tvx 3236	0.0	0.0	0.00
L.S.D. (P = 0.05)	14.79	23.89	0.03

Table 10: Response of cowpea cultivars to infection by *C. capsici* in 1989 in the field at Samaru

Cowpea cultivar	Stem blotch incidence on: 13/9/89 t_1	28/9/89 t_2	Apparent infection rate (r) per unit/day between t_1 and t_2
Ife Brown	28.3	51.1	0.06652
SAMPEA - 2	20.1	36.7	0.0532
TVx 3236	0.0	0.0	0.0
L.S.D. (P = 0.05)	6.68	18.21	0.03

1989 respectively. There were significant differences (P=0.05) between the incidences of brown blotch in the three cultivars.

In 1988 the disease incidence on 30 September and apparent rate of infection between 20 and 30 September in Ife Brown plants were significantly (P=0.05) higher than those in SAMPEA-2 or TVx 3236. The reactions of the three varieties to brown blotch were distinctly different from each other. Thus, Ife Brown had the highest incidence of the disease while TVx 3236 remained free of any of the external symptoms of the disease (Plates III and IV); the reaction of SAMPEA-2 was between the two extremes. Similarly, the apparent infection rate in the last 10 days of September 1988 was 0.09 per unit/day in Ife Brown, 0.007 per unit per day (7.3% that in Ife Brown) in SAMPEA-2 and zero in TVx 3236.

In 1989 the incidence of brown blotch in Ife Brown increased from 28% on 13 September, to 51% on 28 September, the corresponding figures for SAMPEA-2 were 20 and 37% (Table 10) again TVx 3236 carried no symptoms of the disease. However, the apparent infection rates between 13 and 28 September in Ife Brown and SAMPEA-2 were not significantly different from each other.

4.7 Assessment of Yield Losses in Cowpea Due to Brown Blotch

The results obtained for yield losses in cowpea due to brown blotch in 1986 are presented on Table 11. The different frequencies of fungicidal sprays generated different levels of disease in 1986 during which Ife Brown variety was used for the study. Thus, at 8 weeks after sowing the disease incidence varied from 0% for the plots that received the best control of the disease to 58.2% in the plots that received no spray. The results indicate that brown blotch attack was associated with 44% grain yield loss in the plots that received no fungicidal treatment (Table 11). The data presented in Table 11 and Figure 10 show that the yield obtained in the plots with best brown blotch control (T_1) was about 44% higher than that of the unsprayed check.

The data obtained in 1987 are presented on Tables 12 and 13 for varieties IT 83S-742-11 and IT 83S-728-13, respectively. The incidence of brown blotch was low on the two varieties, especially for IT 83S-728-13. However, these low incidences (10-25% and 5-15%) were still associated with 10% and 36% yield losses in varieties IT 83S-742-11 and IT 83S-728-13, respectively.

The results obtained in 1989 are presented on Tables 14 and 15 for varieties IT 86D-1056 and IT 86D-1057 respectively. The Tables show that varying the frequency

Table 11: Relationship between the incidence of brown blotch and grain yield of Ife Brown plants sprayed at different frequencies in 1986

Treatment	Brown blotch incidence (%) on: 24/9/86	8/10/86	Grain yeild (kg plot)	Grain yield (t/ha)	Grain yield loss (%)
T_1	0.0	0.0	2.68	1.78	0.00
T_2	25.3	29.9	2.33	1.55	12.92
T_3	28.3	38.95	1.88	1.25	29.78
T_4	42.0	55.33	1.85	1.23	30.90
T_5	50.41	50.40	1.83	1.22	31.46
T_6	58.2	68.90	1.50	1.00	43.82
L.S.D. (P = 0.05)	14.59	13.59	0.31	0.21	43.82

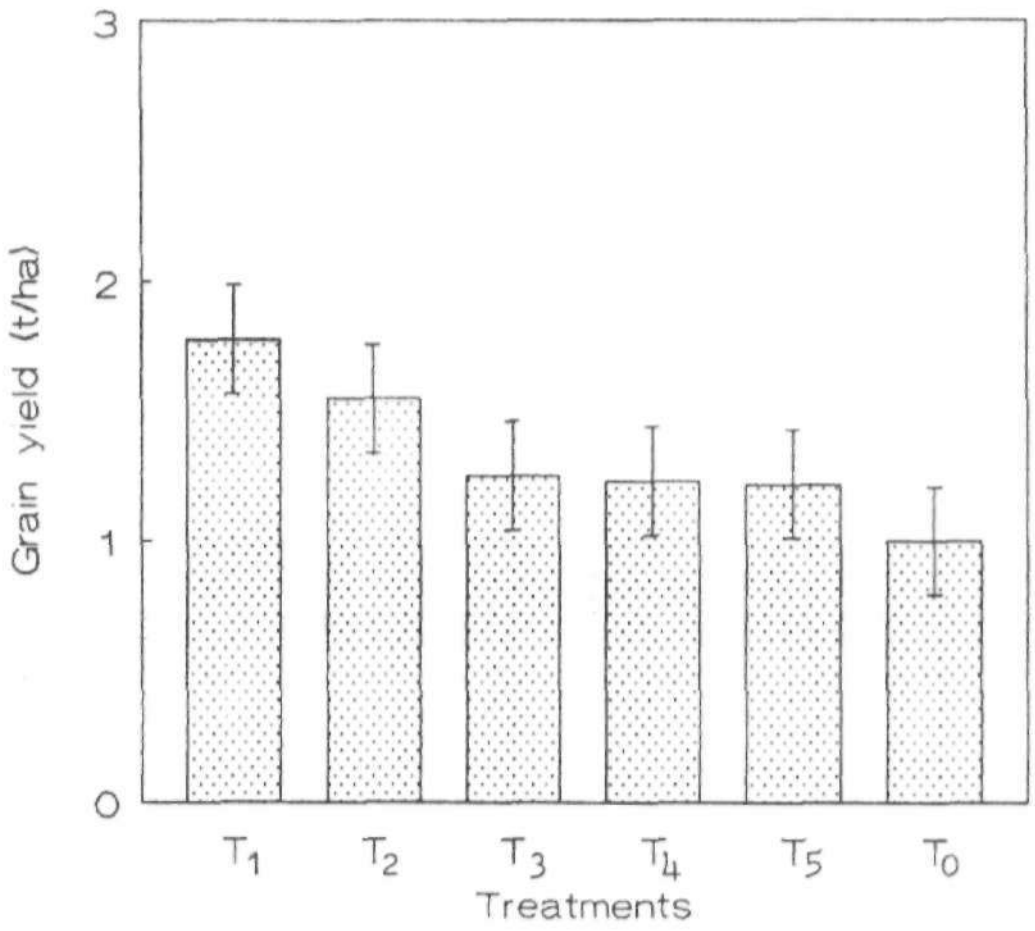


Fig. 10. Crop loss in cowpea due to brown blotch in Ife Brown in 1986

T_o = no spray; T_1 = sprayed twice a week;

T_2 = sprayed weekly; T_3 = sprayed biweekly;

T_4 = sprayed every 3 weeks; T_5 = sprayed every 4 weeks

Table 12. Relationship between the incidence of brown blotch and grain yield of variety IT 83S - 742 - 11 sprayed at different frequencies in 1987

Treatment	Brown blotch incidence (%)	Grain yield (kg/plot)	Yield loss (%)
T_1	0.0	2.43	0.00
T_2	10.0	2.22	8.46
T_3	20.0	2.27	6.58
T_4	20.0	2.20	9.47
T_5	20.0	2.33	4.12
T_o	25.0	2.18	10.3

Table 13: Relationship between the incidence of brown blotch and grain yield of variety IT 83S - 728 - 13 sprayed at different frequencies in 1987

Treatment	Brown blotch incidence (%)	Grain yield (kg/plot)	Yield loss (%)
T_1	0.0	2.4	0.00
T_2	5.0	2.3	4.16
T_3	10.0	2.23	7.08
T_4	10.0	2.20	8.30
T_5	10.0	2.00	16.66
T_6	15.00	1.53	36.25

Table 14. Relationship between severity of brown blotch on different parts and yield of variety IT 86 D - 1056 sprayed at different frequencies in 1989

Treatment	Severity of brown blotch on:[a]				Grain yield kg/plot)	Grain yield (t/ha)	Grain yield loss (%)
	Stem	Flower cushion	Pod	Peduncle			
T_1	1.0	1.0	1.0	1.0	1.75	2.3	0.0
T_2	2.0	2.0	2.0	2.0	1.63	2.18	5.22
T_3	3.3	3.3	4.0	2.7	1.39	1.86	19.13
T_4	4.7	4.7	6.0	2.7	1.40	1.85	19.57
T_5	5.3	5.3	6.7	4.7	1.08	1.44	37.39
T_0	6.7	6.0	7.3	7.3	1.09	1.45	36.96
L.S.D (P = 0.05)	1.72	1.43	2.10	1.27	0.36	0.48	

a: Disease severity at 8 weeks after sowing using a scale of 1-10 - where 1 means no disease and 10 maximum disease score.

Table 15. Relationship between severity of brown blotch on different plant parts and yield of variety IT 86 - D - 1057, sprayed at different frequencies in 1989

Treatment	Severity of brown blotch on:[a]				Grain yield (kg/plot)	Grain yield (t/ha)	Grain yield loss (%)
	Stem	Flower cushion	Pod	Penducle			
T_1	1.0	1.0	1.0	1.0	1.47	1.96	0.00
T_2	2.0	2.0	2.0	2.0	1.39	1.86	5.10
T_3	2.7	3.3	4.7	2.7	1.27	1.70	13.26
T_4	2.7	4.7	5.3	3.3	1.23	1.64	16.33
T_5	4.0	4.7	6.7	5.3	1.11	1.48	24.48
T_0	5.3	5.3	8.0	7.3	1.07	1.43	27.04
L.S.D (P = 0.05)	1.58	1.76	1.27	1.58	0.40	0.53	

a: Disease severity scores taken at 8 weeks after sowing using a scale of 1-10 in which 1 means no disease and 10 is the maximum disease score

of foliar-applied fungicides generated different severities of the disease on different plant parts of the two varieties used.

Brown blotch was associated with the low yield obtained in the plots in which the disease was not controlled. Plants of IT86D-1056 that received good disease control (T_1) had clean, well-filled pods (Plate V), while the plants that received no fungicidal spray had heavily infected pods (Plate VI). Figure 11 shows clearly the differences in grain yield obtained for the different treatments. The yield of 1.45 t/ha obtained from the unsprayed plot was significantly (P=0.05) lower than that of 2.3 t/ha obtained from the plots that received the best control of the disease and led to about 37% loss in yield.

Similarly, data presented in Table 15 and Figure 12 in respect of variety IT86D-1057, show that the unsprayed plot yielded 1.43 t/ha while the plots that had the least disease severity produced 1.96 t/ha; although the difference in weight was not significant statistically. However, the grains from the unsprayed plots were of lower quality (i.e. they were wrinkled) than those from the plots with the least disease.

Table 16 shows the correlation coefficents for disease severity on the different plant parts and grain yield. The correlation between disease severity on any



Plate V: Plot of IT86D - 1056 sprayed twice a week (T_1) showing healthy brown blotch-free pods; the peduncle is naturally brown.



Plate VI: Plot of IT86D - 1056 which was not given foliar-applied fungicidal spary (T_0) arrow shows severe brown blotch lesions on the pods.

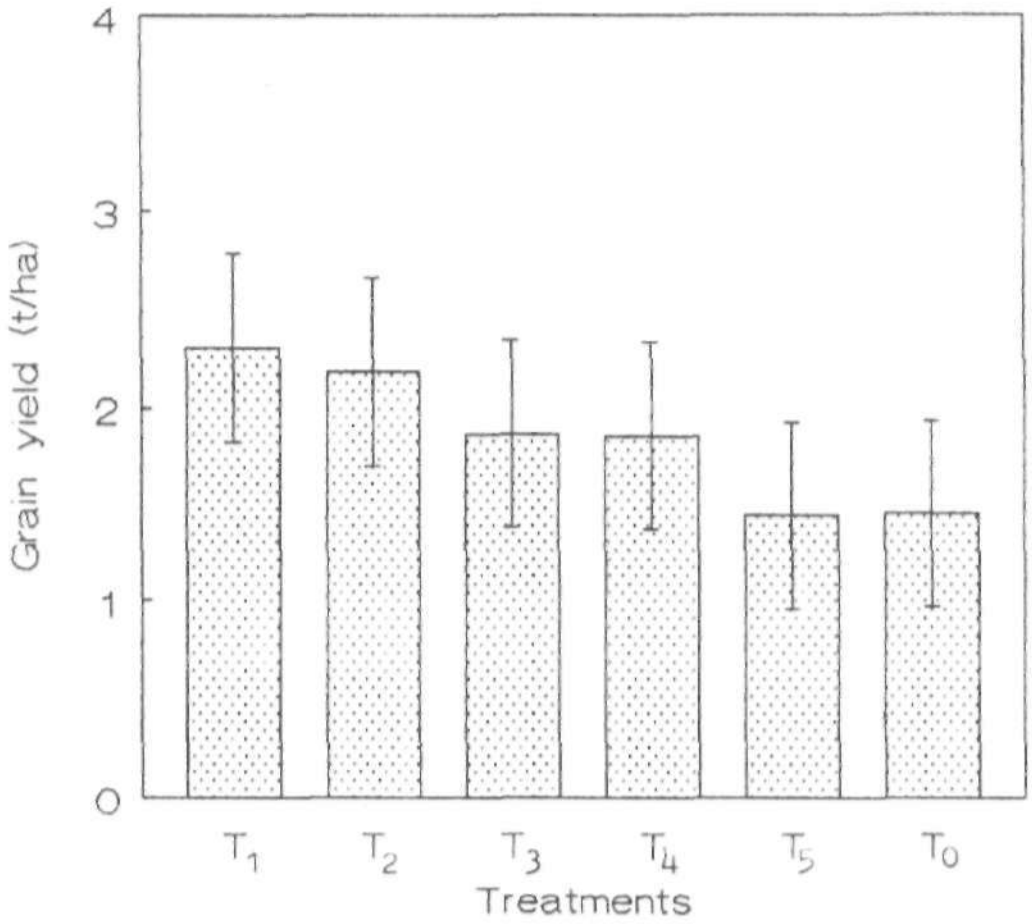


Fig. 11. Crop loss in cowpea due to brown blotch in IT 86D-1056 in 1989

T_0 = no spray; T_1 = sprayed twice a week;

T_2 = sprayed weekly; T_3 = sprayed biweekly;

T_4 = sprayed every 3 weeks; T_5 = sprayed every 4 weeks

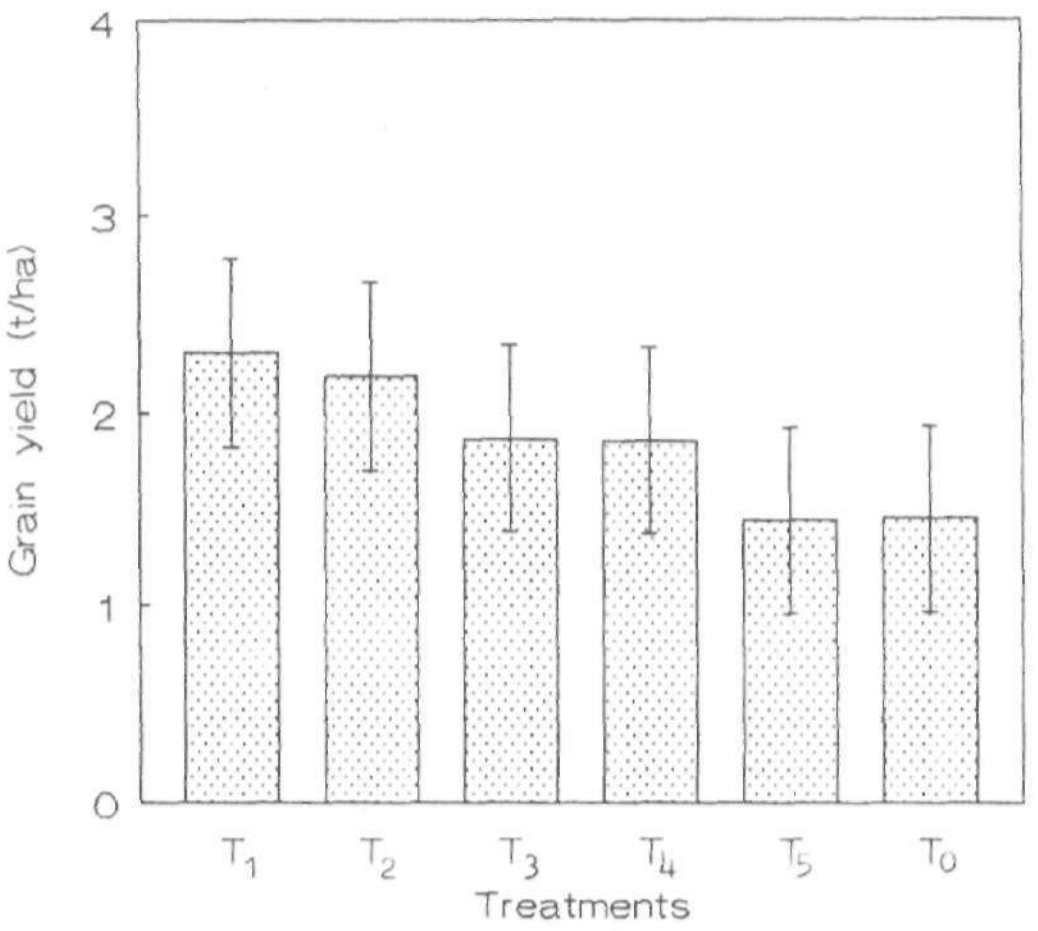


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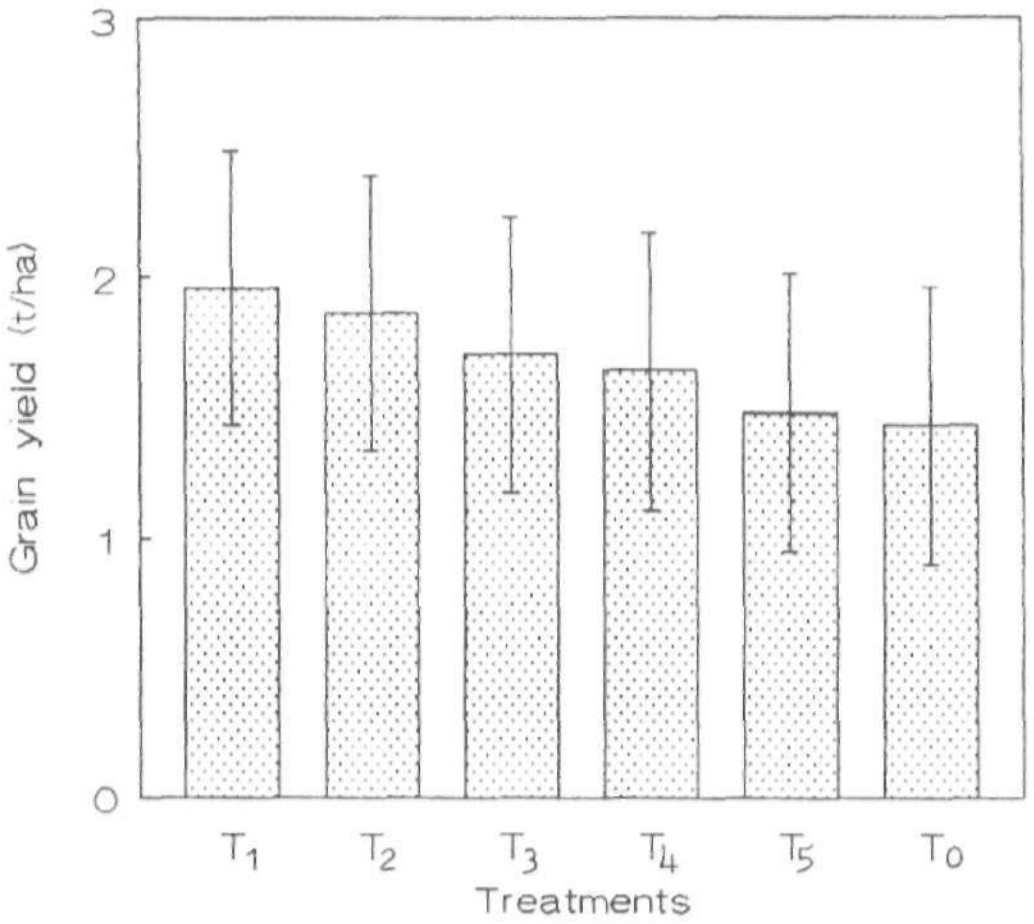


Fig. 12. Crop loss in cowpea due to brown blotch in IT 86D-1057

T_0 = no spray; T_1 = sprayed twice a week;

T_2 = sprayed weekly; T_3 = sprayed biweekly;

T_4 = sprayed every 3 weeks; T_5 = sprayed every 4 weeks

Table 16. Correlation coefficient among the disease severity scores at 8 weeks after sowing on different plant parts and between respective severity scores and grain yield of variety IT86D-1056

Stem	Stem blocth severity	Flower cushion blotch severity	pod blotch severity	Peduncle blotch severity	Grain yield (per plot)
Stem blotch-severity	1.0	0.97	0.896	0.801	-0.49
Flower cushion-blotch severity		1.0	0.894	0.869	-0.548
Pod blotch severity			1.0	0.779	-0.576
Peduncle blotch-severity				1.0	-0.489

of the plant parts and grain yield was negative and significant. The highest negative correlation (r=-0.576) was between pod blotch severity and grain yield.

CHAPTER FIVE

DISCUSSION

The results of the present study have shown that both growth and sporulation of the cowpea brown blotch pathogen, Colletotrichum capsici, were greatly influenced by temperature. The optimum temperature (25^{0}C) for radial growth on potato dextrose agar incorporated with streptomycin was the same for sporulation of the fungus. These results are almost similar to those obtained by Misra and Mahmood (1960) and Solanki et al. (1974) who reported optimum temperature of 25^{0}C for conidial germination of the Capsicum isolate of C. capsici. However, the results differ from those of Singh et al. (1977) who found that the optimum temperature for growth of Capsicum isolate of the fungus was 30^{0}C.

Metereological data obtained from the Institute for Agricultural Research, Samaru, showed that mean minimum and maximum air temperatures during the growing season (July-October) were 21.5 and 31.3 ^{0}C, respectively. This indicates that in general, the air temperatures during the growing season in Samaru are conducive for both growth and sporulation of the fungus.

Plants aged 1-6 weeks which were inoculated with the conidial suspension of the brown blotch pathogen were infected at all inoculum densities tested in the present study. However, plants aged 3 to 6 weeks at the time of

inoculation were not as severely affected as those inoculated at 1 or 2 weeks of age. In addition, younger plants exposed to high inoculum densities were killed while older plants, though severely affected, did not die even at the highest inoculum density (3.67×10^6 conidia per ml). These results agree with those of Coronel and Chaves (1975) who found that reactions of 5-15 day old French bean plants to anthracnose (C. lindemuthianum) were similar in all the five cultivars inoculated and that they suffered higher disease severity than 35 day-old plants which were the least susceptible, even when inoculated with the highest inoculum concentration. Mature plant resistance has been reported for French bean anthracnose (Wheeler, 1982). It has also been reported that young green fruits of Capsicum annuum were more susceptible to anthracnose of chilli pepper than mature fruits (Asuti and Suhardi, 1986).

Cowpea sown in the third week of July or the first week of August had both higher disease incidence and higher apparent infection rate than later-sown cowpea crops. These results corroborate those of Emechebe (1984) who reported that early-sown cowpea had higher severity of brown blotch than later-sown cowpea.

The high and frequent rainfall in July and early August creates conducive environment (high relative humidity and relatively cool temperatures) for the

development of primary lesions of brown blotch on cowpea. This probably explains why there was high incidence of infection and high apparent infection rate of the disease in the early-sown crops. These results agree with those obtained in Colombia where it was found that excessive rainfall reduced yield of French beans sown in October or November due to high incidence of anthracnose (C. lindemuthianum) on bean (CIAT, 1976).

Although the grain yield from late-sown unsprayed plots were low, the seeds were of higher quality than those from early-sown plots. Thus, farmers in Northern Guinea savanna who cannot afford the high cost of fungicide can plant medium maturity cowpea in the second week of August and still obtain modest yields of grains, the quality of which would be much higher than those of earlier sown crops. The high quality seeds thus obtained can be sown the following year, thereby reducing the initial infection on the crop which normally comes from seed-borne inoculum.

The results of the present study reveals that C. capsici sporulated faster on the tender tissues of the petioles and flower cushions than the more woody stem tissues. The temperatures in the polyethylene chamber (21^{0}C to 39^{0}C) and wire mesh cage (21 to 38^{0}C) which were lower than the range in the glasshouse (24 to 50^{0}C) apparently favoured sporulation of the pathogen at least

one day earlier on stem, petiole and flower cushion in the former environment than in the latter. This agrees with Emechebe (1986) who found that day/night temperature regimes of 35/26^{0}C and 28/23^{0}C (similar to temperatures in the polyethylene chamber and wire mesh cage) was conducive to brown blotch pathogenesis in a highly susceptible cowpea variety such as Ife Brown, which was used for the present study. This result also agree with the data in the present study with respect to sporulation of C. capsici in vivo which revealed that the optimum temperature for the sporulation of C. capsici is 25^{0}C. The data obtained for latent period (5-16 days for the different plant parts) is also of practical significance in the timing of the application of fungicides for the control of brown blotch. The interval of spray of fungicides against brown blotch should be between 5-14 days. This agrees with the report by Alabi and Emechebe (1990) that foliar application of fungicides at 7-14 days interval controlled brown blotch of cowpea.

The results of this study suggest that once there is enough rainfall for the establishment of brown blotch, the critical factor necessary for the development of the disease appears to be relative humidity. However, rainfall is still important for brown blotch development, not only because relative humidity depends on it but because rainsplash and wind-driven droplets of rain are

important in the spread of brown blotch from plant to plant (Emechebe,1981a).

The high amount of rainfall preceeding the inoculation of the plants (that is before or at 4 weeks after planting did not have as much effect on the disease compared to the high rainfall that occurred after inoculation, such as that recorded at 7 WAP in 1989 with the corresponding high relative humidity which was associated with the highest weekly increase in disease incidence in 1989. This result corroborated those of Sindhan (1983) who found that for French bean anthracnose, a pre-inoculation moist period increase infection but was not as effective as post-inoculation moist period for the initiation and development of the disease.

The results also show that brown blotch development occurs at relative humidities as low as 67% although much higher values appear more favourable to the disease, as evident by the data obtained in the two years of study. These results are similar to those of Sartorato (1988) who recorded that high relative humidity (>70%) favoured the development of anthracnose of bean (Phaseolus vulgaris).

Varieties Ife Brown, SAMPEA-2 and TVx 3236 had marked differences in their reaction to brown blotch. This study confirms the earlier report by Okpala (1981)

that Ife Brown was highly susceptible and SAMPEA-2 was moderately susceptible since both varieties had high incidences of the disease. TVx 3236 was not affected in all the three years of study which means it is resistant to the isolate of C. capsici at Samaru. This variety was, however, found to be susceptible to another isolate of C. capsici Ik-19 in the southern part of Nigeria (Emechebe, 1986).

Crop loss due to brown blotch in cowpea ranged from 5 to 44% depending on the susceptibility of the variety and the year the trial was conducted. The other fungal diseases of cowpea were practically absent on the varieties used; as such the damage by brown blotch was responsible for the observed yield losses. The weather conditions also affect the varietal susceptibility. Losses obtained in this study are lower than those of Emechebe (1986) and Okpala (1981) who recorded 75% and 100% yield losses respectively due to brown blotch in susceptible varieties. The higher losses recorded in their studies may be due to other factors such as highly favourable environmental conditions, confounding effects of other cowpea diseases and differences in susceptibility of varieties.

From the results obtained in this study, brown blotch can be considered as one of the limiting factors to cowpea production in Nigeria. Consequently,

considerable benefit can be derived from its control. The total elimination of the disease may not be possible due to evolution of new races of the pathogen and the subsequent loss of resistance in cowpea cultivars, but measures to reduce its impact can be taken. The use of fungicides alone for controlling brown blotch may be inpractical for the majority of cowpea growers in Nigeria. Hence this study has shown that at Samaru the use of resistant varieties, planting of medium maturing varieties at the second or third week of August were effective in controlling brown blotch. However, cowpea growers who plant susceptible varieties early will be required to spray biweekly four times, against brown blotch starting at 6 weeks after planting in order to obtain fairly good grain yields.

SUMMARY AND CONCLUSIONS

Colletotrichum capsici (Syd.) Butler and Bisby was cultured on potato dextrose agar incorporated with streptomycin (PDAS) at 15, 20, 25, 30 and 40°C and its radial mycelia growth and sporulation were measured. The growth and sporulation of the fungus occurred between 20 to 35°C and neither growth nor sporulation occurred at 15 and 40°C. The optimum temperature for both growth and sporulation was 25°C.

The mean minimum and maximum air temperatures in Samaru during the growing season, July to October were 21.5 and 31.5°C respectively. Thus the air temperatures during the growing season in Samaru are condusive for both growth and sporulation of the fungus.

The effect of plant age and inoculum density on severity of brown blotch was investigated in the glasshouse and in the field. In the glasshouse the spore suspension ranged from 4.16×10^5 to 3.75×10^6. Cowpea plants aged 1 to 6 weeks were inoculated to run-off with the conidial suspension the inoculation period in plants of various ages was found to be between 2 to 3 days.

However, at low inoculum densities the severities of the disease on plants aged 1 or 2 weeks at the time of inoculation were significantly (P=0.01) higher than those in plants inoculatd at 3 to 6 weeks of age. At higher

inoculum densities there were no significant differences in disease severities in plants of all ages. Mature plant resistance is being shown to C. capsici by cowpea plants.

The latent period of C. capsici was determined in the glasshouse, polyethylene chamber placed on the table in the laboratory and wire mesh cage, subsequent to artificial inoculation of 8-week old plants. Different latent periods for the fungus were obtained on stems, pods, peduncles, flower cushions and petioles thus, latent period varied from 5-6 days for the petioles to 13 to 16 days for the stem. The practical significance of this result is that the interval of spray of fungicides for the control of brown blotch should be between 5-16 days but for practical purpose, the current recommendation of spraying at 7-14 days interval has been effective.

The effects of four sowing dates on the incidence of infection, apparent infection rat of brown blotch and grain yield of cowpea variety, Ife Brown were studied in 1986, 1987 and 1989. Early sowing resulted in higher incidence of infection and higher infection rate of brown blotch in cowpea. The disease incidence was low in unsprayed plots sown at second week of August in the three years of study. However, yields of late sown plots were lower in quantity but were of higher quality than

those of early-sown plots, regardless of the fungicidal spray treatment. From this study it can be concluded that sowing cowpea seeds at the second or third week of August will not only reduce the amount of money spent on production, since no fungicidal spray will be required, but will also give good quality grain yield that can be used for planting in the following year. This would, however depend on the ceasation of rainfall and the earliness of the variety.

The relationships betweeen the weekly rainfall, relative humidity and the weekly increase in disease incidence were studied. It was found that once there was enough rainfall for brown blotch of cowpea to establish on the crop, the disease progresses with increase in relative humidity until the plant matures. The decline in the amount of rainfall is not as critical as the decline in the relative humidity value. Thus, the progress of brown blotch on cowpea is more dependent on relative humidity than rainfall.

Cowpea grain yield loss due to brown blotch was assessed in the field using different varieties in 1986, 1987 and 1989. Different amounts of disease were generated by spraying the plants at different frequencies with a tank mixture of benomyl and mancozeb. At 4 weeks after sowing, the plants were sprayed-inoculated at flowering. The results showed that brown blotch severity

was negatively correlated with grain yield in cowpea. There was 5 to 44% loss in grain yield in plots that had the highest disease severity. The disease also affected the quality of grain produced; the seeds from plots with high disease severity being wrinkled.

From the results of this study, brown blotch can be considered as one of the limiting factors to cowpea production in Nigeria. Consequently, considerable benefit can be derived from its control. The total eradication of the disease may not be possible but a combination of control measures which requires among other things, the use of resistant varieties or planting of non-photosensitive medium maturing varieties at second or third week of August can be effective in the control of this disease. However, cowpea growers who plant susceptible varieties early will be required to apply fungicide sprays aginst brown blotch biweekly starting at 6 weeks after sowing in order to obtain fairly good grain yields.

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APPENDIX

Appendix 1: Analysis of variance for the effect of temperature on growth of C. capsici

Source	DF	Sum of Squares	Mean Square	F value
A (Temp.)	5	12155.00	2430.99	9386.10**
B (Radial growth)	4	2858.17	714.54	2758.85**
AB	20	2277.15	113.858	439.16**

** highly significant at P= 0.01

Appendix 2: Analysis of variance for the effect of temperature on sporulation of C. capsici

Source	DF	Sum of squares	Mean Square	F value
Replicate	2	0.000411	0.000206	0.47
Temperature	5	87.126388	17.4252	3985.45**
Error	10	0.043722	0.00437	

** highly significant at P=0.01

Appendix 3: Analysis of variance for the data on effect of plant age and inoculum density on severity of cowpea brown blotch in the glasshouse at 10 days after inoculation

Source	DF	Sum of squares	Mean Square	F value
Rep.	2	0.06349	0.03175	0.328
Plant Age (pa)	5	99.2064	19.84	205*
Inoculum density (x)	6	795.429	132.57	1369.73**
pa x X	30	76.5714	2.55	26.371**
Error	82	7.9365	0.0967	

* significant at P=0.05
** significant at P=0.01

Appendix 4: Analysis of variance for the effect of plant age and inoculum density on severity of cowpea brown blotch in the field

Source	DF	Sum of squares	Mean Square	F value
A Plant age	4	102.53	25.63	7.93**
B Inoculum density	2	22.2	11.10	3.43*
AB Plant age x Inoculum density	8	19.47	2.43	0.75
Error	15	48.50	3.23	

* Significant at P=0.05

** significant at P=0.01

Appendix 5: The weekly rainfall and average weekly relative humidity during 1985 planting season

Weekly after planting	Weekly rainfall(mm)	Average weekly relative humidity (%)		
		Morning (10 a.m)	Evening (4 p.m)	Mean
0	22.4	90	81	85
1	84.8	86	72	79
2	71.1	87	69	78
3	29.4	83	68	76
4	70.4	79	79	77
5	33.3	77	68	73
6	51.8	82	69	76
7	51.4	79	70	75
8	50.6	82	73	78
9	25.7	74	60	67

Appendix 6: The weekly rainfall, and average weekly relative humidity during 1989 planting season

Week after planting	weekly rainfall(mm)	Average weekly relative humidity (%)		
		Morning	Evening	Mean
0	47.5	82	65	74
1	75.4	83	68	75
2	36.8	81	74	78
3	22.8	82	76	79
4	46.0	82	68	75
5	45.3	80	69	75
6	20.7	81	68	74
7	58.3	84	71	78
8	29.6	74	64	69
9	7.4	79	64	72

Appendix 7: Analysis of variance for relationship between brown blotch and frequency of spray in 1986 on Ife Brown.

Source	DF	Sum of squares	Mean Square	F value
Replicate	3	734.61	244.869	2.61
Treatment	5	8741.26	1748.253	18.61**
Error	15	1409.49	93.966	

Appendix 8: Analysis of variance for relationship frequency of spray and yield in 1986 on Ife brown

Source	DF	Sum of squares	Mean square	F value
Replicate	3	0.06	0.019	0.98
Treatment	5	1.56	0.313	16.20**
Error	15	0.29	0.019	

Appendix 9: Analysis of variance for the relationship between frequency of spray and yield in 1989 for variety IT86D-1056

Source	DF	Sum of squares	Mean square	F value
Replicate	2	0.36	0.182	2.59
Treatment	5	1.99	0.398	5.69*
Error	10	0.70	0.070	

Appendix 10: Analysis of variance for the relationship between frequency of spray and yield in 1989 for variety IT86D-1057

Source	DF	Sum of squares	Mean square	F value
Replicate	2	0.09	0.047	0.55
Treatment	5	0.55	0.110	1.29
Error	10	0.86	0.086	

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