INFLUENCE OF DIFFERENT CROPPING SYSTEMS, PLANT AGE AND VARIETAL DIFFERENCES ON INCIDENCE AND SEVERITY OF BACTERIAL PUSTULE OF COWPEA (*Vigna unguiculata* (L.) WALP) CAUSED BY *Xanthomonas campestris* pv. *vignicola* (BURKHOLDER) DYE.

By

Jotham O. Ouko

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

of

MASTER OF SCIENCE IN AGRICULTURE

PRESENTED TO CROP SCIENCE DEPARTMENT,

FACULTY OF AGRICULTURE,

UNIVERSITY OF NAIROBI.

NAIROBI

JUNE, 1987

DECLARATION:

I declare that this thesis is my original work and has not been presented for a degree in any other University.

Date ....4/3/88..... Name J.O. Ouko

Signature .....J.O. Ouko..

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

Date 8/3/88..... Dr. R.A. Buruchara

Signature .....

Date ..30/3/88..... Prof. D.M. Mukunya

Signature .....

ACKNOWLEDGEMENTS

I would like to express my thanks to my University Supervisors Dr. R.A. Buruchara and Prof. D.M. Mukunya for their guidance. My sincere vote of thanks particularly to Dr. Buruchara who spared his time out of his busy schedule to visit and supervise this work at Matuga Agricultural Research Station. His invaluable advice was taken and incorporated into this study. I would also like to thank the Director of Research, Ministry of Agriculture who sponsored this study and the Officer In Charge, Matuga Agricultural Research Station, who put at my disposal the facilities to undertake the study.

Finally, I wish to record my deep appreciation to Mr. J. Awino and Mr. S. Bimbuzi for field and laboratory assistance, the Chemist, Government Chemist's Department, Mombasa, for allowing me to use their laboratory facilities and Ms. Monica Folingi for her keen typing.

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ABSTRACT

Cowpea bacterial pustule caused by Xanthomonas campestris pv. vignicola is one of the factors limiting cowpea production in Kenya especially in high rainfall areas. The present studies were undertaken to determine the effects of three different cropping systems and plant age on disease development and evaluate plant varietal reactions following inoculation with the pathogen. A preliminary attempt was also made to detect possible existence of pathogenic variation.

The disease was found to have an incubation period of between seven and eight days when cowpea plants 21-25 days old were foliar sprayed with a suspension of the pathogen. Disease spread within and between plants was shown to be least when cowpeas were grown as a relay crop after maize during the long rains and when grown as an intercrop with maize during the short rains. During the short rains disease incidence observed eight days after inoculation was 52.5% in cowpea-maize intercrop, 57.5% in cowpea pure culture and 57.0% in cowpea-maize relay crop. Forty days after inoculation this increased to 62.5%, 75.0% and 91.25% respectively. During the long rains disease incidence observed eight days after inoculation was 45.0% in cowpea-maize relay

crop, 8.75% in cowpea pure culture and 5.0% in cowpea-maize intercrop which 16 days after inoculation increased to 68.75%, 100% and 100% respectively.

Rainfall was observed to be important in disease development and spread as there was more disease and a faster rate of spread with increased amount of rainfall. Cowpea pure culture and cowpea-maize intercrop planted during the long rains when about 748.9mm of rainfall was received had severe and faster disease spread compared to cowpea-maize relay crop which had least disease and was on the ground when only 63.5mm of rain was received. Cowpea-maize relay crop during short rains had more disease and it was on the ground when the long rains started. During the experimental period there was no significant variation in relative humidity and daily temperatures between the treatments and hence they could not influence the differences observed in disease development and spread.

Young plants were observed to have less disease than old plants when plants were inoculated at the same time but at different ages. During the short rains, plants 6, 4, 2 and 0 weeks old at inoculation had 100%, 82.5%, 60% and 0% disease incidence respectively 40 days after inoculation. However, there were no differences in disease development with increased

rainfall. Disease severity also varied with age. Plants 6, 4, 2 and 0 weeks old attained severity scores of 4.5, 3.75, 2.5 and 1.0 during short rains and severity scores of 5.0, 5.0, 3.5 and 2.0 during long rains respectively.

Varietal reactions to the pathogen varied but 5 varieties were found resistant, 14 moderately resistant while 4 were susceptible when grouped arbitrarily in mean disease severity classes where score 1-2.0 = resistant; 2.1-3.0 = moderately resistant; and 3.1-4.0 = susceptible. One of the 5 resistant varieties never developed any disease symptoms. Some variation was noted in the virulence of the 2 pathogen isolates tested on 22 cowpea varieties. The Mbita Point isolate was more virulent than the Mtwapa isolate.

1. INTRODUCTION

Cowpea (Vigna unguiculata (L.) Walp.) is one of the most important legume crops grown in Tropical Africa which accounts for 70% of the crop produced in the world (Summerfield et al., 1974). The crop is grown for its greer leaves, green pods and grain. Cowpea seed has a protein content of about 27.5% and forms a siginificant source of phosphorous, iron and some water soluble vitamins (Bressani and Elias, 1980). This makes it a good source of supplementary protein to diets based on cereal grains and starchy foods.

In Kenya cowpea is grown mostly in the arid and semi arid areas which cover about 80% of the country (Anon., 1978b). The leading area under the crop is Eastern Province which had 57,097 hectares planted in 1983 (Anon., 1984). Coast Province had 5,320 hectares under the crop in 1982 (Anon., 1983b). The crop is also grown in the low lying areas around Lake Victoria. Cowpea is cultivated in these areas mostly as an intercrop with maize, root crops, simsim and other food crops although pure stands can be found in garden plots. This may have varied implications on disease development.

The crop is popular both for vegetable and grain consumption. However, there are a number of production constraints which have kept yields considerably low. Yields of upto 1.7 tons

of grain per hectare have been obtained in research plots at the Coast Province while farmers obtain yields as low or less than 0.5 tons per hect. (Anon., 1978a).

The major constraints affecting production of the crop especially in the wetter areas are pests and diseases. One of the diseases which has come into prominence recently is the cowpea bacterial pustule caused by Xanthomonas campestris pv. vignicola (Burkholder) Dye. The disease affects foliage, pods and sometimes the stems. Leaves become discoloured with spots and become unattractive, thus lowering their value as a vegetable crop. Grain yields are also reduced as severe infections lead to yellowing of leaves and in some cases premature defoliation.

2. LITERATURE REVIEW

2.1. Disease history and distribution

Bacterial pustule was first described in 1975 in Nigeria (Williams, 1975) where it had been observed to be widespread. It was next recorded in Tanzania (Patel, 1978), Kenya (Anon., 1978b; Kaiser and Ramos, 1979) and Mozambique (Dhindsa and Mondjane, 1984). It is a disease which is restricted only to Africa (Patel, 1981) as no records are available from outside the continent. However, Singh and Allen (1979) noted that the disease may occur in Brazil although no records exist to confirm its occurence. In Nigeria, the disease is rated among the top six major diseases of cowpea and is considered the most serious bacterial disease of the crop (Anon., 1977). It is considered to be of intermediate economic importance in Mozambique where the crop is of primary importance (Dhindsa and Mondjane, 1984).

Kaiser and Ramos (1979) suggested that the disease was introduced into Kenya through germplasm imported from International Institute of Tropical Agriculture (I.I.T.A.), Nigeria, during the 1970's for experimental purposes. In screening trials of the introduced germplasm at Coast Agricultural Research Station, Mtwapa, many lines were found to be susceptible (Anon., 1978b). Infections ranged from light to medium. At National Dryland Farming

Research Station, Katumani, the same report indicated that some plots had infections which were rated as not severe. Kaiser and Ramos (1979) reported that the disease intensity was low although many varieties were infected at one site at the Coast Province.

Recent screening trials at Coast Agricultural Research Station have shown the disease to be prevalent on the varieties grown. No work has been done to determine the importance and the distribution of the disease since the IITA cultivars were distributed to some farmers after the initial observation trials in Kenya.

2.2. Disease symptoms

The disease symptoms begin as tiny, dark, water soaked spots on the lower leaf surface. The spots later enlarge to become circular spots 1.0 to 3.0 millimetres in diameter (Williams, 1975). Young lesions appear on the lower leaf surface as dark, water soaked pustules and on the upper leaf surface as dark brown necrotic spots. With time, the lesions enlarge and become dry and sunken at the centre but remain water soaked around the margin.

The above symptoms described in West Africa are similar to those described in East Africa by Kaiser and Ramos (1979) and Patel (1981). They observed the pustules to increase in size from 1.0 to 4.0 millimetres and remained more or less circular on the lower leaf surface

while on the upper leaf surface the lesion centres were surrounded by a yellow halo which became necrotic and sunken. Singh and Allen (1979) noted that heavily infected leaves turn yellow and fall and susceptible varieties could loose most of their leaves before maturity. This is significant since both leaves and grains are the edible components of the plant. Inoculated plants showed symptoms 8 days after inoculation as pustules became observable as water soaked, transluscent and raised on the under side of the leaf surface (Patel, 1981).

A related disease of cowpea, bacterial blight, incited by a strain of the organism causing bacterial pustule also exhibit similar symptoms which only differ in size. In bacterial blight symptoms, dead spots tend to be large and irregular as the lesions later merge (Singh and Allen, 1979).

2.3. The Pathogen

Bacterial pustule disease of cowpea is incited by Xanthomonas campestris pv. vignicola (Burkholder) Dye. The organism was first described in the United States of America by Burkholder in 1944 as the cause of bacterial blight of cowpea. The bacterial pustule pathogen is a strain of the bacterial blight pathogen as both have been shown to have similar cultural, physiological and biochemical properties and host range but can be distiguished by

symptoms induced on inoculated cowpea leaves (Patel, 1981).

As the incitant of bacterial blight, the pathogen prefers vascular tissues while as a causal of bacterial pustule it prefers parenchymatous tissues (Shekhawat et al., 1977). The organism belongs to the phytopathogenic Xanthomonads, a group which Dye (1962; 1963; 1966) found to be remarkably uniform as shown by their cultural, physiological and biochemical characters. Members of the genus only differ in the range of host plants to which they are pathogenic. Similarities of pathogenic species have been reported in other genera and in some cases has resulted in confusion over classification and identification of plant pathogenic bacteria (Young et al., 1978).

When Kaiser and Ramos undertook laboratory characterisation tests, they concluded that the difference in symptom expression of bacterial blight and bacterial pustule is not sufficient to warrant designating them as different species but should be considered as strains. They described the organism as an aerobe, gram negative motile rods with a single monotrichus flagellum. It formed yellow colonies on nutrient agar in 3 days at 25°C. It oxidised galactose, mannitol, raffinose, sucrose and xylose but not lactose. It utilised citric acid as sole source of carbon. It formed hydrogen sulfide but did not reduce nitrate to nitrite or produce ammonia. It produced amylase, gelatinase but not

β-glucosidase. It was actively lipolytic but was unable to rot potato slices. It also did not induce hypersensitive reaction when infiltrated into tobacco leaves.

The above tests were comprehensive enough and can serve as a basis for identifying or verifying an isolate of the pustule bacteria. In 1962, Dye suggested mininal tests required in the diagnosis of Xanthomonas. These tests included gram reaction, slime formation, gelatin hydroysis, action in milk and hydrogen sulfide production.

2.4. Host range

The pathogen is known to have a wide host range. Kaiser and Ramos (1979) found the organism to be pathogenic to the foliage of several legume species in green house inoculation tests. Their work showed moderate pathogenicity in Arachis hypogaea, Cajanus cajan, Dolichos lablab, Glycine max, Macroptilium lathyroides, Phaseolus acutifolius, P. lunatus and Vigna radiata. It was found highly pathogenic to P. vulgaris, Pisum sativum, V. aconitifolia, V. vulgaris, V. umbellata and V. unguiculata. In all cases typical pustule symptoms developed on leaves while cankers developed on the stems in severe cases.

Jindal and Patel (1980) and Jindal et al., (1981) in their study of variability of 10 Xanthomonads isolated from different grain legumes found the pathogen to be cross infective and had overlapping host range but all

were found aggressive on natural hosts. The strains causing bacterial blight and bacterial pustule were pathogenic to both cowpea and Phaseolus bean. Similar symptoms described on cowpea occured on mungbean (Phaseolus aureus) although raised lesions on mungbean were observed on the upper surface of infected leaves (Patel and Jindal, 1972).

Vakili (1977) found Xanthomonas isolates from diseased cowpea fields in Puerto Rico to be pathogenic to both cowpea and beans. Xanthomonas bacteria were also isolated from bacterial pustules of Phaseolus vulgaris, P. coccineus, P. lunatus and Glycine max but no bacterial pustule symptoms were observed on cowpea.

The above findings imply that the pathogen can easily be spread among different legumes grown in proximity to each other and can be a means of perpetuating the disease especially where perennial legumes are grown.

2.5. Host varietal reaction

Varietal reactions following plant inoculations depend on the pathogenicity of the organism and the host genotype. Methods used in inoculating plants and environmental conditions under which they are kept may also play a role in determining the subsequent reactions. While screening cowpea germplasm for bacterial pustule

resistance, Williams (1977) inoculated test plants by spraying with ground infected leaves collected from the field. The inoculated plants were sprinkle irrigated to create water splash which enhanced disease development and spread. The screened lines were graded as either resistant or susceptible.

Patel (1981) inoculated the cowpea lines tested by Williams by leaf infiltration method with a suspension of pure culture of the bacterial pustule pathogen. He confirmed Williams' results and also identified three different types of reactions, thus distiguishing two types of resistant reactions. The reactions were brown hypersensitive reaction (BHR) which was observed within 24-76 hours after inoculation; resistant (R) reaction where small eruptions appeared 10-15 days after inoculation, but secondary spread was very limited; and susceptible (S) reaction where typical pustule symptoms developed within 8 days after inoculation.

No records exist of any screening work on bacterial pustule pathogen in Kenya although identification of resistant varieties could be useful in further breeding work. Initial screening of IITA lines were for performance and adaptability of the introduced germplasm.

2.6. Pathogenic variability

Recent work has shown the existence of pathogenic

variability among isolates of the bacterial pustule pathogen. Patel (1981), working with cowpea lines identified as resistant to bacterial pustule at IITA, found that many of them were susceptible when grown and tested at Ilonga in Tanzania. This indicated existence of strains having different pathogenicity from those at IITA. Working with ten bacterial isolates from Tanzania and Nigeria, Patel detected existence of three races using four cowpea lines. He based his groupings on leaf reactions of inoculated test plants by leaf infiltration method. He suggested that race one may be prevalent in West Africa while races two and three may be prevalent in East Africa. He proposed the following varieties to be adapted as standard differentials: TVu 1190, TVu 1630, TVu 43, TVu 134 and Prima.

Given the wide range of climate, ecology and cropping systems under which cowpea is grown in Kenya, there is a possibility of having pathogenic variation. There is thus a need to investigate this characteristic of the pathogen since control of most bacterial diseases are dependent on a good understanding of pathogen variability, host resistance and consequent development of resistant cultivars. At IITA, several varieties have been identified which show multiple disease resistance including bacterial pustule (Anon., 1976; Singh and Allen, 1981).

Breeding work at IITA on specific resistance to bacterial pustule pathogen showed that 2 gene pairs are involved and the mechanism of resistance involves epistasis where gene B suppreses gene A (Anon., 1976). Evidence on resistant varieties hence tends to indicate that some sources of resistance are specific thus necessitating thorough varietal screening in different environments before being recommended.

2.7. Epidemiology

2.7.1. Mode of infection and dissemination

No bacterial pathogen of cowpea has been studied in detail (Singh and Allen, 1981). Development of bacterial pustule of cowpea like other bacterial diseases is little understood.

As in the case with most plant pathogenic bacteria, X. campestris pv. vignicola enters the host plant through natural openings like stomata and through wounds. Primary inoculum can be from plant debris or through seed as the pathogen has been shown to be seed-borne (Kaiser and Ramos, 1979). In newly planted areas, seed transmission is more important as a source of inoculum. Kaiser and Ramos found seeds harvested from inoculated pods to be frequently discoloured and shrivelled. These workers reisolated the bacterium from internal tissues of

surface sterilised seeds collected from pods which were inoculated with the pathogen. Typical bacterial symptoms developed on primary leaves of 5-10% of germinating seedlings from which the pathogen was reisolated.

Various workers in Nigeria have observed the disease to be more adapted to the wet savanah zones (Williams, 1975). The disease has been observed to be more prevalent and also spread more rapidly during frequent heavy rains (Singh and Allen, 1979). This suggests that rain splash plays a significant role in inoculum dissemination. At IITA, Nigeria, overhead irrigation is used to maintain and promote the disease development (Williams, 1977).

Mode of pathogen survival during adverse conditions other than through seed have not been investigated. However, it is suggested by Singh and Allen (1981) that crop residue left in the field can serve as inoculum source although the duration of the bacterium survival on plant debris is unknown.

2.7.2. Factors affecting disease development

Factors affecting bacterial pustule development are little understood. The only factor known to favour disease development is high rainfall. Frequent heavy rainfall has been observed by Williams (1977) to help increase the rate of disease spread from plant to plant.

It is therefore assumed that water in the form of rain splash is necessary for disease spread. Disease epidemics are therefore likely to occur during periods of high rainfall. This factor might have a major implication when cowpea is grown in wet areas.

Cowpea in the tropics is grown in a multiple cropping system (Summerfield et al., 1974; Steele and Mehra, 1980; Hamblin, 1980; Ezueh, 1982). The system plays a role in cultural control of disease. Evidence is gradually accumulating to show that intercropping often leads to decreased disease and pest incidences. The effect of intercropping cowpea with cereals on severity and rate of spread of pathogens is that disease may either be enhanced or reduced (Anon., 1977; Singh and Allen, 1981). Odhiambo (1985) reckons that whatever modern agricultural systems are developed for tropical regions, crop heterogeneity in terms of intercropping is a basic requirement in order to confer genetic stability and stable crop performance. However, no investigations have been carried out on the effect of cowpea intercropping systems on development of bacterial pustule.

Work with cowpea bacterial blight, a disease incited by a strain of the same organism has shown that plant age at time of inoculation had some effect on disease development although there was no evidence that older

plants were less susceptible than younger plants (Allen et al., 1981). The effect of plant age on bacterial pustule infection and development has not been studied although it is essential information in understanding disease development. It is also a useful pre-requisite in advising on control measures and having an insight into the probable effects of the disease on yields.

The foregoing literature review indicates that despite the work that has been done elsewhere little has been done on bacterial pustule in Kenya. The present work was therefore undertaken to study some aspects of the disease. These included (a) determination of disease development under different cropping systems; (b) determination of host susceptibility when inoculated at different ages; (c) determination of plant varietal reactions and (d) a preliminary attempt to detect the existence of pathogenic variability within Kenya.

3. MATERIALS AND METHODS

3.1. Site description

The present study was carried out at Matuga Agricultural Research Station, Kwale District, Coast Province. The station is about 20 km South of Mombasa, off Mombasa - Lunga-Lunga road and 6 km from the sea. It lies at an altitude of 120m above sea level and is located at latitude 4° 15' South and longitude 39° 37' East.

The station receives an average annual rainfall of about 1000mm. Rainfall is bimodal with long rains occuring between April and June while the short rains occur between October and December. The long rains account for more than 50% of the total annual rainfall received while the short rains account for about 20%.

Soils are medium grained sands (Magarini sands) of good drainage but deficient in organic matter and essential nutrients (Michieka et al., 1978).

This study was carried out over two seasons; the short rains of 1985 and the long rains of 1986, covering the months of October, 1985 to September, 1986.

3.2. Source of isolates

One of the isolates of Xanthomonas campestris pv.

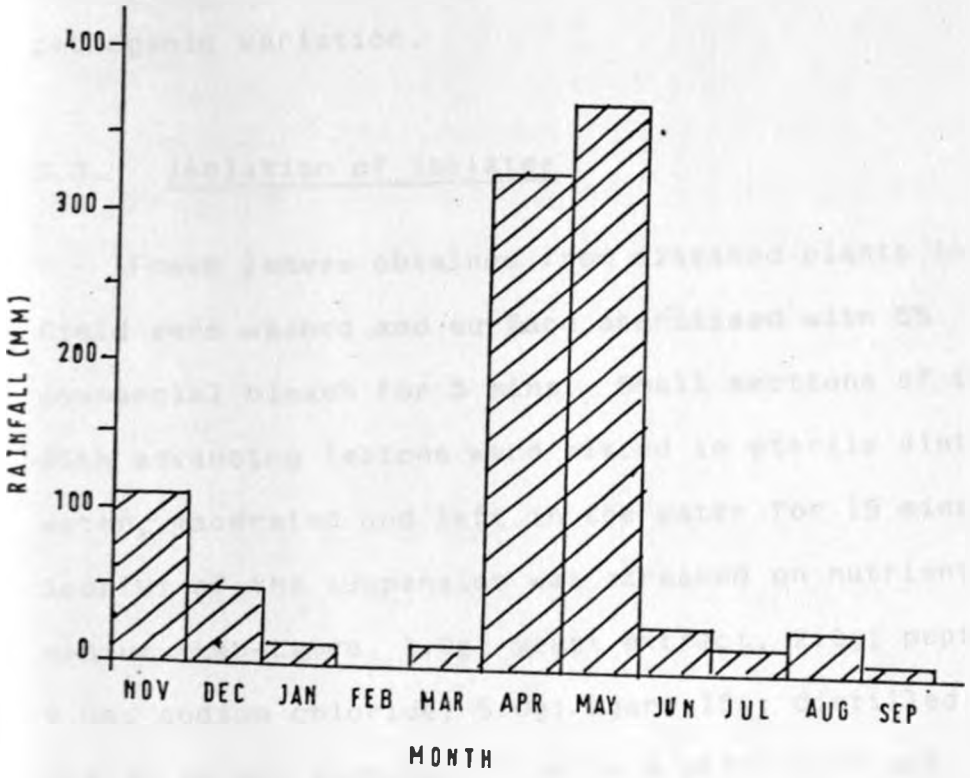

FIG. 1 RAINFALL DISTRIBUTION AT MATUGA RESEARCH STATION DURING EXPERIMENTAL PERIOD (NOV 1985 TO SEP 1986)

vignicola was obtained from naturally infected cowpea leaves at the experimental plots of the Coast Agricultural Research Station, Mtwapa, and another from the Internatinal Centre of Insect Physiology and Ecology (ICIPE), Field station at Mbita Point in South Nyanza. The Mtwapa isolate was used in the determination of disease development under different cropping systems , determination of host susceptibility at different ages and determination of host varietal reactions. The Mbita Point isolate was used in comparison with the Mtwapa isolate to determine pathogenic variation.

3.3. Isolation of isolates

Fresh leaves obtained from diseased plants in the field were washed and surface sterilised with 5% commercial bleach for 5 mins. Small sections of tissues with advancing lesions were placed in sterile distilled water, macerated and left in the water for 15 mins. A loopful of the suspension was streaked on nutrient agar medium (Lab-Lemco, 1.0g; yeast extract, 2.0g; peptone, 5.0g; sodium chloride, 5.0g; agar, 15g; distilled water, 1.0 l; pH approximately 7.40)in a petri dish and incubated at 25-30°C for 3 days. Single colonies were transfered to nutrient agar slants and subjected to two colony transfers for purification.

The pathogenicity of the isolates obtained was

confirmed by inoculating the isolated organism into a local cowpea variety by leaf infiltration method using a syringe. A diluted suspension of the bacterial growth was injected into leaflets at the first trifoliate leaf stage on either side of the midvein. The casual organism was reisolated after 14 days from leaves showing similar symptoms as original plant host. The symptoms produced by the organism on the original host were similar to those produced under artificial inoculation thus confirming pathogenicity in accordance with Koch's postulates.

Laboratory characterisation tests were undertaken to attempt to verify the identity of the casual organsim using tests recommended by Dye (1962) and Kaiser and Ramos and compare with documented descriptions. A sample was also sent to the Commonwealth Mycological Institute (C.M.I.) for verification.

Cultures of the isolated organism were maintained on nutrient agar slants at 4°C. The cultures were renewed every 2-3 months during the duration of the experiments.

All tests were carried out following methods described by Cowan and Steel (1974) and 48 hour old cultures were used except where specified.

3.4. Isolate characterisation

3.4.1. Gram reaction

The method used was that of Preston and Morrel (1962). A bacterial suspension was smeared over a glass slide and air dried. Ammonium oxalate - crystal violet was applied for half a minute and then washed off thoroughly with Lugol's No.1 iodine solution. Lugol's iodine solution was applied for half a minute before washing off with acetone - iodine then applying acetone - iodine for half a minute. This was washed off with water before counterstaining with weak carbol fuchsin for half a minute. The slide was then washed with water and blotted to dry before examination.

3.4.2. Hydrogen sulfide production

The organism was grown in nutrient broth (beef extract, 10.0g; peptone, 10.0g; Nacl, 5.0g; water, 1l; pH 7.4) poured into tubes. Lead acetate papers inserted between the cap and tube were used as indicators. The papers were observed daily for 7 days for blackening.

3.4.3. Nitrate reduction to nitrite

The method used was that of Bachmann and Weaver

(1947). A medium consisting of peptone, 10.0g; beef extract. 30g; KNO3, 10g; water, 1l was dispensed into 1.0ml tubes and heated to 37°C in a water bath. These were inoculated with 2 loopsfull of 6 hour old cultures. After 15 minutes, a drop, each of sulphanilic acid and dimethyl-α-naphthylamine reagents, was added. Observations were made after 15 minutes for any colour change.

3.4.4. Gelatin hydrolysis

The method used was as described by Cowan (1974). Plates of gelatin agar (gelatin, 4g; water, 50 ml; nutrient agar, 1l) were spot inoculated with a loopful of bacterial suspension and incubated at 25-30°C for 3 days. The plates were then flooded with mercuric chloride solution.

4.4.5. Methyl red reaction

Glucose phosphate medium (peptone, 5g; K_2HPO_4, 5g; water, 1 l and glucose, 5g) in tubes was inoculated with the bacterial suspension and incubated at 25-30°C for 5 days. Two drops of methyl red solution were added to determine the final pH.

3.4.6. Starch hydrolysis

Plates containing starch agar composed of yeast

extract, 5g; peptone, 5g; Lab-lemco (Oxoid), 5g; water, 1l; potato starch (Difco), 1% (W/V) were spot inoculated with bacterial suspension. The plates were incubated at 25-30°C for 5 days then flooded with dilute Lugol's iodine solution.

3.5. Inoculum preparation

Inoculum was prepared by streaking a loopful of bacterial suspension on nutrient agar and incubating at 25-30°C for 48 hours. The growth was washed off into 9.0ml sterile water blanks and mixed thoroughly. These were serially diluted upto 10^{-10} dilution. Using a Varian 634 spectrophotometer with wavelength set at 620nm, the absorbance of each dilution was determined from the bacterial suspension.

From each dilution, 0.1ml was transfered onto petri dishes containing nutrient agar and evenly spread using a sterile bent glass rod. The petri dishes were incubated at 25-30°C and observed after 3 days to record the number of colonies which contained 300 or less bacterial colonies. The number of bacterial cells per ml. was determined by multiplying the number of colonies on the plates by each dilution. The absorbance values were ploted against the log 10 values of the estimated number of cells to obtain a standard

curve used to determine inoculum concentration. A concentration of 10^6 - 10^8 bacterial cells per ml was used in all inoculations.

3.6. Inoculation procedure

Inoculation was done using a Solo motorised mist blower (Plate 1) late in the afternoon. Inoculum was sprayed onto plants until runoff was attained. The plants were sprinkle irrigated using a hose-pipe connected to a water tap a day before spraying if there were no rains the previous day. In case of insufficient rainfall this kind of irrigation was continued.

3.7. Disease assessments

Disease incidence was assessed as defined by James (1974) as the number of plants infected in each plot expressed as a percentage of total number of plants observed. Observations were made every four days. Observations started immediately after the symptoms were observed or 8 days after inoculation depending on which came first. Twenty plants were observed in the middle 4 rows leaving 2 rows on either side as guard rows in each plot. Five plants were observed on each row.

Disease severity defined by James (1974) as the area of tissue affected by disease was also

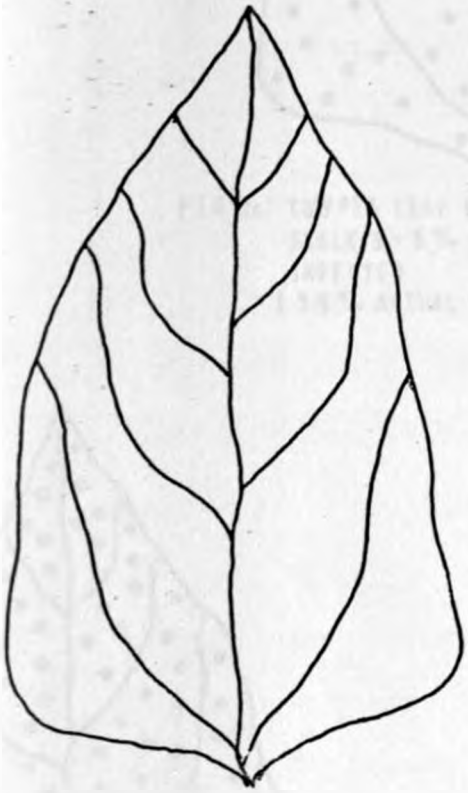

FIG. 2a. COWPEA LEAF DISEASE SEVERITY
SCALE 1 = NO SYMPTOMS

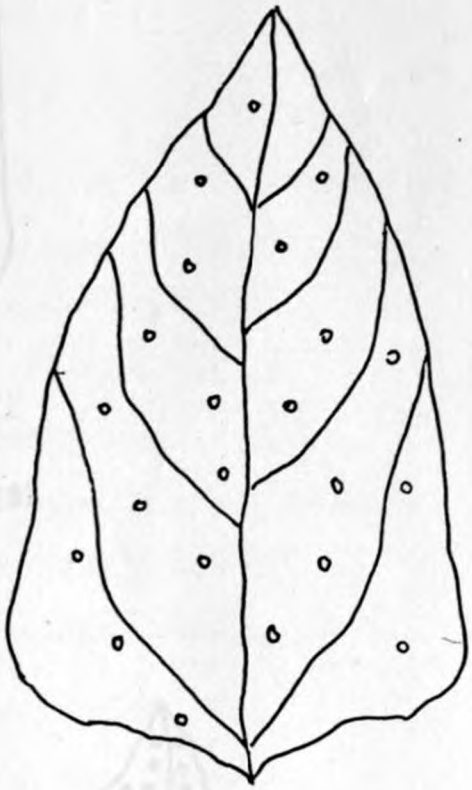

FIG. 2b. COWPEA LEAF DISEASE SEVERITY
SCALE 2 = LESS THAN 5% OF LEAF INFECTED
[0·5 % ACTUAL LEAF AREA INFECTED

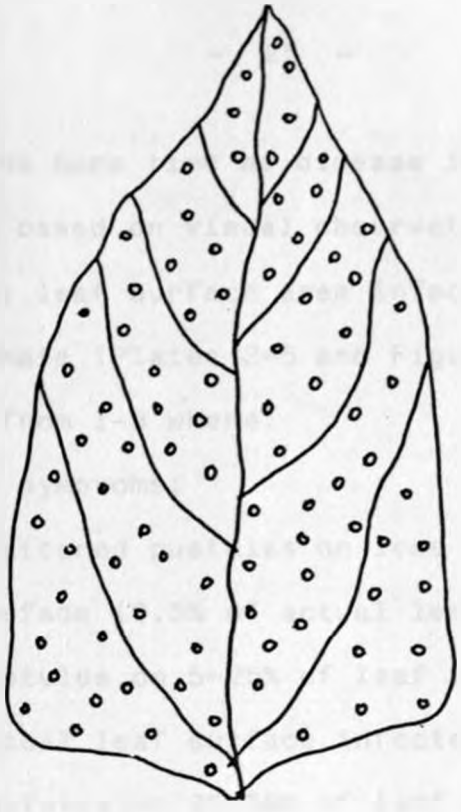

FIG:2c: COWPEA LEAF DISEASE SEVERITY SCALE 3 = 5 % TO 25 % OF LEAF INFECTED [2·5 % ACTUAL LEAF AREA INFECTED]

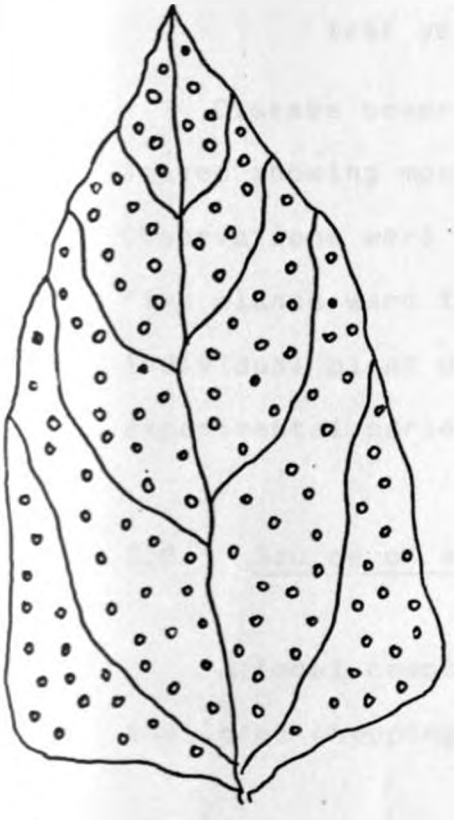

FIG.2d: COWPEA LEAF DISEASE SEVERITY SCALE 4 = 25 % — 50 % OF LEAF INFECTED [5 % OF ACTUAL LEAF AREA INFECTED]

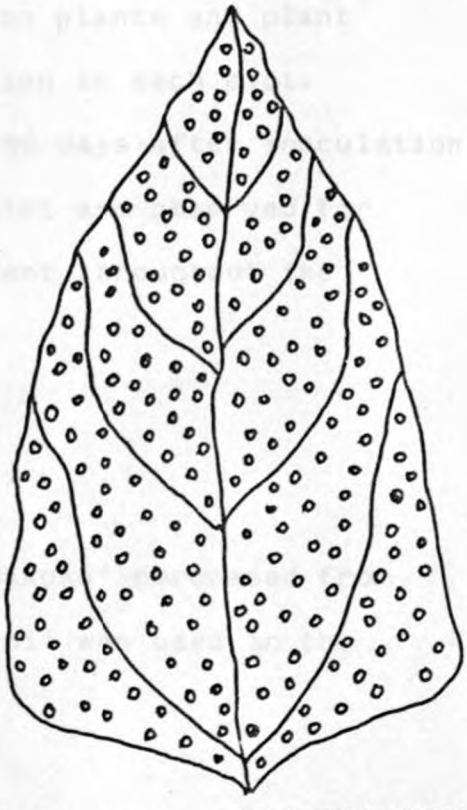

FIG.2e: COWPEA LEAF DISEASE SEVERITY SCALE 5 = MORE THAN 50% OF LEAF INFECTED [MORE THAN 10% ACTUAL LEAF AREA INFECTED]

assessed at the same time as disease incidence. A scale was developed based on visual observations and calculation of actual leaf surface area infected to form an unbiased estimate (Plates 2-5 and Figure 2). The scale ranged from 1-5 where:

1 = No symptoms;

2 = Scattered pustules on less than 5% of leaf surface (0.5% of actual leaf surface infected);

3 = Pustules on 5-25% of leaf surface (2.5% of actual leaf surface infected);

4 = Pustules on 25-50% of leaf surface (5% of actual leaf surface infected); leaf yellowing;

5 = Pustule on more than 50% of leaf surface (more than 10% of actual leaf surface infected); leaf yellowing; leaf defoliation occuring.

Disease severity was scored on plants and plant leaves showing most severe infection in each plot. Observations were continued upto 40 days after inoculation. Five plants were tagged in each plot and observed for individual plant disease development throughout the experimental period.

3.8. Source of seeds

A local cowpea cultivar 'Kimakoko' purchased from the local shopping centre (Ngombeni) was used in the

determination of disease development under different cropping systems, in the determination of the effect of plant age on disease development and together with other varieties in the other experiments. The seeds of the variety which are brown to red in colour were sold to the shop by local farmers. The variety is a semi-erect type which is suitable for vegetable (leaves) and grain production. Seeds used were selected both for uniformity in colour and size.

Thirteen out of the 15 varieties used in the experiment to determine host varietal reactions were obtained from Coast Agricultural Research Station (CARS), Mtwapa. These were original collections of Katumani and IITA materials introduced into the Coast Province under the Grain Legume Improvement Programme in the 1970's. However, the wild type was obtained from Royal Botanic Gardens, Wakehurst Palace, England while the local cultivar was purchased locally. The other varieties included ER 1-1, 332, Machakos 66, MAK - 1/39/1/B, 238, Kangau, Vita 1, Vita 5, 9533, TVu 310, TVu 410, TVx 66-24, Katumani 80 and 233.

In the experiment to determine the pathogenic variability, the varieties listed above (except the wild type) were used in addition to 7 others obtained from ICIPE, Mbita Point collection. The 7 from Mbita Point were ICV 1, ICV 6, IT 83D-442, IT 82D-889, IT 83S-850,

HB 48/E 10 and 419.

Coast composite maize seed, purchased from the local Kenya Seed Company agent was used in the inter-cropping experiment.

3.9. Determination of disease development under different cropping systems

The objective of this study was to determine the effect of growing cowpeas under different cropping systems on disease development in the field and examine possible relationships between some environmental factors (i.e. temperature, rainfall and relative humidity) and disease progress. The trials were conducted during the short rains of 1985 and long rains of 1986. A local cowpea variety (described in section 3.7) and the Coast Composite maize were used in the experiment. No fertilizer was applied at planting but maize was later top-dressed at knee height with Calcium Ammonium Nitrate (C.A.N.) fertilizer at the rate of 150kg per hectare. No disease control was done but Diazinon was applied at 20ml per 20l spray tank regularly to control foliage and flower pests as was necessary.

A completely randomised block design with 3 cropping systems as treatments replicated 4 times was used in November 1985 (short rains) and April 1986 (long

rains). The cropping systems (treatments) were:

(a) Pure stand cowpea planted at a spacing of 60x30cm;

(b) Cowpea - maize intercrop planted at a spacing of 30x30cm for cowpea and 90x60cm for maize. 2 rows of cowpea were planted between maize rows;

(c) Cowpea - maize relay crop at a spacing similar to (b) but cowpea planted later when the maize crop was mature in February (for short rains cropping season) and July (for long rains cropping season) 1986.

Plot sizes were 4.8m x 4.0m each consisting of nine rows of cowpea in pure stand and ten rows of cowpea in the cowpea-maize intercrop and relay crop between six rows of maize. Paths of 1.0m each were left between plots.

Plants in the first two treatments (cowpea pure stand and cowpea-maize intercrop) were inoculated 21 days after planting during the short rains season using the method described in section 3.5. The long rains season crops was also inoculated 21 days after planting but a second inoculation was carried out after two weeks since disease establishment was found to be low. Cowpea-maize relay crop was inoculated 25 days after planting for the short rains crop and 24 days after planting for the long rains crop.

Assessments of disease incidence and severity were done every four days starting eight days after inoculation. Records of temperature and relative humidity were taken daily and averaged every four days to coincide with days of plant disease evaluation. Temperatures were recorded daily at 9.00 a.m. and 3.00 p.m. using a whirling hygrometer of Brannan thermometers. Relative humidity was read off from the slide scale accompanying the hygrometer.

3.10. Determination of the effect of plant age on disease development

The trial was designed to examine the effect of plant age at time of inoculation on disease development in a local commonly grown cowpea cultivar. The objective was to determine the plant's developmental stage at which it is most susceptible to the pathogen.

A local cowpea cultivar (Kimakoko) was used. The plants were spaced at 60x30cm in plots of 4.8m x 4.0m. Five middle rows were used in the experimental evaluations ignoring 2 guard rows on either side.

The experiment was laid out as a randomised complete block design with 4 treatments replicated 4 times. Planting was done every 2 weeks with each planting constituting a treatment. At the time of inoculation

the plants were 6, 4, 2, 0 weeks old i.e. the oldest plants were 6 weeks while the youngest were planted on the day of inoculation. The youngest plants were therefore not inoculated. No further inoculation was done.

The short rains crop was planted from 25th November, 1985 to 9th January, 1986. The long rains crop was planted from 10th April, 1986 to 22nd May, 1986. No fertilizer was applied but foliage and flower pests were controlled as necessary using Diazinon at the rate of 20ml per 20 lit. spray tank and sprayed to runoff.

Evaluation for disease incidence and severity was done every 4 days as outlined in section 3.5. Days to 50% flowering and 50% pod formation were also recorded.

3.11. Determination of host varietal reaction

The objective of this trial was to screen available cowpea germplasm and determine their reactions to the bacterial pustule pathogen. Six plants were grown in 21 plastic pots filled with unsterilised soil mixed with well rotten goat manure in a ratio of 3:1. Each pot contained the same cowpea cultivar and each cultivar was grown in 2 pots. The plants were inoculated when 21 days old using the method described in section 3.5.

Water was put into the pots a day before inoculation and after inoculation water was sprinkled over the plants frequently.

Evaluation started 8 days after inoculation and lasted 20 days. The plant reactions were recorded as disease severity scores as described in section 3.6. The experiment was repeated 3 times between March, 1986 and June, 1986.

The 15 cowpea lines screened in the experiment included ER 1-1, 332, Machakos 66, wild variety, local variety (Kimakoko), 238, Kangau, 9533, TVu 310, TVu 410, TVx 66-24, Vita 1, Vita 5, Katumani 80 and 233.

3.12. Determination of pathogenic variability

An attempt was made to study pathogen variability using two isolates, one collected from Mbita Point at the shores of Lake Victoria and another from Mtwapa in Coast Province. The two sites are some of the major cowpea growing areas in Kenya and have variable climatic conditions. The tests were meant to detect occurance of pathogenic variability amongst the isolates. Varietal reaction to inoculation with the two isolates was studied in 22 cowpea lines locally available at the Coast Province and Mbita Point.

An experiment using a split plot design in which the 2 isolates were the main plots and the 22 cowpea accessions were the subplots was laid out in the field during the month of July, 1986. The experiment was replicated four times and each plot measured 2.4m x 1.8m. The plants were inoculated 21 days after planting using the method described in section 3.5.

Evaluation for disease incidence and severity started 8 days after inoculation and lasted upto 20 days after inoculation as described in section 3.6.

The cowpea lines tested were ER 1-1, 332, Machakos 66, MAK - 1/39/1/B, 238, Local variety (Kimakoko), Kangau, Vita 1, Vita 5, 9533, TVu 310, TVu 410, TVx 66-24, Katumani 80, 233, ICV 1, ICV 6, IT 83D-442, IT 82D-889, IT 83S-850, HB 48/E10 and 419.

4. RESULTS

4.1. Characterisation of the pathogen

The cowpea bacterial pustule pathogen was found to be gram negative. It produced pale yellow growth on nutrient agar after 3 days at 25°-30°C. The yellow pigment was non-water soluble and tended to change with age of culture. The colonies produced on nutrient agar were convex. Hydrogen sulphide production test gave a strong positive reaction after 7 days. The pathogen was unable to reduce nitrate to nitrite. Nutrient gelatin was liquified and potato starch also hydrolysed. Methyl red reaction varied and showed lack of consistency as results were slightly acidic to alkaline.

In all cases disease symptoms appeared 7-8 days after inoculation. The symptoms initially appeared as tiny, raised, dark green spots on the lower surface of leaves. No water soaking was visible on the initial spots but appeared 2-3 days after symptom appearance. Necrotic spots then appeared on the upper leaf surface as the spots enlarged. The centre of the spots became dry, sunken and a circular halo developed. The pustules which tended to remain circular enlarged to diameters between 1.0 and 4.0 millimetres. Their enlargement depended on the prevailing environmental conditions.

Under wet conditions the lesions enlarged faster than under dry conditions. With time the old pustules became water soaked at the margins although necrotic centres remained dry and surrounded by a halo.

The above bacteriological and pathological properties verified that the pathogen was Xanthomonas campestris pv. vignicola. This was in agreement with results of samples sent to Commonwealth Mycological Institute (C.M.I.), Surrey, England, where they also identified the organism as Xanthomonas campestris pv. vignicola.

4.2. Disease development under different cropping systems

Among the environmental factors recorded, only rainfall showed variation over the experimental period (figure I). Relative humidity and daily temperatures showed few differences between the treatments over the short rain and the long rain crops (Table I and Appendix Tables 1-4). They showed no significant differences.

TABLE I: Mean Relative Humidity and Temperatures under different cropping systems during the 1985 short rains and the 1986 long rains at Matuga.

Cropping system	Mean relative humidity (%)		Mean Temperature (°C)	
	Short rains	Long rains	Short rains	Long Rains
Cowpea pure stand	64.56	76.64	29.92	26.16
Cowpea-maize intercrop	64.36	77.14	29.80	25.60
Cowpea-maize relay crop	68.31	69.32	30.33	26.36
S.E ±	2.07	2.64	0.36	0.23
C.V. (%)	9.45	10.67	3.44	2.70

4.3.1. Disease incidence

Bacterial pustule incidence results showed similar patterns for crops grown during both short and long rains. Cowpea pure stand and cowpea-maize intercrop were not significantly different from each other but both were different from cowpea-maize relay crop ($P = 0.01$) in both seasons.

Mean disease incidence in the different cropping systems varied from 60.55% to 75.36% during the short rains. It was lowest in the cowpea-maize intercrop and highest in the cowpea-maize relay crop. During the long rains the mean disease incidence varied from 57.77% to 78.41%. It was lowest in cowpea-maize relay crop and highest in cowpea pure stand (Table 2).

Disease spread varied between the different cropping systems and between the short and long rains. Disease incidence 8 days after inoculation varied from 52.5% to 57.5% during the short rains and 5.0% to 45.0% during the long rains (Appendix Tables 5 and 6).

During the short rains disease incidence eight days after inoculation was almost similar among the treatments being 52.5% in cowpea-maize intercrop, 57.0% in cowpea-maize relay crop and 57.5% in cowpea pure culture. Disease incidence increased progressively in

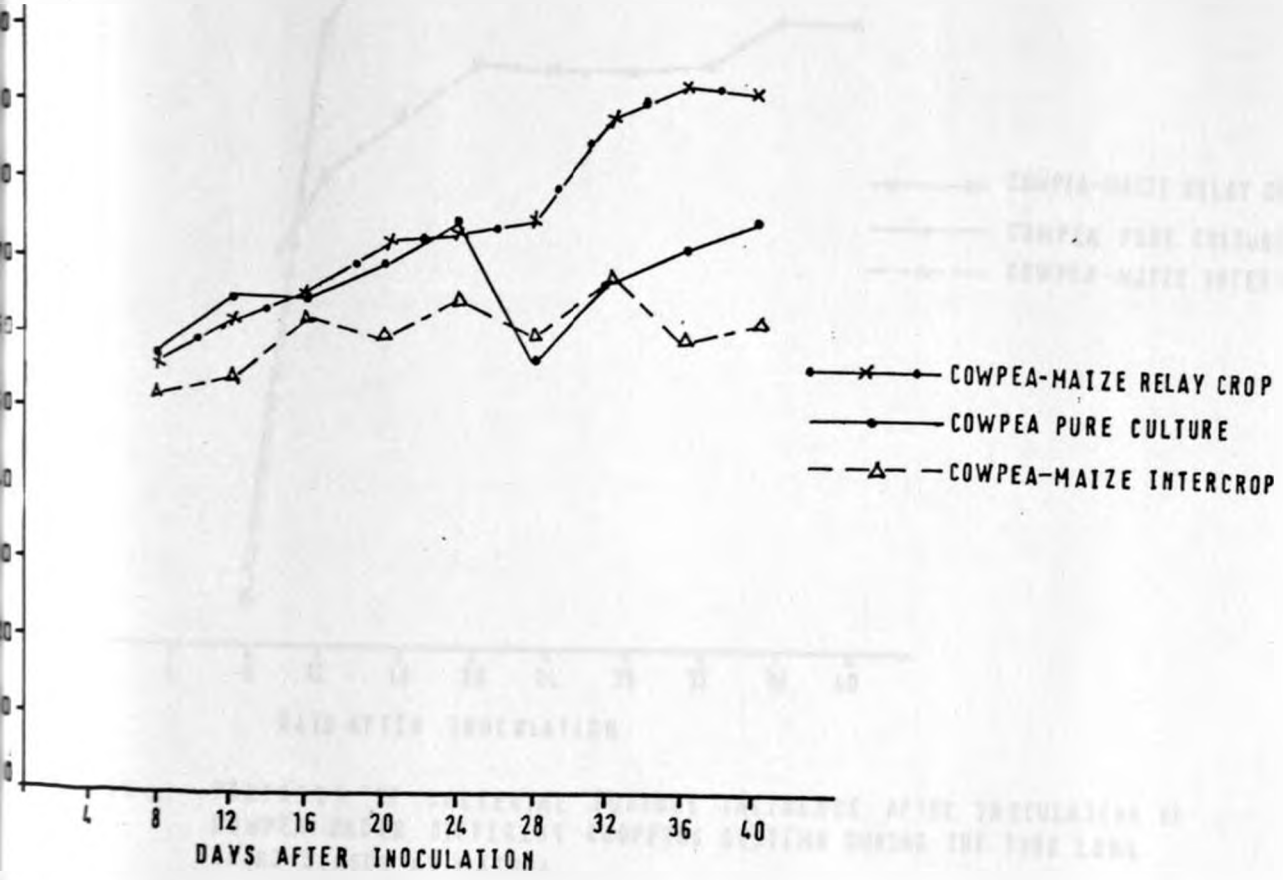

FIG 3 PROGRESS OF BACTERIAL PUSTULE INCIDENCE AFTER INOCULATION OF COWPEA UNDER DIFFERENT CROPPING SYSTEMS DURING THE 1985 SHORT RAINS SEASON AT MATUGA.

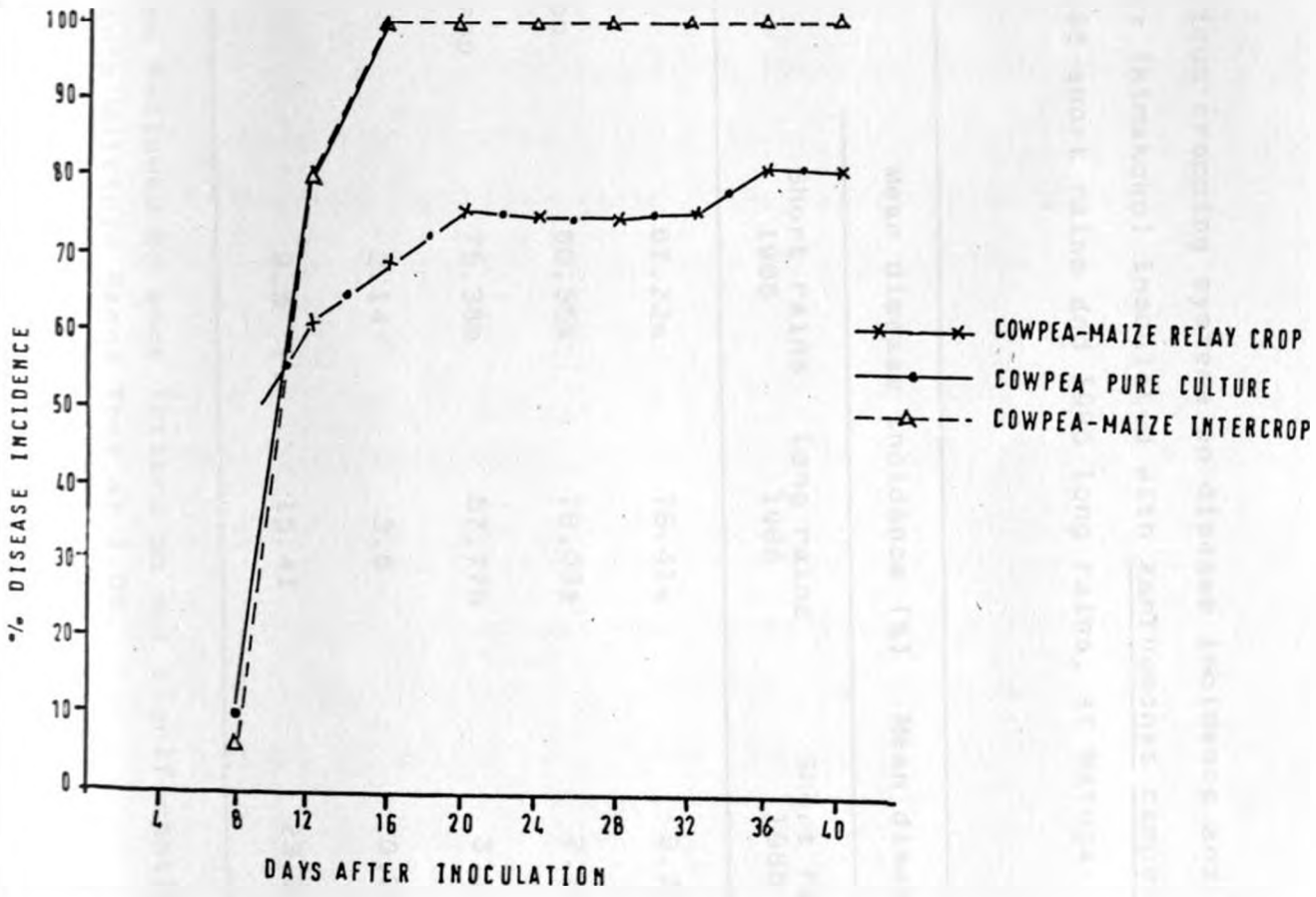

FIG. 4 PROGRESS OF BACTERIAL PUSTULE INCIDENCE AFTER INOCULATION OF COWPEA UNDER DIFFERENT CROPPING SYSTEMS DURING THE 1986 LONG RAINS SEASON AT MATUGA.

TABLE 2: Effect of various cropping systems on disease incidence and severity on a local cowpea variety (kimakoko) inoculated with Xanthomonas campestris pv. vignicola during the 1985 short rains and 1986 long rains, at Matuga.

Cropping system	Mean disease incidence (%)		Mean disease severity scores	
	Short rains 1985	Long rains 1986	Short rains 1985	Long rains 1986
Cowpea pure culture	67.22a	78.41a	3.22a	3.80a
Cowpea-maize intercrop	60.55a	78.03a	2.77a	2.77a
Cowpea-maize relay crop	75.36b	57.77b	3.11a	3.11a
S.E ±	2.14	3.6	0.24	0.13
C.V. (%)	9.5	15.41	23.81	11.41

[a] Means in same column followed by same letters do not significantly differ as determined by Duncan's Multiple Range Test at 1.0%.

Disease incidence was higher during the long rains (78.41%) than the short rains (75.36%). Cowpea-maize intercrop system had the lowest disease incidence during the short rains while cowpea-maize relay crop had the highest disease incidence. Rate of disease spread was faster during long rains where 100% disease incidence was observed only 16 days after inoculation while during short rains the highest incidence observed was 92.0% forty days after inoculation. Faster spread was therefore observed in cowpea-maize relay crop only during short rains while during the long rains it was observed in both cowpea pure culture and cowpea-maize intercrop.

4.2.2. Disease severity

The disease severity scores showed variation between the treatments and between the 2 seasons. However, there were no significant differences between the treatments in both short rains and long rains (Table 2).

The short rains had mean disease severity ranging from scale 2.77 to 3.22. Observations eight days after inoculation showed cowpea pure culture had a severity score of 3.0 while the other 2 treatments had a severity score of 2.0 each (Appendix Table 7). Cowpea pure culture attained a severity score of 4, the highest score in the season only after 20 days while both cowpea-maize intercrop and cowpea-maize relay crop had

a severity score of 3.0 at the same period. Cowpea-maize intercrop attained highest severity score (scale 4) 24 days and cowpea-maize relay crop 36 days after inoculation. There was therefore a faster disease severity increase in cowpea pure culture than in any other treatment during the short rains (Fig. 5).

During the long rains, cowpea pure culture again developed severe disease symptoms faster than the other cropping systems (Appendix Table 8 and Fig. 6, although the initial severity score was lower than in the others. Cowpea pure culture also recorded the highest disease severity score of 4.75 during the season. The average severity scores ranged from 1 to 4.75 between the treatments over the whole season. Cowpea pure culture attained a disease severity score of 4.25 only 16 days after inoculation while cowpea-maize intercrop and cowpea-maize relay crop had a score of 3.0 and 3.25 respectively at the same period.

Disease severity development exhibited by plants in the different cropping systems were comparable over the 2 seasons with plants in cowpea-pure culture showing faster severity development. However, higher severities were observed during the long rains than the short rains. Disease severity progress was also faster during long rains than short rains in all the three cropping systems.

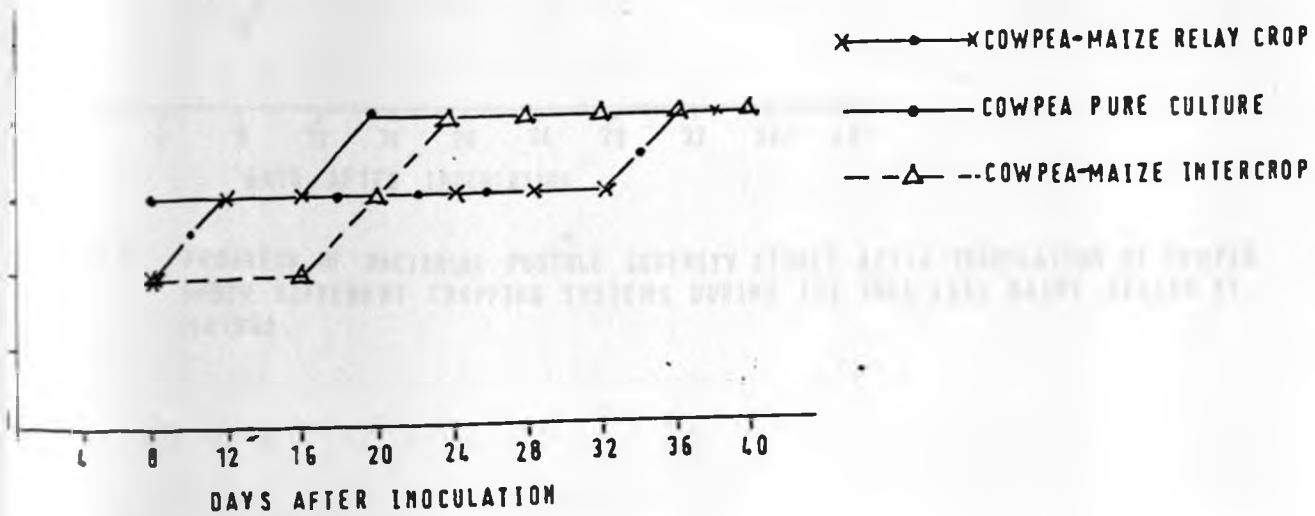

FIG. 5 PROGRESS OF BACTERIAL PUSTULE SEVERITY SCORES AFTER INOCULATION OF COWPEA UNDER DIFFERENT CROPPING SYSTEMS DURING THE 1985 SHORT RAINS SEASON AT MATUGA.

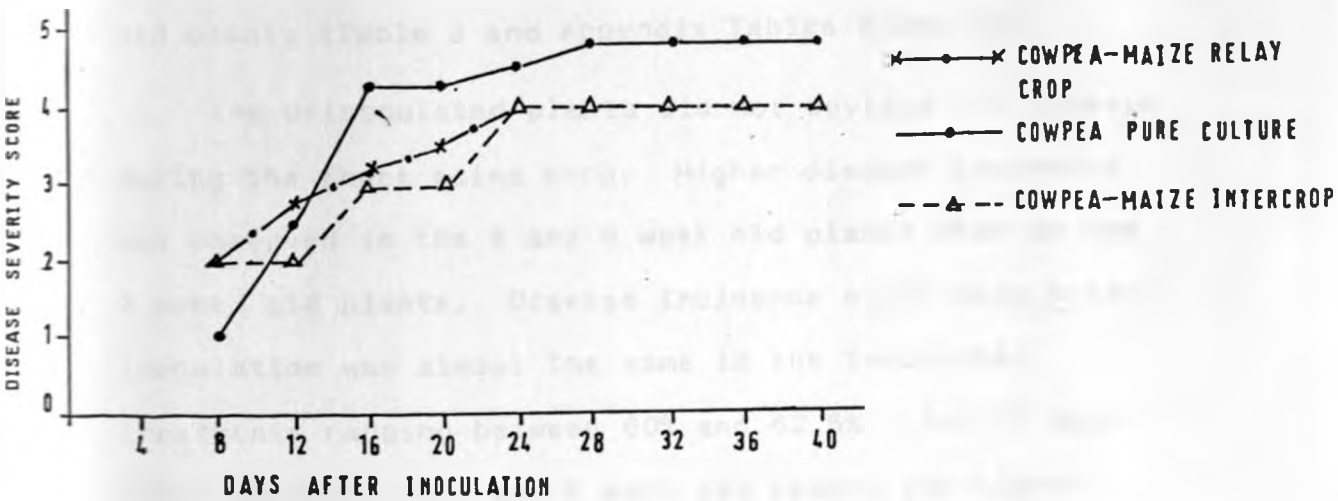

FIG. 6. PROGRESS OF BACTERIAL PUSTULE SEVERITY SCORES AFTER INOCULATION OF COWPEA UNDER DIFFERENT CROPPING SYSTEMS DURING THE 1986 LONG RAINS SEASON AT MATUGA.

4.3. Effect of plant age on disease development

4.3.1. Disease incidence

Uninoculated plants (0 days old at inoculation) were significantly different from the other treatments during both short and long rains. During the long rains, plants inoculated when 2, 4 and 6 weeks old were not significantly different from each other. However, during the short rains, uninoculated and 2 week old plants were significantly different from 4 and 6 week old plants (Table 3 and Appendix Tables 9 and 10).

The uninoculated plants did not develop the disease during the short rains crop. Higher disease incidence was observed in the 4 and 6 week old plants than in the 2 week old plants. Disease incidence eight days after inoculation was almost the same in the inoculated treatments ranging between 60% and 62.5%. But 20 days after inoculation 4 and 6 week old plants had higher disease incidences than 2 week old plants, being 90%, 85% and 70% respectively. Forty days after inoculation 2 week old plants showed 60% infection, 4 week old plants, 82.5% and 6 week old plants, 100%. Disease spread in inoculated plants was higher in older plants than in younger plants.

TABLE 3: Effect of plant age on % bacterial pustule incidence on a local cowpea variety (Kimakoko) inoculated with Xanthomonas campestris pv. vignicola over two seasons.

Age of plant at inoculation (weeks)	Mean % disease incidence	
	Short rains 1985	Long rains 1986
0	0.0a	32.59a
2	59.77b	83.47b
4	85.0c	88.49b
6	84.54c	88.68b
S.E. ±	2.74	2.55
C.V. (%)	11.90	12.54

[a] Means in same column followed by same letters do not significantly differ as determined by Duncan's Multiple Range Test at 1.0%.

During the long rains season, the inoculated plants got infected although disease incidence was much higher and rate of spread was faster in the inoculated plots (Appendix Table 10). Disease incidence 8 days after inoculation was 0% in uninoculated plots, 42.5% in 4 week old plants and 91.25% in 6 week old plants. But 12 days after inoculation, the uninoculated plants still had no infection while the other 3 treatments had attained maximum infections of 100%. The uninoculated plants attained a maximum disease incidence of 56.25% observed 36 days after inoculation. Infection could be due to cross contamination from the adjoining plots.

Disease incidence was higher and rate of spread faster during the long rains than during short rains in all the treatments.

4.3.2. Disease severity

Disease severity scores showed that uninoculated plants were significantly different from plants inoculated when 2, 4 and 6 weeks old. However, plants 4 and 6 weeks old when inoculated were not significantly different from each other (Table 4, Appendix Table 11 and 12).

During the short rains, disease severity 8 days after inoculation ranged from 1.0 in uninoculated plants

to 2.75 in 4 week old plants. Two and six week old plants had disease severity scores of 2.0 and 2.25 respectively at the same period. There was a gradual rise in disease severity and 20 days after inoculation 2, 4 and 6 week old plants had severity scores of 2.5, 3.75 and 3.25 respectively. Maximum severity score observed in 2 weeks old plants was 2.5 which occured 12 days after inoculation. In 4 week old plants, the maximum severity score was 3.75 and this was observed 12 days after inoculation while in 6 week old plants the maximum severity score was 4.5 observed 32 days after inoculation. There was therefore a trend of older plants showing more severe infections.

TABLE 4: Effect of plant age on bacterial pustule severity on a local cowpea variety inoculated with Xanthomonas campestris pv. vignicola over two seasons.

Age of plant at inoculation (weeks)	Mean disease severity score	
	Short rains, 1985	Long rains, 1986
0	1.0a	2.08a
2	2.06b	3.33b
4	3.20c	3.92c
6	3.54c	4.05c
S.E. ±	0.13	0.16
C.V. (%)	18.72	18.56

[a] Means in same column followed by same letters do not significantly differ as determined by Duncan's Multiple Range Test at 1.0%.

The long rains crop showed similar trends as in the short rains. Disease severity score 8 days after inoculation was 1.0 in uninoculated plants, 2.0 in 2 week old plants, 2.25 in 4 week old plants and 2.5 in 6 week old plants. Twenty days after inoculation 0, 2, 4 and 6 week old plants had mean disease severity scores of 2.0, 3.24, 4.25 and 4.75 respectively. Maximum disease severity scores observed were 2.0 in uninoculated plants, 3.5 in 2 week old plants and 5.0 in both 4 and 6 week old plants. The maximum severity in 6 week old plants was observed earlier (24 days after inoculation) than in 4 week old plants (28 days after inoculation).

4.4. Host varietal reaction

Mean disease severity scores among the different varieties varied from 1.3 to 3.3. Some varieties showed significant differences among them (Tables 5 and 6). The varieties were grouped into 3 classes depending on disease severity reactions observed 20 days after inoculation. There were 3 resistant varieties which fell in the class with disease severity scores of 1.0 -2.0. Nine varieties were grouped as moderately resistant in the class with disease scores of 2.1-3.0. The class with disease score of 3.1-4.0 considered to be susceptible had 3 varieties.

TABLE 5: Classification of 15 cowpea varieties based on their disease severity reactions when inoculated with Xanthomonas campestris pv. vignicola observed 20 days after inoculation*.

1.0-2.0	2.1-3.0	3.1-4.0
Wild variety	ER 1-1	Local variety (Kimakoko)
9533	332	Vita 1
Kangau	238	TVu 310
	TVx 66-24	
	Vita 5	
	TVu 410	
	Katumani 80	
	233	
	Machakos	

* Mean disease severity classes 1.0-2.0 = resistant; 2.1-3.0 = moderately resistant; 3.1-4.0 = susceptible.

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TABLE 6: Reactions of 15 cowpea varieties inoculated with Xanthomonas campestris pv. vignicola observed 20 days after inoculation.

Variety	Disease severity
ER 1-1	2.66d
332	2.66d
Wild variety	1.33a
238	2.66d
Local variety (Kimakoko)	3.33f
Kangau	2.00c
Vita 1	3.33f
9533	2.00b
TVu 310	3.33f
TVx 66-24	2.66d
Vita 5	2.33c
TVu 410	3.0c
Katumani 80	3.0c
233	2.66d
Machakos 66	2.66d
S.E. ±	0.27
C.V. (%)	17.74

[a] Means in same column followed by same letters do not significantly differ as determined by Duncan's Multiple Range Test at 1.0%.

Varietal reactions varied although all showed symptoms 8 days after inoculation. ER 1-1, Wild variety, TVx 66-24, Vita 5 and 233 had slower symptom development as compared to the other varieties. Between 12 and 15 days after inoculation, varieties ER 1-1, 233, TVu 410, Vita 1, TVu 310 and Machakos 66 exhibited some leaf yellowing but no trifoliate leaves were shed although primary leaves dropped earlier than in the other varieties. There was rapid secondary disease spread on some of the moderately resistant varieties especially TVu 410 and Katumani 80.

Local variety, Vita 1 and TVu 310 showed some leaf defoliation 20 days after inoculation.

Pustule sizes were more or less uniform in the initial stages but tended to enlarge faster on susceptible varieties. However, they remained small on the wild type.

4.5. Pathogenic variability

4.5.1. Disease incidence

Disease incidence varied between 0 and 100% although most of the cowpea varieties had disease incidences ranging between 45.0% and 100% with only one variety having no infection (Table 7). There were significant differences in disease incidence between the 2 isolates and also between some of the varieties.

However, there was no interation between the isolates and the varieties. The Mtwapa isolate had a lower disease incidence than the Mbita Point isolate with most of the cowpea varieties. Variety HB 48/E 10 showed resistance to both isolates.

TABLE 7: Disease incidence of 22 cowpea varieties inoculated with two isolates of *Xanthomonas campestris* pv. *vignicola* observed 20 days after inoculation.

Variety	% Disease incidence	
	Mtwapa isolate	Mbita Point isolate
ER 1-1	75.0	77.5
332	62.5	100
Machakos 66	70.0	100
Mak/1/39/1/B	67.5	77.5
238	85.0	95.0
Local variety (Kimakoko)	95.0	100
Kangau	47.5	97.5
Vita 1	75.0	90.0
9533	47.5	80.0
TVu 310	67.5	90.0
TVx 66-24	70.0	92.5
Vita 5	45.0	85.0
TVu 410	77.5	82.5
Katumani 80	97.5	87.5
233	70.0	92.5
ICV 1	67.5	77.5
ICV 6	60.0	67.5
IT 83 D-442	85.0	97.5
IT 82 D-889	95.0	97.5
IT 83 S-850	75.0	97.5
HB 48/E 10	0.0	0.0
419	65.0	72.5
S.E. $\pm$	2.36	6.49
C.V. (%)	27.10	22.92

4.5.2. Disease severity

There were no significant differences in disease severity between the 2 isolates but there were significant differences between some of the varieties. Only one variety showed no symptoms among the 22 cowpea varieties used. Mean disease severity ranged from 1.0 to 3.25 indicating a range between resistant and susceptible (Table 8).

TABLE 8: Disease severity reactions of 22 cowpea varieties inoculated with two isolates X. campestris pv. vignicola.

Variety	Disease severity	
	Mtwapa isolate	Mbita Point isolate
ER 1-1	2.25cd	3.25a
332	2.25cd	2.75a
Machakos 66	2.5acd	3.0a
MAK/1/39/1/B	2.25cd	3.25a
Local variety (Kimakoko)	3.25a	2.75a
Kangau	2.0cd	2.5acd
Vita 1	3.0a	2.75a
9533	2.0cd	1.75cd
TVu 310	3.0a	2.5acd
TVx 66-24	2.25cd	2.5acd
Vita 5	2.5acd	3.0a
TVu 410	3.25a	3.0a
Katumani 80	2.5 acd	2.75a
233	2.25cd	2.5acd
ICV 1	2.25cd	2.5acd
ICV 6	2.25cd	2.75a
IT 83 D-442	2.0cd	2.25ac
IT 82 D-889	2.5acd	2.5acd
IT 83 S-850	2.5acd	3.a
HB 48/E 10	1.0b	1.0b
419	2.0cd	2.25cd
S.E. ±	0.099	0.174
C.V. (%)	28.07	20.09

[a]Means in same column followed by same letters do not significantly differ as determined by Duncan's Multiple Range Test at 1.0% .

5. DISCUSSION

Cowpea bacterial pustule has come into prominence recently since its introduction from West Africa in the 1970's. Susceptible varieties have their leaves discoloured and defoliated thus making them less attractive for consumption as a vegetable and also lowering the grain yield. The disease is more prevalent during heavy rains. Although the disease is becoming important, little information is available on its spread in the country. In this study, work was undertaken to determine the effect of cropping systems and the effect of plant age on the disease development. Varietal reactions to the disease and preliminary studies on pathogenic variability were also attempted.

Results of the laboratory characterisation and pathogenicity tests verify the test pathogen in the genus Xanthomonas. The tests used are some of those recommended by Dye (1962) in the diagnosis of Xanthomonas. The results obtained agreed with those outlined by Buchanan and Gibbons (1974) and those reported by Kaiser and Ramos (1979) with some East African isolates of the cowpea bacterial pustule pathogen. Results of bacterial samples sent to the Commonwealth Mycological Institute, England, also verified that the organism was Xanthomonas campestris pv. vignicola (Burkholder) Dye.

Incubation period of the cowpea bacterial pustule pathogen was between 7 and 8 days when plants 21-25 days old were foliar sprayed with a pure culture of the pathogen. This was consistent in this study irrespective of prevailing environmental conditions. Patel (1981) also observed pustule symptoms to develop 8 days after inoculation and similar results have also been obtained in later work with bacterial blight of cowpea caused by a strain of the bacterial pustule pathogen (Prakash and Shivashankar, 1982).

Bacterial pustule spread has been shown in this study to be lowest when cowpea was grown as an intercrop with maize during the short rains and when grown as a relay crop with maize during the long rains. The disease spread was high when cowpea was grown as a pure stand or relay crop with maize during the short rains and when grown as a pure stand and an intercrop with maize during the long rains.

Bacterial pathogens usually enter into plants through natural openings and wounds, multiply and then spread either on same plant or from plant to plant. Spread depends on ability of the pathogen to move to new sites which may on the other hand depend on epidemiological factors like physiology of host plant and the prevailing environmental conditions. Faster disease spread in cowpea pure culture over both seasons

could be due to the ease with which bacterial cells were able to move to adjoining cowpea plant rows from infected plants. It may be that maize plants between the cowpea plant rows in the cowpea-maize intercrop served as a barrier which hindered movement of bacterial cells to adjoining cowpea rows, thus causing delay in spread. Disease spread in cowpea-maize mixture is more open to cowpea plants within rows and on same plant but restricted between rows due to the maize barrier.

The study also showed rainfall to be an important environmental factor which influenced bacterial pustule disease severity and spread. During the long rains, cowpea pure stand and cowpea-maize intercrop had severe and faster disease spread than cowpea-maize relay crop. The 2 treatments were observed between April and early July, 1986 when a total of 748.9mm of rainfall was received. The cowpea in the relay crop which had less disease was on the ground during long rains when only 63.5mm of rainfall was received (Fig. 1). Cowpea-maize intercrop had least disease during the short rains although it was grown at the same period as cowpea pure culture when a total of 172.5mm of rainfall was received. The cowpea-maize relay crop of the short rains season had more disease as it was on the ground for a period of time (April, 1986) when the long rains started.

Rainfall influences both initiation and development of bacterial diseases. Bacterial pathogens are usually disseminated in water drops splashed by rain and in rain water moving from surface of infected tissues to healthy ones (Agrios, 1970). Increased water uptake in plants from the soil enhances stomatal openings thus creating avenues for bacterial entry. Once in the plant the bacteria multiply while at the same time dispersal intensifies with increased rains.

Evidence is gradually accumulating to the effect that intercropping can be effectively used to decrease disease and pest incidences by slowing disease and pest infestations (Anon., 1983 a; Odhiambo, 1985). The effect of high rainfall on spread and intensity of bacterial pustule has been documented in Nigeria (Williams, 1975; Singh and Allen, 1979). Dudley (1948) has also shown bacterial blight of cowpea to be spread by field moisture propelled by wind.

Cowpea in Tropical Africa is grown mostly in a mixed cropping system, the crop combination varying from area to area (Hamblin, 1980, Steele and Mehra, 1980, Allen et al., 1981). However, in the Coast Province, it is grown as an intercrop with maize, simsim, cassava and other food crops. There are 2 rainy seasons in the Province, hence a multiple cropping systems can be practised either as a pure crop, intercrop

or relay crop. The fact that cowpea bacterial pustule has been shown to increase with increased rainfall may have some serious implications on cowpea growing in high rainfall areas. Already, cowpea is being considered by some farmers as a high risk crop because of high pest and disease incidences (Anon., 1986). The habit by farmers in the Coast Province to plant cowpea as a second crop during the long rains can be explained by the above findings that there is less bacterial pustule when cowpea is grown as a relay crop with maize during the long rains and as an intercrop with maize during the short rains.

There was no variation in relative humidity and daily temperatures and hence they did not appear to influence disease development in this study. Relative humidity is considerably high during the whole year due to the location of Matuga near the ocean (Michieka et. al., 1978). Throughout this study, RH was above 64%. Temperature fluctuations were also low with mean temperatures ranging between 20°-30°C. This range is favourable for bacterial growth hence the little influence on disease development.

The study on the effect of plant age showed that older plants and older leaves on young plants tended to have higher infection rates than younger plants and young leaves. This was exhibited by younger plants developing less disease symptoms than older plants.

Younger leaves also had less disease symptoms. The effect of rainfall was noted when uninoculated plants became infected during the long rains whereas similar plants showed no symptoms during the short rains. Various workers have shown that older plants develop more disease than young plants but offer no explanations as to why (Last, 1959; Coyne et al., 1973; Kauffman et al., 1973; Ekpo and Saettler, 1976; Allen et al., 1981).

Increased disease intensity with increased age could be due to several reasons including environmental and physiological factors. The bacterial pustule pathogen enters plants mostly through natural openings of which stomata are the most abundant. Entry by bateria is not through force but occurs when stomata are open. Stomatal closure occurs in response to increased water loss and young leaves at the top of cowpea plants are likely to be more exposed to more windy conditions than the lower dense foliage. Windy conditions result in increased transpiration and stomatal closure and hence reduced avenues for bacterial entry.

Available infection sites may also explain increased disease with age of the plant as older plants have more dense foliage. This may have resulted in older plants having more bacterial colonies established. Increased foliage also creates an ecoclimate independent of that

above the crop. This creates conditions such as high humidity within the plant canopy which are suitable for bacterial multiplication and subsequent host infection.

Young leaves on older plants were observed to get infected as plants got older. At the time of inoculation only leaves which had formed were subject to the supplied inoculum. These leaves could therefore only be infected by spread of inoculum from contaminated or infected plant parts or from other diseased plants.

Increased disease infection with increased age may affect the quality of seed more than reduction in grain yield. Observations showed infection to reach the peak about the time of flower formation. The disease has been shown to be seed transmitted (Kaiser and Ramos, 1979), and plants infected when between 2 and 6 weeks old will mostly serve as a source of inoculum for the subsequent crops. Plants grown from infected seed may end up being infected in the early seedling stages and their growth and subsequent yields drastically reduced in addition to reduced seed quality. Combining the knowledge on the effect of cropping system, rainfall and plant age, planting time can be manipulated to enable the crop to escape infection

The varietal reactions varied from resistant to susceptible in two of the experiments undertaken. One

variety HB 48/E 10 was found to have no disease symptoms when observed 20 days after inoculation. Four other resistant varieties had infections which affected less than 2.5% of actual leaf surface. Fourteen moderately resistant varieties had infections between 2.5 and 5% of actual leaf surface infected while 4 susceptible varieties had more than 5% of actual leaf surface infected.

Patel (1981), Anon. (1976), and Singh and Allen (1980) have identified some varieties resistant to bacterial pustule and some with multiple disease resistance. The varieties identified as resistant and moderately resistant can be used for future work as sources of resistance in improving popular local cowpea varieties. It may also be of interest to test the same material in other agro-ecological regions and observe their reactions.

There is indication of possible existence of pathogenic variation within the X. campestris pv. vignicola in the country. The Mbita Point isolate was more virulent than the Mtwapa isolate on most of the tested cowpea lines. However, variety HB 48/E 10 was resistant to both isolates which forms a good reason for its use in future work in addition to other varieties

Existence of pathogenic variation has been observed elsewhere using a set of differentials with isolates from various parts of the tropics (Vakili, 1977; Patel, 1981). Although the use of only two isolates may be

too few to make firm references from the above findings, there is indication that races might be existing in the country. Further work is however necessary to confirm this conclusion.

6. CONCLUSIONS

Cowpea bacterial pustule has come into prominence recently since its introduction from West Africa and might present a major constraint to cowpea production. Planting of cowpeas during the long rains as a relay crop with maize has been shown to have less disease than when cowpeas are planted as an intercrop with maize or as a pure stand. Heavy rains which occur during the main season encourage faster disease development.and spread. During the short rains cowpeas should be planted as an intercrop with maize as this system has less disease than cowpeas planted in a pure stand.

Older cowpea plants and plant parts have been shown to develop more disease symptoms than younger plants and plant parts. This results in a build up of inoculum when the plants enter the reproduction phase. As the disease is seedborne, seeds from the infected crop may be infected and this is bound to affect the proceeding crop. Use of host resistance offers best solution to disease problems and the identified resistant varieties offer potential solution to bacterial pustule if they can be used to incorporate the resistance into varieties with acceptable characteristics.

Variation in virulence between the 2 isolates screened against several cowpea varieties calls for

further research to be undertaken to evaluate more isolates against some recommended differentials. Once the range of available pathogenic variation has been determined, more cowpea germplasm can be screened based on these findings.

Further work is also suggested to determine the yield losses which might occur when plants are inoculated at different ages. The effect of disease on grain yield can also be investigated on the different cropping systems which have been shown to influence disease development.

. PLATES

Plate 1. Inoculating cowpea with Xanthomonas campestris pv. vignicola by spraying using a Solo model motorised mist blower

Plate 2. Cowpea leaf disease severity scale 2

Plate 3. Cowpea leaf disease severity scale 3

Plate 4. Cowpea leaf disease severity scale 4

Plate 5. Cowpea leaf disease severity scale 5

Plate 6. Experimental plots to determine the effect of cropping systems on bacterial pustule development during the 1986 long rains season at Matuga

Plate 5. Cowpea leaf disease severity scale 5

Plate 6. Experimental plots to determine the effect of cropping systems on bacterial pustule development during the 1986 long rains season at Matuga

Plate 7. Experimental plots of cowpea pure culture and cowpea-maize intercrop during the 1986 long rains season at Matuga

Plate 8. Some cowpea plants infected with bacterial pustule disease in the experiment to determine effect of cropping systems on disease development

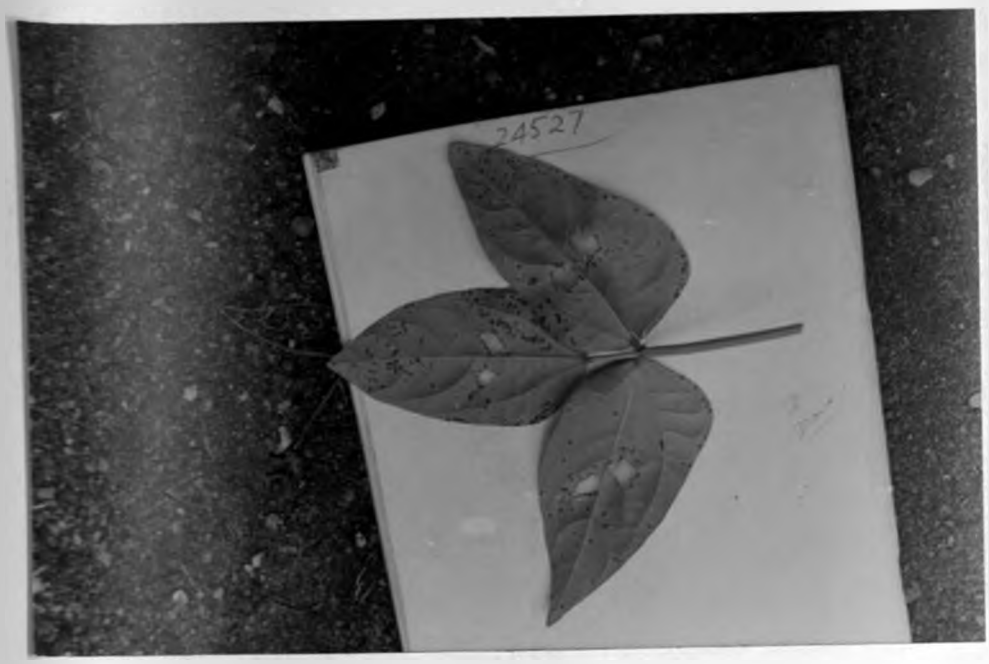

Plate 9. Bacterial pustule symptoms observed on a leaf early after symptom appearance

Plate 10. Bacterial pustule symptoms observed on a leaf during heavy rains

Plate 11. Bacterial pustule symptoms observed late during heavy rains

Plate 12. Experimental plots of cowpea to determine effect of plant age on disease development

8. REFERENCES

Agrios, G.N. 1970, Plant Pathology. Academic Pres Inc. New York. 629 pp.

Allen, D.J., Emechebe, A.M., Ndimande, B. 1981. Identification of resistance in Cowpea disease of the African Savanas. Trop. Agric. (Trinidad) 58: 267-274.

Allen, D.J. Nebane, C.L.N, Raji, J.A. 1981. Sceening for resistance to bacterial blight in Cowpea. Trop. Pest Managemnet 27: 218-224.

Anon. 1976. IITA, Annual report for 1975, Ibadan, Nigeria.

Anon. 1977. IITA, Annual report for 1976, Ibadan, Nigeria.

Anon. 1978a. Coast Agricultural Research Station, Annual report for 1978.

Anon. 1978b. University of Nairobi, Cowpea Improvement project, 1st Annual report.

Anon. 1983a. IITA, Annual report for 1982, Ibadan, Nigeria.

Anon. 1983b. Coast Province, Annual report, Ministry of Agriculture.

Anon. 1984. Eastern Province, annual report, Ministry of Agriculture.

Anon. 1985. IITA, Annual report for 1984, Ibadan, Nigeria.

Anon. 1986. IITA, Annual report and Research Highlights for 1985.

Bachman, B., Weaver, R.H. 1947. A quick microtechnique for the detection fo the reduction of nitrates to nitrites by bacteria. J. Bacteriology 54:28

Bressani, R., Elias, L.G. 1980. Nutritional value of legume crops for humans and animals. In Advances in legume Science (Eds. Summerfield R.J., Bun ting A.H.). Royal Botanic Gardens, Kew, and Ministry of Agriuclture Fisheries, and Food, London.

Buchanan R.E., Gibbons, N.E. (Eds.) 1974. Bergey's Mannual of Determinative Bacteriology, 8th edition. Williams and Wilkins Co. Baltimore, 1246 pp.

Burkholder, W.H. 1944. Xanthomonas vignicola sp nov. pathogenic on cowpea and beans. Phytopathology 34:430-432.

Cowan, S.T. 1974. Cowan and Steel's mannual for identification of medical bacteria. Cambridge Univ. Press 2nd ed. 238 pp.

Coyne, D.P., Schuster, M.L., Hill, K. 1973. Genetic control of reaction of common blight bacterium in bean (Phaseolus vulgaris) as influenced by plant age and bacterial multiplication J. Am. Soc. Hort. Sci. 98: 94-99.

Dhindsa, P., Mondjane, P. 1984. Index of plant diseases and associated organisms of Mozambique. Trop . Pest Management 30: 407-429.

Dudley, P. 1948. Bacterial canker of cowpeas in Oklahoma. Phytopathology, 38: 572.

Dye, D.W. 1962. The inadequancy of the usual determinative tests for the identification of Xanthomonas spp. New Zealand J. of Sci. 5: 393-416.

Dye, D.W. 1963. Comparative study of the biochemical reactions of additional Xanthomonas spp. New Zealand J. of Sci. 6: 483-486.

Dye, D.W. 1966. Cultural and biochemical reactions of additional Xanthomonas spp. New Zealand J. of Sci. 9: 913-919.

Ekpo, E.J.A., Saetlier, A.W. 1976. Pathogenic Variation in Xanthomonas phaseoli and X. phaseoli var fuscans. Plant Dis. Reptr. 60: 80-83.

Ezueh M.T. 1982. Effects of planting dates on pest infestation yield and harvest quality of cowepa (Vigna unguiculata), Expl. Agric. 18: 311-318.

Hamblin, J. 1980. Breeding legumes for improved system of crop protection. In Advance in legume science (Eds. Summerfield, R.J., Bunting, A.H.). Royal Botanic Gardens, Kew, and Ministry of Agriculture, Fisheries and Food, London.

James, W.C. 1974. Assessment of plant disease and losses. Ann. Rev. Phytopath. 12: 27-48.

Jindal. J.K., Patel, P.N. 1980. Variability in Xanthomonads of grain legumes. I. Pathogenic behaviour and taxonomy. Phytopath. Z. 99: 332-356.

Jindal, J.K., Patel, P.N., Khan, A.M. 1981. Variability

in Xanthomonads of grain legumes. II. Pathogenic variability in X. phaseoli mungbean strain, X. vignicola and X. phaseoli var sojense. Phytopath. Z. 100: 1-9.

Kaiser, J.N., Ramos, A.H. 1979. Two bacterial diseases of cowpea in East Africa. Plant Dis. Reptr. 63: 304-308.

Kauffman, H.E., Reddy, A.P., K., Hseith, S.P.Y., Merca, S.D. 1973. An improved technique for evaluating resistance of rice varieties to Xanthomonas oryzae. Plant Dis. Reptr. 57: 537-541.

Last, F.T. 1959. Leaf infection of cotton by Xanthomonas malvacearum (E.F. SM) Dowson. Ann. App. Biol. 47: 647-657.

Michieka, D.O., Van de Pouw, J.A., Vleeshouwer, J.J. 1978. Soils of Kwale-Mombasa-Lunga Lunga area. Kenya soil survey, Ministry of Agriculture.

Odhiambo, T.R. 1985. Pest problems as a constraint to agriculture in tropical Africa. In pesticide management in East and South Africa. Proceedings of a regional workshop, 10-15 March, Nairobi, 1985 .

Patel, P.N. 1978. Bacterial pustule disease of cowpea in Tanzania: pathogenic variability and host resistance. 3rd Int. Congr. Plant Pathol. Abst. pap. P. 72 Munich, Aug. 1978.

Patel, P.N. 1981. Pathogenic variability and host resistance in bacterial pustule disease of cowpea in Africa. Tropical Agric. (Trinidad), 58: 275-280.

Patel, P.N., Jindal, J.K. 1972. Bacterial leaf spot and halo blight disease of mungbean (Phaseoli aureus) and other legumes. Indian Phytopathology, 25: 517-526.

Patel, P.N., Singh, D. 1984. New bacterial blight resistant vegetable cowpeas in India. Trop. Grain Leg. Bulletin, 29: 14-18

Prakash, C.S., Shivashanker, R.G. 1982. Evaluation of cowpea genotypes for resistance to bacterial blight. Trop. pest Management, 28:131-135.

Preston, N,W., Morrel, A. 1962. Reproducible results with Gram stain. J. path. Bact. 84: 241

Russel, G.E. 1978. Plant breeding for pest and disease resistance. Butterworths and Co. 485 pp.

Sabet, K.A. 1959. Studies in the bacterial diseases of Sudan crops. III. On the occurence, host range and taxonomy of the bacteria causing leaf blight disease of certain leguminous plants. Ann. Appl. Biol. 47: 318-331.

Shekhawat, G.S., Patel, P.N., Raj, S. 1977., Histology of cowpea plants infected with Xanthomonas vignicola. Z. Pflanzenkr. pfanzansuchutz, 84: 547-558.

Singh, S.R., Allen, D.J. 1979. Cowpea pests and diseases. IIIA Ibadan, Nigeria.

Singh, S.R., Allen, D.J. 1980. Pests, diseases, resistance and protection in cowpeas. In advances in legume science (Eds. R.J. Summerfield and A.H. Bunting) Royal Botanic Gardens, Kew and Ministry of Agriculture, Fisheries and Food, London.

Steele, W.M., Mehra, K.L. 1980. Structure, evolution and adaptation to farming systems and environments in Vigna. In advances in legume science. (Eds. R.J. Summerfield and A.H. Bunting), Royal Botanic Gardens, Kew and Ministry of Agriculture, Fisheries and Food, Lodon.

Summerfield, R.J., Huxley, P.A., Steele, W.M. 1984. Cowpea (Vigna unguiculata (L) Walp. Field Crop Abstr. 27:301-312.

Vakili, N.G. 1977. Pathogenicity of Xanthomonas. Strains causing bacterial blight and pustule of edible legumes in puert. Rico. Tropical Grain Legume Bulletin. 8: 33-38.

Williams, R.J., 1975. Diseases of cowpea (Vigna unguiculata (L) Walp) in Nigeria. PANS 21: 253-267.

Williams, R.J. 1977. Identification of multiple disease resistance in cowpeas. Trop. Agric. (Trinidad) 54: 53-59.

Young, J. M. Dye, D.W., Bradbury, J.F. Panagopoulos, C.G., Robbs, C.F. 1978. A proposed nomeclature and classification for plant pathogenic bacteria. N.Z. Journal of Agricultural Research 21: 153-177.

8. APPENDIX TABLES

Appendix 1: Mean relative humidity (%) recorded under different cropping systems during the 1985 short rains season at Matuga*.

Days after inoculation	Mean RH (%)		
	Cropping System		
	Cowpea Pure culture	Cowpea-maize intercrop	Cowpea-Maize relay crop
8	67.75	68.50	58.42
12	70.25	68.00	71.69
16	63.41	63.25	62.75
20	67.75	68.25	73.00
24	66.85	68.15	57.38
28	58.43	58.50	74.13
32	58.50	50.50	80.94
36	65.75	66.16	66.80
40	62.38	60.00	69.75

* Mean daily relative humidity averaged over 4 days. Cowpea pure culture and cowpea-maize intercrop were planted in November, 1985 and observed up to early February, 1986 while cowpea in the relay crop was planted in February, 1986 and observed upto April, 1986.

Appendix 2: Mean relative humidity (%) recorded under different cropping systems during the 1986 long rains season at Matuga*.

Days after inoculation	Mean RH (%)		
	Cropping System		
	Cowpea pure culture	Cowpea-maize intercrop	Cowpea-maize relay crop
8	87.78	88.51	58.42
12	88.80	88.86	65.63
16	79.78	81.43	64.13
20	74.75	78.04	76.19
24	75.13	75.04	76.07
28	66.63	66.82	82.25
32	68.51	69.57	66.59
36	71.88	72.59	66.76
40	76.50	73.94	67.84

* Mean daily relative humidity averaged over 4 days. Cowpea pure culture and cowpea-maize intercrop were planted in April, 1986 and observed upto July, 1986 while cowpea in the relay crop was planted in July. 1986 and observed upto September, 1986.

Appendix 2: Mean relative humidity (%) recorded under different cropping systems during the 1986 long rains season at Matuga*.

Days after inoculation	Mean RH (%)		
	Cropping System		
	Cowpea pure culture	Cowpea-maize intercrop	Cowpea-maize relay crop
8	87.78	88.51	58.42
12	88.80	88.86	65.63
16	79.78	81.43	64.13
20	74.75	78.04	76.19
24	75.13	75.04	76.07
28	66.63	66.82	82.25
32	68.51	69.57	66.59
36	71.88	72.59	66.76
40	76.50	73.94	67.84

* Mean daily relative humidity averaged over 4 days. Cowpea pure culture and cowpea-maize intercrop were planted in April, 1986 and observed upto July, 1986 while cowpea in the relay crop was planted in July. 1986 and observed upto September, 1986.

Appendix 2: Mean relative humidity (%) recorded under different cropping systems during the 1986 long rains season at Matuga*.

Days after inoculation	Mean RH (%)		
	Cropping System		
	Cowpea pure culture	Cowpea-maize intercrop	Cowpea-maize relay crop
8	87.78	88.51	58.42
12	88.80	88.86	65.63
16	79.78	81.43	64.13
20	74.75	78.04	76.19
24	75.13	75.04	76.07
28	66.63	66.82	82.25
32	68.51	69.57	66.59
36	71.88	72.59	66.76
40	76.50	73.94	67.84

* Mean daily relative humidity averaged over 4 days. Cowpea pure culture and cowpea-maize intercrop were planted in April, 1986 and observed upto July, 1986 while cowpea in the relay crop was planted in July. 1986 and observed upto September, 1986.

Appendix 3: Mean temperature (°C) recorded under different cropping systems during the 1985 short rains season at Matuga*.

Days after inoculation	Mean temperature (°C)		
	Cropping System		
	Cowpea pure culture	Cowpea-maize intercrop	Cowpea-maize relay crop
8	30.09	30.01	32.80
12	29.44	29.06	30.18
16	30.22	30.31	31.06
20	29.87	29.53	29.58
24	28.94	28.88	31.60
28	30.40	30.12	29.89
32	30.44	30.43	27.68
36	29.90	30.07	29.87
40	-	-	-

* Mean daily temperature averaged over 4 days. Cowpea pure culture and cowpea-maize intercrop were planted in November, 1985 and observed upto February, 1986 while cowpea in the relay crop was planted in February, 1986 and observed upto April, 1986.

Appendix 4: Mean temperatures (°C) recorded under different cropping systems during the 1986 long rains season at Matuga*.

Days after inoculation	Mean temperatures (°C)		
	Cropping Systems		
	Cowpea pure culture	Cowpea-maize intercrop	Cowpea-maize relay crop
8	25.83	25.6	26.91
12	24.34	24.21	26.23
16	27.29	26.73	26.11
20	25.82	24.54	25.97
24	26.76	26.40	26.08
28	26.57	26.21	25.01
32	26.97	26.53	27.06
36	26.68	26.24	26.96
40	25.22	24.86	26.98

* Mean daily temperatures averaged over 4 days. Cowpea pure culture and cowpea-maize intercrop were planted in April, 1986 and observed upto July, 1986 while cowpea in the relay crop was planted in July, 1986 and observed upto September, 1986.

Appendix 5: Effect of different cropping systems on % disease incidence on a local cowpea cultivar (Kimakoko) inoculated with *Xanthomonas campestris* pv. *vignicola* during the 1985 short rains season at Matuga*.

Days after inoculation	Average % disease incidence		
	Cropping Systems		
	Cowpea pure culture	Cowpea-maize intercrop	Cowpea-maize relay crop
8	57.0	52.5	57.0
12	65.0	55.0	62.5
16	65.0	62.5	65.0
20	70.0	60.0	72.5
24	75.0	65.0	73.5
28	57.5	60.0	75.0
32	67.5	67.5	88.75
36	72.5	60.0	92.5
40	75.0	62.5	91.25

* Cowpea pure culture and cowpea-maize intercrop were planted in November, 1985 and observed upto February, 1986 while the cowpea in the relay crop was planted in February, 1986 and observed upto April, 1986.

Appendix 6: Effect of different cropping systems on % disease incidence on a local cowpea cultivar (Kimakoko) inoculated with Xanthomonas campestris pv. vignicola during the 1986 long rains season at Matuga*.

Days after inoculation	Average % disease incidence		
	Cropping Systems		
	Cowpea pure culture	Cowpea-maize intercrop	Cowpea-maize relay crop
8	8.75	5.0	45.0
12	78.75	80.0	61.25
16	100.0	100.0	68.75
20	100.0	100.0	76.25
24	100.0	100.0	75.0
28	100.0	100.0	75.0
32	100.0	100.0	76.25
36	100.0	100.0	81.25
40	100.0	100.0	81.25

* Cowpea pure culture and cowpea-maize intercrop were planted in April, 1986 and observed upto July, 1986 while cowpea in the relay crop was planted in July, 1986 and observed upto September, 1986.

Appendix 7: Effect of different cropping systems on disease severity on a local cowpea cultivar (Kimakoko) inoculated with Xanthomonas campestris pv. vignicola during the 1985 short rains season at Matuga*.

Days after inoculation	Average disease severity score		
	Cropping Systems		
	Cowpea pure culture	Cowpea-maize intercrop	Cowpea-maize relay crop
8	3	2	2
12	3	2	3
16	3	2	3
20	4	3	3
24	4	4	3
28	4	4	3
32	4	4	3
36	4	4	4
40	4	4	4

* Cowpea pure culture and cowpea-maize intercrop were planted in November, 1985 and observed upto February, 1986 while cowpea in the relay crop was planted in February, 1986 and observed upto April, 1986.

Appendix 8: Effect of different cropping systems on disease severity on a local cowpea cultivar (Kimakoko) inoculated with *Xanthomonas campestris* pv. *vignicola* during the 1986 long rains season at Matuga.

Days after inoculation	Average disease severity score, Cropping Systems		
	Cowpea pure culture	Cowpea-maize intercrop	Cowpea-maize relay crop
8	1.0	2.0	2.0
12	2.5	2.0	2.75
16	4.25	3.0	3.25
20	4.25	3.0	3.5
24	4.5	4.0	3.75
28	4.75	4.0	4.0
32	4.75	4.0	4.0
36	4.75	4.0	4.0
40	4.75	4.0	4.0

* Cowpea pure culture and cowpea-maize intercrop were planted in April, 1986 and observed upto July, 1986 while cowpea in the relay crop was planted in July, 1986 and observed upto September, 1986.

Appendix 9: Effect of plant age on % disease incidence of bacterial pustule on cowpea during the 1985 short rains season at Matuga.

Days after inoculation	Average % disease incidence			
	Age of plants at inoculation (weeks)			
	0	2	4	6
8	0	60.0	62.5	62.5
12	0	67.5	82.5	65.0
16	0	57.5	92.5	72.5
20	0	70.0	90.0	85.0
24	0	60.0	95.0	87.5
28	0	57.5	90.0	85.0
32	0	55.0	90.0	97.5
36	0	60.0	85.0	100
40	0	60.0	82.5	100

Appendix 10: Effect of plant age on % disease incidence of bacterial pustule on cowpea during the 1986 long rains season at Matuga.

Days after inoculation	Average % disease incidence			
	Age of plants (weeks) at inoculation			
	0	2	4	6
8	0.0	42.5	88.75	91.25
12	0.0	100.0	100.0	100.0
16	17.5	100.0	100.0	100.0
20	35.0	100.0	100.0	100.0
24	42.5	100.0	100.0	100.0
28	48.75	100.0	100.0	100.0
32	50.0	100.0	100.0	100.0
36	56.25	100.0	100.0	100.0
40	41.25	100.0	100.0	100.0

Appendix 11: Effect of plant age on disease severity of bacterial pustule on cowpea during the 1985 short rains season at Matuga.

Days after inoculation	Average disease severity score*			
	Age of plant at inoculation (weeks)			
	0	2	4	6
8	1.0	2.0	2.75	2.25
12	1.0	2.5	3.75	2.75
16	1.0	2.5	3.75	3.25
20	1.0	2.5	3.75	3.5
24	1.0	2.5	3.75	3.5
28	1.0	2.5	3.75	3.5
32	1.0	2.5	3.75	4.5
40	1.0	2.5	3.75	4.5

Appendix 12: Effect of plant age on disease severity of bacterial pustule on cowpea during the 1986 long rains season at Matuga.

Days after inoculation	Average disease severity score*			
	Age of plant at inoculation (weeks)			
	0	2	4	6
8	1.0	2.0	2.25	2.5
12	1.0	2.25	3.75	4.25
16	1.5	2.75	4.25	4.5
20	2.0	3.25	4.25	4.75
24	2.0	3.25	4.5	5.0
28	2.0	3.25	5.0	5.0
32	2.0	3.50	5.0	5.0
36	2.0	3.50	5.0	5.0
40	2.0	3.5	5.0	5.0