

BACTERIAL BLIGHTS OF BEANS

(PHASEOLUS VULGARIS L.)

CAUSED BY XANTHOMONAS PHASEOLI

(SMITH) DOWSON, AND

X. PHASEOLI VAR. FUSCANS (BURK) DOWSON

IN KENYA .

BY

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## ABSTRACT:

Xanthomonas phaseoli (Smith) Dowson, and X. phaseoli var. fuscans (Burk) Dowson were isolated from blighted bean plants collected during a survey in small scale farms in Kenya. Common and fuscous blights of beans were prevalent in all bean growing areas, but reached epidemic proportions in a few areas such as Meru, Kitui, Machakos, Kakamega, Embu, Trans-nzoia and Muranga. Common blight was severe in Meru with 12.9% incidence; Machakos, 11.0% and Kiambu and Muranga with 9.6% incidences each. Fuscous blight had higher incidences in Meru, 16.7%; Machakos, 12.0% and Kiambu and Muranga 9.6% each. In 54 small scale farms surveyed, the incidence of fuscous blight was 90.7% and that of common blight 83.3%.

The symptoms of common and fuscous blight of beans were found similar, leaves and pods showed water soaked spots in all cases. Common and fuscous blight showed irregular necrotic patches surrounded by yellow to brown margins. Brick-red longitudinal lesions were found on stems with water soaking at the nodes. Greenhouse symptoms were similar to those in the field except minor differences in 'Mwezi-moja' and 'Contender' beans. Isolates of X. phaseoli var.

fuscans caused small pinhole lesions on the undersurface of the leaves of 'Mwezi-moja'. The leaves were distorted and had chlorotic lesions. On 'Mwezi-moja', X. phaseoli caused large irregular watersoaked lesions on the undersurface of the leaves and chlorosis, covering the whole leaf on the upper surfaces. On 'Contender', X. phaseoli var. fuscans produced small necrotic lesions, that were raised and clustered together in a small area with no water soaking, but X. phaseoli produced dark, green water soaked flat lesions on the pods.

The identity of X. phaseoli var. fuscans was confirmed by the production of a brown pigment on nutrient agar, while X. phaseoli produced a yellow pigment.

The host-range of Xanthomonas phaseoli included Dolichos lablab, Phaseolus vulgaris, and P. lunatus; and that of X. phaseoli var. fuscans was restricted to D. lablab, P. vulgaris, P. lunatus and Vigna unquiculata.

Out of the 96 bean entries, tested for susceptibility to Xanthomonas phaseoli and X. phaseoli var. fuscans, only 12 were identified as resistant. The following entries possessed resistance to X. phaseoli: NB 107, NB 116, NB 178, NB 207, NB 1154, NB 1208, NB 1362, NB 2306, and GLP x 92. Resistance to X. phaseoli var fuscans was found in NB 107, NB 116, NB 134, NB 178, NB 207, NB 1154, NB 1208, NB 1362, NB 2234 and NB 2306.

Control measures recommended for common and fuscous blight of beans were crop rotation, use of certified seed and growing of resistant varieties.

## 1. INTRODUCTION:

### 1.1. Bean production in Kenya:

Bean (Phaseolus vulgaris L.) is the most important pulse crop in Kenya (1,36). It is grown over an area approximately 300,000 ha during two annual rainy seasons, the first beginning from March to June and the second starting from October to December. During 1974/75 crop season  $763.5 \times 10^3$  ha were planted to beans (Appendix 1).

Most beans are grown in mixed stands with other crops such as maize, sorghum, millet, cowpeas, pigeon peas, potatoes, cotton and cassava. For the 1974/75 crop season, 93.7% of the hactarage grown to beans in Kenya was in mixed stands (47).

The main varieties of dry beans grown by most farmers are "Canadian wonder", "Rose coco", "Iwezi-moja", and "Red harricot" (35).

Bean yields are generally low with a national average below 500 kg/ha (36). During 1975-77, the United Nations Food and Agricultural Organization (F.A.O.) estimated annual world bean production at 12.4 million tons. Latin America produced 4.7 million tons and was the most important region in

bean production followed by Eastern Africa that produced 0.8 million tons (12). In Kenya beans are grown in all the provinces (Appendix 2 and 3). Current varieties of beans are developed from types originally found in Central America. They require relatively high temperatures (above 10°C) for effective growth. Beans need free draining and moist soil throughout the growing period (1). The distribution of bean growing in Kenya is given in Appendix 2. Machakos, Kirinyaga and Kitui are currently the greatest producers of beans in Kenya (46).

#### 1.2. Beans as sources of protein in diets:

Due to the fast growth in human population the supply of adequate and the right foodstuffs is becoming a great problem to countries, especially in the developing world. One of the problems in the developing countries, such as Kenya, is the production of high quality protein in sufficient amounts to meet human demand. Since animal proteins are relatively more expensive than plant proteins, legumes such as beans are important in Kenyan Agriculture for the supply of relatively inexpensive plant proteins, for both rural and urban populations. Beans contain 22% protein (57).



Beans can be utilized in a variety of ways in Kenya. Most rural people boil them in water together with either maize, vegetables, bananas or potatoes. When cooked and fried, beans can be used with "Ugali".

### 1.3. Bean production problems:

In Kenya, bean yields are generally low with a national average below 500 Kg/ha (36). The major problems in bean production include uneven rainfall distribution, poor cultural practices and destruction by pests and diseases. The important bean growing areas include Machakos and Kitui (Appendix 2) which are in the dry marginal areas. Proper timing of planting and weed control are quite indispensable in these areas. The provision of good quality planting seeds free from diseases has been indicated as a major problem in Kenya (52). The low bean production figures given in Appendix 3 are as a result of lack of certified planting seeds (35).

The bean plant is attacked by numerous pests. The insect pests include bean fly (Melanogromyza phaseoli Coq.), american bollworm (Heliothis armigera Hb.), spotted borer (Chilo partellus Swinh.), bean aphid (Aphis fabae Scop.) and bean bruchid (Acanthoscelides obtectus Say). These insect pests can be controlled by the application of several

insecticides in the market.

The principal pathogens of beans are Colletotrichum lindemuthianum, Uromyces phaseoli, Isariopsis griseola, Pseudomonas phaseolicola, Xanthomonas phaseoli, X. phaseoli var. fuscans and bean common mosaic virus. These pathogens are wide spread and although varying in intensity in place and season, are collectively, together with the insect pests, responsible for much crop loss every year (6).

Bacterial diseases of beans are becoming severe in most areas of the world (36, 49). Though not well studied in Kenya, bean diseases caused by phytopathogenic bacteria have been reported to occur and are considered to be one of the major causes of yield fluctuations in Kenya (1, 36, 38, 39).

Kenya is very much dependent on the export of green beans and dry canned beans to various European Markets. Bacterial diseases of beans reduce yields and spoil quality. The green beans export figures have been rising since 1969. In 1969 and 1970, Kenya exported six tonnes respectively to Switzerland, and in 1971 eleven tonnes to France (28). The export of green beans earns Kenya considerable foreign exchange.

#### 1.4. Objectives of the study:

To support a stable productive Agriculture, crops have to be protected from pests and diseases. The Kenya Government has stated officially that the improved nutritional status of the population is one of its most important policy objectives and the Government has further stated that the promotion of bean production is a crucial aspect of this goal of improved nutrition (52). To generate information on common blight and fuscous blight of bean in Kenya, this study was undertaken. The objectives were:

1. To determine the incidence, prevalence and severity of common blight and fuscous blight of food beans in Kenya.
2. To characterize the symptoms of common blight and fuscous blight of beans in Kenya.
3. To study the host range of Xanthomonas phaseoli and X. phaseoli var. fuscans isolates in Kenya.
4. To screen bean lines and cultivars maintained at the National Bean Germplasm Bank at Kabete, Kenya, for resistance to common blight and fuscous blight.

The unavailability of information on bean common and fuscous blights in Kenya - created need for these investigations. If realized, these objectives should aid our Agriculture in increasing bean production.

## 2: LITERATURE REVIEW:

### 2.1. Introduction:

Beans suffer from several bacterial diseases but the important ones are common blight caused by Xanthomonas phaseoli (Smith) Dowson, fuscous blight caused by X. phaseoli var. fuscans (Burk) Dowson, halo blight caused by Pseudomonas phaseolicola (Burk) Dowson, and wilt caused by Corynebacterium flaccumfaciens (Hedges) Dowson (41).

Common blight of beans has been reported in several parts of the world (49). The disease was described for the first time by Beach in 1892 (67).

### 2.2. Geographical distribution of bean Xanthomonads:

Bacterial blights of beans caused by phytopathogenic Xanthomonads have been reported from most areas of the world. In Africa, common blight of beans caused by Xanthomonas phaseoli has been reported in Central African Republic, Egypt, Ethiopia, Kenya, Malagasy, Malawi, Mozambique, Nigeria, Zimbabwe, Somalia, South Africa, Sudan, Tanzania, Uganda, Zambia and Morocco. Fuscous blight caused by X. phaseoli var. fuscans has been reported in most of these countries including Tanzania, Uganda and Kenya (13).

### 2.3. Symptomatology of bean bacterial blights:

In the field symptoms of common blight and fuscous blight are similar (60). Symptoms appear on the leaves, pods and stems. On the leaves it causes irregular, red to brown spots surrounded by yellow margins. The spots may coalesce forming irregular patches. Severely infected leaves are shed prematurely (59). On the pods water soaked greasy spots are formed. On the stems longitudinal brown necrotic lesions are noticed. Wilting and flagging of the top foliage occurs quickly followed by top-necrosis in severe infections (49).

After artificial inoculations, symptoms appear as watersoaked spots on the leaves. The tissues between and around some of these spots become flaccid, expand and subsequently develop into large necrotic patches surrounded by yellow boarders. Fully blighted leaflets turn brittle and hang on the plants or drop off. Infected pods shrivel and are watersoaked. Seeds from severely infected pods are shrivelled. In general, both common and fuscous blight of beans exhibit identical symptoms (30, 41). Logan (30) using an atomizer to inoculate 10 bean plants found that the symptoms of Xanthomonas phaseoli and X. phaseoli var. fuscans were similar, but those of X. phaseoli var. fuscans were more intense.

## 2.4. Etiology of bean xanthomonads:

### 2.4.1. Nomenclature.

The nomenclature of bacterium causing common blight of beans has been of interest since its discovery in 1892. Smith named the causal organism of the disease as Bacillus phaseoli E.F. Smith. After investigating the cultural characteristics of the organism in 1901, he transferred it to the genus Pseudomonas. In 1905 he changed the genus to Bacterium, and later on to Phytomonas (67). Dowson (7) placed the casual bacterium under the genus Xanthomonas. When referring to common blight of beans, the following are used as synonyms of the bacterium; Bacillus phaseoli Smith, Pseudomonas phaseoli Smith, Bacterium phaseoli Smith, Phytomonas phaseoli Smith, and Xanthomonas phaseoli (Smith) Dowson.

When Burkholder (8) reported fuscous blight bacterium, he considered it a variety of Xanthomonas phaseoli, hence X. phaseoli var. fuscans. Unlike X. phaseoli, X. phaseoli var. fuscans produces a brown pigment in several culture media.

### 2.4.2. Morphology and physiology:

Both Xanthomonas phaseoli and X. phaseoli var. fuscans are single celled straight rods, motile by a polar flagellum. They are gram-negative. Growth

on agar media is usually yellow, but X. phaseoli var. fuscans browns the agar media. The bacteria exhibit respiratory metabolism, negative or weak oxidase reaction and positive catalase reaction. Acid is produced from small amounts of many carbohydrates but not from rhamnose, inulin, adonitol, dulcitol, inositol or salicin and rarely from sorbitol. Starch is hydrolysed, but nitrates are not reduced. Hydrogen sulphide is produced from nutrient agar medium. Indole is not produced. Asparagine is not utilised as a source of carbon and nitrogen. They are strictly aerobic. Optimum temperatures for growth are between 25 and 27°C (9, 21, 23).

Temperature, relative humidity and culture age influence the cells of X. phaseoli. Survival is better at 5°C than at 20°C and at either temperature it is better at 34% relative humidity than at 20% or 75% relative humidity. Survival at 20°C is poorest at 75% relative humidity (56).

Colony variants in Xanthomonas spp. have been noted to be due to large amounts of polysaccharides in media. Such variants have no capsules. Some species exist as a mixture of mucoid and non-mucoid types and are easily separated by plating on yeast extract dextrose calcium carbonate agar (YDCA) (14).

### 2.4.3. Infection by bean Xanthomonads:

Common blight development is favoured by temperatures greater than 25°C. Relative humidity greater than 90% is essential during inoculation and infection stage (14). The rate of spread of common blight is increased by wind blown rain (42). Sutton and Wallen (60) noted similar epidemiological and ecological relations of Xanthomonas phaseoli and X. phaseoli var. fuscans.

The bacteria causing common blight and fuscous blight of beans are motile and capable of moving to the host in a film of water. Penetration into the host is through natural openings such as stomata, hydathodes and nectaries; through surface wounds and breaks in root hairs, trichomes, and exudate glands. After entry the bacteria invade the intercellular spaces causing a gradual dissolution of the middle lamella. On pods the pathogen enters the sutures of the pods from the vascular system of the pedicel and then passes into the funiculus and to the seedcoat. The micropyle serves as a point of entry into the seed (16, 51, 67).

Soil temperatures are reported to influence the infection rates (31). Water is important for bacterial multiplication in plant tissue. Fog and heavy dew are ideal for blight development (60).



#### 2.4.4. Survival of bean Xanthomonads:

The Xanthomonads survive between crop seasons either in diseased seeds or in the soil in association with crop residues from the preceding season (65). Zaumeyer, et al (67) found that these bacteria can survive on beddings for animals and on farm yard manure. Sutton and Wallen (60), however, could not isolate X. phaseoli from soil in which infected plants had been grown.

Temperatures influence the viability, virulence and physiology of X. phaseoli. In seeds stored at 20 to 35°C, for three years, X. phaseoli was viable and virulent (5). In the laboratory, desiccation and temperature reduce the survival of X. phaseoli. At 20°C and 75% relative humidity, the cells had a shorter viability due to depletion of reserve nutrients through metabolic activity (56).

#### 2.4.5. Spread of bean Xanthomonads:

Common blight and fuscous blight of beans are seedborne and infected seed is important for long distant and local dissemination of the bacteria (67). Seeds are the primary source of inoculum for bacterial blights of beans (60).

Cowpea seed has been suspected to be a possible initial source of introduction of bacterial blight of beans and other edible legumes in the developing countries (61). Xanthomonas phaseoli and X. phaseoli var. fuscans from cowpeas have wide host ranges. They infect Phaseolus vulgaris, P. lunatus, P. acutifolium, P. coccineus and Glycine max (61).

Water is a potential agent in the spread of bacterial blight of beans. Water polluted with X. phaseoli if used for surface irrigation on beans will spread the bacteria (58). Sprinkler irrigation (34) was found to spread bacterial blight of beans in arid climates. The spread from plant to plant is achieved through the slime exudation from infected plants.

Rain splash and wind transfer inoculum from infected plants to healthy plants. Cafati and Saettler (10) suggested the role of Chenopodium album and Amaranthus retroflexus as means of reciprocal secondary spread.

Insect transmission of bacteria by Ceratoma raficormis, Chalcoedermis ebemiums, Diapreps abbreviata, Empoasca spp and Nezema viridula was noted to occur from infected beans to healthy ones (26).

#### 2.4.6. Host range of Xanthomonas phaseoli and X. phaseoli var. fuscans:

Several tests have confirmed host specificity of

several Xanthomonads as very characteristic (30, 48, 51, 63) and therefore this character is used in the determination of the species level. The host specificity was unaltered after repeated passage through P. vulgaris (51). Wernham (63) reported that X. phaseoli and X. phaseoli var. fuscans were restricted in host range to P. vulgaris. Sabet (48), using cross-inoculation technique, noted that X. phaseoli var. fuscans infected Dolichos lablab, P. vulgaris and Vigna unguiculata.

Bergey's manual (7) defines the host range of X. phaseoli to include D. lablab, Lupinus polyphyllus, P. vulgaris; and that of X. phaseoli var. fuscans to D. lablab, P. vulgaris and V. unguiculata. Vakili, et al (61), and Kaiser and Ramos (27) found that Xanthomonads from cowpea were pathogenic to both beans and cowpeas.

#### 2.4.7. Pathogenicity of bean Xanthomonads:

Schuster and Coyne (53) reported new strains of Xanthomonas phaseoli that were highly virulent, from Colombian dry bean seed. These strains were more virulent than the Nebraskan isolate. A Ugandan strain was as virulent as the Nebraskan isolate (54).

In 1948 Wernham (63) used pathogenicity as a specific character of 17 members of the genus Xanthomonas in cross-inoculation tests on 16 taxonomically distinct hosts. Pathogenic variation in X. phaseoli and X. phaseoli var. fuscans on the basis of disease reactions in 6 bean cultivars, showed that X. phaseoli var. fuscans was more virulent than X. phaseoli and susceptibility of the cultivars increased with age (21).

#### 2.5. Control of Xanthomonas blights of beans:

Crop yields by the farmers are far below the experimental station ones. Mukunya and Keya (36) estimated potential yields of up to 1500 Kg/ha but those of the farmers below 500 Kg/ha. Bean diseases caused by phytopathogenic bacteria are considered to be one of the major causes of yield fluctuations in Kenya (36, 52).

Common blight of beans is a disease that causes material losses of yield, and quality in many principal bean producing areas (18, 44, 50). Yield losses by either common blight or fuscous blight is difficult to assess due to the similarity of symptoms of both diseases and the association of the two pathogens in the same host (67). Bacterial blight can be expressed as the percent leaf area affected. In Cauca valley in Colombia yield losses

due to common bacterial blight were estimated at 13.1% (44).

Various control measures may be required before, during or after, the planting season to reduce the losses caused by different bean pathogens. Recommended control measures for common and fuscous blight of beans are; use of disease free seeds, suitable rotations, deep ploughing of plant debris, and use of tolerant cultivars (67).

Seeds can be treated by immersion in a solution of 1:500 Mercuric chloride in 70% Ethyl alcohol and 2% Acetic Acid. This causes some reduction in the germination of the treated seed (43). Use of mercuric compounds in crop protection has been discouraged due to high mammalian toxicity and residues.

Considerable effort has been committed to develop common blight tolerant cultivars through effective inoculation methods, inheritance of disease reactions, development of useful breeding strategies and genotypes, and stage of plant development on bacterial multiplication and symptoms (2). The work of Coyne et al (18) indicated that resistance is conditioned by a few genes whose mean effect is partial dominance. These workers found that transgressive segregation was in all crosses and reaction to X. phaseoli was

inherited quantitatively in the cross of early flowering tolerant line GN Nebr. 1 sel. 27. There was partial dominance for susceptibility at 25 days and nearly complete dominance at 43 days after inoculation. Plants at vegetative stages showed greater tolerance and lower bacterial populations than those with pods. Pathogenic variation in X. phaseoli and X. phaseoli var. fuscans are important in breeding for resistance.

Tolerance to X. phaseoli has been noted in "Jules" a Great Northern dry bean variety (17), PI 169727 and 167379 from Turkey; PI 207262 from Colombia; PI 197687 from Mexico; PI 163117 from India; "Guali" from Colombia and Great Northern Nebraska 1 sel 27, and tepary (Phaseolus acutifolius) showed no symptoms (16). Burkholder and Bullard (9) found Great Northern No. 1 bean resistant to X. phaseoli var. fuscans.

Chemical control of bean blights has been attempted in several places and found to be effective. Copper hydroxide 56%; 40% Potassium (hydroxy-methyl), and Methyl dithio-carbamate gave best control on leaves (62). Protective sprays, for example, Ortho-Cu-53, and Oxy-cu-8L, gave appreciable control (40).

Streptomycin and aureomycin were more effective than terramycin, penicillin, bacitracin and chloromycetin in the control of bacterial blights of beans (32). Resistance to antibiotics by the bacteria has been reported, for example, X. phaseoli and X. phaseoli var. fuscans are resistant to streptomycin (11). Since antibiotics are expensive, their use is of little economic value.

### 3. MATERIALS AND METHODS:

#### 3.1. Survey of bacterial blights of beans in Kenya:

Surveys were conducted in the major bean growing areas of Kenya from October to December 1980 (Appendex 2). Farms were picked at random on specified routes on a map. Beans were examined for presence of bacterial blight symptoms. Suspected samples of leaves, stems, pods or whole plants were collected and preserved for further re-examination and culturing in the Plant Pathology Laboratory of Crop Science Department, Faculty of Agriculture, University of Nairobi.

Disease assessments were done with respect to incidence, severity and distribution. The incidence was determined by counting diseased plants in a 5m<sup>2</sup> plot in each of the farms sampled.

Farmers were interviewed in order to find out the source of their planting seed.

From the laboratory isolations, three types of bacteria causing blight of beans were identified and separated for further laboratory and greenhouse studies.



### 3.2. Laboratory characterization of the isolates:

Isolation from the diseased samples were done on Nutrient Agar (NA) plates. Infected tissues were cut into small pieces, surface sterilized in 70% alcohol for half a minute and placed in a drop or two of sterile distilled water for a few minutes and ooze streaked on the NA plates.

The colonies were purified, separated and designated as follows: Cream white colonies (KBC), Yellow colonies (KBY) and brown colonies (KBB).

Morphological, cultural and biochemical characters of the pure isolates were determined using the methods by Cowan (15), Dye (20), and Hayward (22). All the tests were replicated four times, and unclear results were repeated till there was no doubt as to the reaction.

The isolates were observed for growth in NA, Nutrient broth (NB), tyrosine agar (TA), and yeast extract dextrose calcium carbonate agar (YDCA). On these media, the isolates were examined for growth, colour, form, elevation, margin and pigment production.

Morphology was determined by staining with Methylene blue, Ziehl's carbofuchsin, nigrosin and gram stain. The staining procedures described by Cowan (15) were applied. Motility was observed by

preparing hanging drop, and growth on motility media. The composition of the motility test medium was (peptone 10g, sodium chloride 5g, agar 4g, beef extract 3g, distilled water 1000ml and gelatin 80g) as described by Skerman (55). The staining solutions had the following composition:

Solution A: Basic fuchsin 0.3g  
Ethyl alcohol (95%) 10 ml

Solution B: Phenol 5g  
Distilled water 95ml

Solution A and B were mixed during use.

Mordant A. Tannic acid (10% aqu. solution) 18ml  
Ferric chloride ( $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ )  
(6% aqu. solution) 6ml

Mordant B. Basic fuchsin (0.5% in ethyl alcohol) 0.5ml  
Mordant solution A 3.5 ml  
Concentrated hydrochloric acid 0.5 ml  
Formalin 2.0ml

Mordant A and B were allowed to act on the smears for 7 minutes without heating. This was then washed with distilled water. They were covered with Ziehl's carbolfuchsin for 1 minute, washed with water. The slides were dried and examined. The fine delicate flagella stained red.

The production of hydrogen sulphide was tested by use of filter papers dipped in lead acetate.

These were placed at the mouth of a NA slant culture during incubation. Blackening of the filter paper due to lead sulphide was recorded as a positive reaction.

Nitrate broth consisting of 1% peptone, 0.3% beef extract and 0.1% potassium nitrate was used for the nitrate test. Gas production was detected by foam in the broth. Nitrite test was performed using sulfanilic acid and dimethyl -  $\alpha$  - naphthylamine. Red colour indicated presence of nitrate. Zinc dust was used in case of negative reaction and the nitrite test repeated. Presence of nitrite was shown by pink colour.

Indole production was tested in 1% peptone water medium for 4 to 10 days using Erlich's reagent. Pink colour indicated presence of indole.

Catalase was detected by emulsifying loopfuls of bacterial growth in 10 volumes of 30% hydrogen peroxide on a slide and observed macroscopically for evolution of oxygen.

Oxidase tests were done on a 7cm filter paper in a petri-dish, 2 to 3 drops of 1% tetramethyl phenylenediamine dihydrochloride were placed on the filter paper. The test organism grown on NA was removed with a glass rod and smeared across the surface of the impregnated paper. A positive reaction was shown

by the development of a dark purple colour within 10 seconds. A delayed positive reaction was recorded within 60 seconds.

Levan production was determined on NA containing 4% (W/V) sucrose. A thick slimy growth was considered positive for levan production. The cultures were incubated at 20-22°C for 1 week.

Starch hydrolysis was performed on a 2% starch in NA medium. Iodine was used as the indicator. Blue colour clearing within 5 days at 20°C was considered positive.

Ammonia from peptone was tested by use of Nessler's solution. Production of a brown colour was positive, but a faint yellow colour was considered negative.

Gelatine hydrolysis was detected on nutrient gelatin medium (peptone 5g, Beef extract 3g, Gelatin 120g in 1000ml distilled water) incubated at 20°C.

The cultures were tested for growth in 5%, 6% and 7% sodium chloride. Growth in 0.1% and 0.02% tetrazolium salt was also tested.

Carbon and nitrogen sources were tested by use of the synthetic medium (potassium diphosphate ( $K_2HPO_4$ ) 0.5g, sodium chloride (NaCl) 0.1g, magnesium sulphate ( $MgSO_4 \cdot 7H_2O$ ) 0.2g, distilled water 1000ml and the test source at 10g). Bromothymol blue

indicator was used.

The production of acid and gas from carbohydrates were tested in NB basal medium using bromothymol blue as the indicator. The carbohydrates were added to the NB basal medium at the rate of 5%.

Aerobic requirements were tested by growing the cultures in NA slants covered with sterile liquid paraffin. Absence of growth indicated that air is required for growth as shown in the slants without the liquid paraffin.

Pigment production and rot of potato was performed by cutting cylinders with a 20mm cork borer, rejecting any that were bruised and or diseased. Each cylinder was cut obliquely into a slant. These were left in running water overnight, then sterilised at 115°C for 30 minutes.

All the media used in the study were standardized to a pH of 7.0 to 7.4 and were sterilized in an out-clave at 121°C for 15 minutes, unless otherwise stated. The incubation temperature was

$27 \pm 1^{\circ}\text{C}$  unless where specified otherwise.

The isolates were tested for pathogenicity on beans and tobacco seedling by injecting the bacterial suspension into the leaves (49).

The isolates were identified by tabulating all the characteristics studied. These were compared to those in Bergy's manual (7) and by Hayward (22).

### 3.3. Symptomatology in the greenhouse.

#### 3.3.1. Preparation and standardization of bacterial inocula:

The bacterial suspension was prepared by growing the bacteria in YDCA medium (yeast extract 10g, dextrose 10g, calcium carbonate 2.5g, and agar 20g in 1000ml of distilled water) (49) for 48 hours. This was scraped into 9ml sterile distilled water in a Macartyney bottle.

The suspension was serially diluted and plated on ten NA plates for 24 hours and viable bacterial cell counts done. The method of "Miles and Mizra" was used (33). Each NA plate was subdivided from the bottom of the plate into eight segments, labelling them  $10^{-1}$ ,  $10^{-2}$  and so on, to correspond to the serial dilution. The plates were incubated for 24 hours and each segment containing approximately 20 or more colonies was counted. The number of colonies per ml was then calculated.

#### 3.3.2. Leaf inoculation techniques:

Three leaf inoculation techniques were compared using the bean variety "Red harricot" (NB 84). The 3 techniques were: atomizing the bacterial suspension on the leaves, until the whole surface was wet, using a hand atomizer; injecting the bacterial

suspension into the leaves with a sterile 0.65 x 25mm hypodermic needle; rubbing the surface of the leaves with cotton wool immersed in a carborandum bacterial suspension mixture. The control treatments were inoculated with sterile distilled water. The treatments were completely randomized, and the experiment was repeated 3 times. The parameters recorded were; appearance of initial symptoms, type of symptoms, severity of symptoms produced and damage caused on the leaves.

### 3.3.3. Seed inoculation techniques:

Seeds of "Red harricot" were used to test the best seed inoculation technique. In the first method, intact seeds were soaked in a bacterial suspension for 1, 2, 3, 6 and 12 hours and planted in pots in the greenhouse. In the second method, the seeds were scratched using a sharp sterile razor blade. The cotyledons were exposed at the back of the seed. The seeds were soaked in a bacterial suspension for 1, 2, 3, 6 and 12 hours and then planted in pots in the greenhouse. Disease development was compared in all these treatments and the best method and timing selected for use in the susceptibility studies.

### 3.3.4. Symptoms produced by *Xanthomonas phaseoli* and *X. phaseoli* var. *fuscans* in the greenhouse:

Several sets of experiments, aimed at differentiating the symptoms of common blight and fuscous

blight of beans in the greenhouse, were performed. The inoculation techniques of atomizing, injecting and rubbing the primary leaves of beans were used. Several bean cultivars were used. The bacterial suspension was adjusted to  $1 \times 10^7$  cells per ml. All the plants were observed daily for symptom development. Records on the nature of infection and the type of symptoms produced by each isolate were noted.

#### 3.4. Host range studies with *Xanthomonas phaseoli* and *X. phaseoli* var. *fuscans*:

The materials tested for the host range were obtained from the East African plant quarantine station, Muguga, and the East African Seed Company in Nairobi, Kenya. The plant species tested included legumes, vegetables and cereals. The plants were grown in plastic pots and inoculated 3 weeks later with the bacterial suspension. The experiment was completely randomized and replicated 4 times. Reactions of the inoculated plants were recorded daily for 60 days after inoculation.

#### 3.5. Susceptibility studies:

The bean varieties tested were obtained from the collection maintained at the National Bean Germplasm Bank, Kabete; except HLR, GLP 16, and



GLP x 92 which were obtained from Mr. Kinyua, G.K. at the National Horticultural Research Station, Thika, Kenya. HLR, GLP16 and GLP x 92 are known to be resistant to halo blight caused by Pseudomonas phaseolicola.

A procedure to test the large number of bean materials available was designed. Seeds were scratched using a sharp sterile razor blade so that the cotyledons were exposed at the back of the seed. Exactly 10 seeds of each entry were soaked into the bacterial suspension in a McCartney bottle. The bacterial inocula were adjusted to  $1 \times 10^8$  cells per ml. The seeds were soaked for 2 hours. They were planted into 15cm polythene tubes, and placed on the greenhouse benches at random, and each entry was replicated 4 times. Observations were made at 7, 14 and 21 days after germination. This allowed a large number of entries to be tested in a relatively short period.

The disease reaction was based on a scale similar to that of Yoshii, et al (64). The number of lesions, the lesion size and severity were all taken into account. On this scale, 1 represented highly resistant entries and 5 highly susceptible ones.

The scale is explained below:

- 1 = No visible symptoms.  
Reisolations possible.  
Highly resistant entry.
- 2 = Slight small lesions on 1-5% of the leaves.  
Resistant entry.
- 3 = Moderate number of lesions of various sizes,  
some leaves chlorotic.  
Intermediate entry.
- 4 = Severe and many large lesions on most  
leaves, pronounced chlorosis and necrosis.  
Susceptible entry.
- 5 = Very severe infection, plants chlorotic,  
necrotic and defoliated.  
Highly susceptible entry.

### 3.6. Seed borne transmission test:

Seed samples of the bean cultivars "Longtom" and "Mwezi-moja" were planted in the greenhouse. The seedlings were observed for any common and fuscous blight symptoms.

In another set of experiment, seeds from severely infected plants in the greenhouse were harvested and after 2 months storage, planted in pots in the greenhouse. All plants with symptoms of common and fuscous blight were counted.

### 3.7. Greenhouse maintenance of experimental material:

Diammonium phosphate (D.A.P.) fertilizer was mixed with all soil mixtures used in the growing of experimental plants. The soil mixtures were sterilized by treatment with Methyl bromide, covered with a polythene sheet and left to rest for 3 days before use.

The seedlings were watered as necessary. The temperatures in the greenhouse varied between 15°C in the cold weather to 30°C or more in the hot weather. Occasionally, water was sprinkled on the greenhouse floor and benches to increase humidity.

The plants were sprayed against white flies with Rogor E or Rogor L40.

#### 4. RESULTS:

##### 4.1. Survey for bacterial blight of beans in Kenya:

Bacterial blights of beans caused by Xanthomonas phaseoli (Smith) Dowson, and X. phaseoli var. fuscans (Burk) Dowson were found distributed in all the districts surveyed in Kenya (Table 4.1). Halo blight caused by Pseudomonas phaseolicola (Burk) Dowson, was noted during laboratory isolations from the survey samples. Disease severity differed from district to district. It was difficult to differentiate common blight from fuscous blight by field symptoms. The bacterial blights were prevalent throughout the major bean growing areas.

The common bean varieties grown by the small scale farmers were "Rose coco", "Canadian wonder", "Red harricot", "Mwezi-moja", "Mexican 142", "Black bean" and "Kabithari". In most cases the farmers were growing mixed varieties of those mentioned above and other types they could not specify. The small scale farmers obtained their planting seeds from 1 or 2 of the following sources: the local markets, the previous harvest, or from neighbours.

Table 4.1. Distribution of common and fuscous blights of beans in 54 small scale farms in Kenya:

INCIDENCE		
<u>DISTRICT/LOCATION</u>	<u>COMMON BLIGHT</u>	<u>FUSCOUS BLIGHT</u>
<b>Kirinyaga</b>		
Ngariama	H	H
Gichugu	L	L
Ndia	L	L
Kianyaga	-	-
<b>Embu</b>		
Manyatta	H	H
Siakago	L	L
Kagaari	M	M
<b>Meru</b>		
Nithi	L	L
South Imenti	H	H
North Imenti	L	L
Keria	H	H
Abogeta	H	H
Nkuene	L	L
Gaitu	L	L
Kirua	-	L
Kiriga	-	L

Table. 4.1 cont'd.

INCIDENCE		
<u>DISTRICT/LOCATION</u>	<u>COMMON BLIGHT</u>	<u>FUSCOUS BLIGHT</u>
Trans-nzoia		
Kitale Research Station	H	H
Moi's Bridge	-	M
Nakuru		
Lake Naivasha	H	H
Kakamega		
Vihiga	H	H
Muhiga	L	L
Western Agric, Research Station	-	-
Uasin-Gishu		
Timboroa	-	-
Kiambu		
Gatundu	L	L
Kamunyaka	M	M
Magina	L	L
Kieni	H	H
Kabuku	-	-
Ngoiliba	H	H

Table 4.1. cont'd.

INCIDENCE		
<u>DISTRICT/LOCATION</u>	<u>COMMON BLIGHT</u>	<u>FUSCOUS BLIGHT</u>
Muranga		
Mweiga	H	H
Gatura	H	H
Kibaru	H	H
Kiriti	M	H
Ichichi	L	L
Nyandarua		
Kipipiri	H	H
Nyahururu	L	L
Subukia	L	L
South Nyanza		
Kasipul	L	L
Kanyada	L	L
Kanyada F.T.C.	L	L
Kisii		
Research Station	L	L
Wanjare	L	L
Keumbu	-	-

Table. 4.1. cont'd:

INCIDENCE		
<u>DISTRICT/LOCATION</u>	<u>COMMON BLIGHT</u>	<u>FUSCOUS BLIGHT</u>
<b>Kitui</b>		
Matinyani	H	H
Changwithya	H	H
Mulango	H	M
<b>Machakos</b>		
Mukaa	H	H
Konza	H	H
Masil	-	H
Matungulu	L	L
Kangundo	M	M
Ukia	L	M
Katamani	L	L
<b>Nairobi</b>		
Nairobi West	H	H
Prison farm		

- = No disease noted

L = Low incidence (less than 10%)

M = Moderate incidence (between 10-30%)

H = High incidence (greater than 30%)



Most farmers were using their own seeds saved from the previous harvest, except in Kitui district where all the farmers got the planting seeds from the local markets.

Of the 54 small scale farms sampled, 41 were in Eastern and Central provinces, the 2 major bean growing areas. The other 13 small scale farms were distributed in the Rift Valley, Nyanza and Western provinces of Kenya (Table 4.2.). Common blight and fuscous blight were found in all the small scale farms surveyed (Figure 1). Very severe infections of common blight and fuscous blight were noted in Meru, Kakamega, Muranga and Machakos districts. It was found that 37.0% and 35.3% of the farms scored high incidences of common and fuscous blight infections respectively (Table 4.3).

"Monel", "Mwezi-moja" and "Canadian wonder" beans were more severely infected. The symptoms in the field were similar. The infected plants showed varying degrees of infections on the stems, leaves and pods.

The symptoms consisted of necrotic lesions with yellow to brown margins on the leaves. Some leaves showed necrotic lesions from the edges. This type of symptom was associated with very severe disease incidence. From isolations in the laboratory analysis this was caused by Xanthomonas phaseoli var. fuscans. Systemically infected leaves were deformed.

FIGURE 1: DISTRIBUTION OF BACTERIAL BLIGHTS OF BEANS IN KENYA.

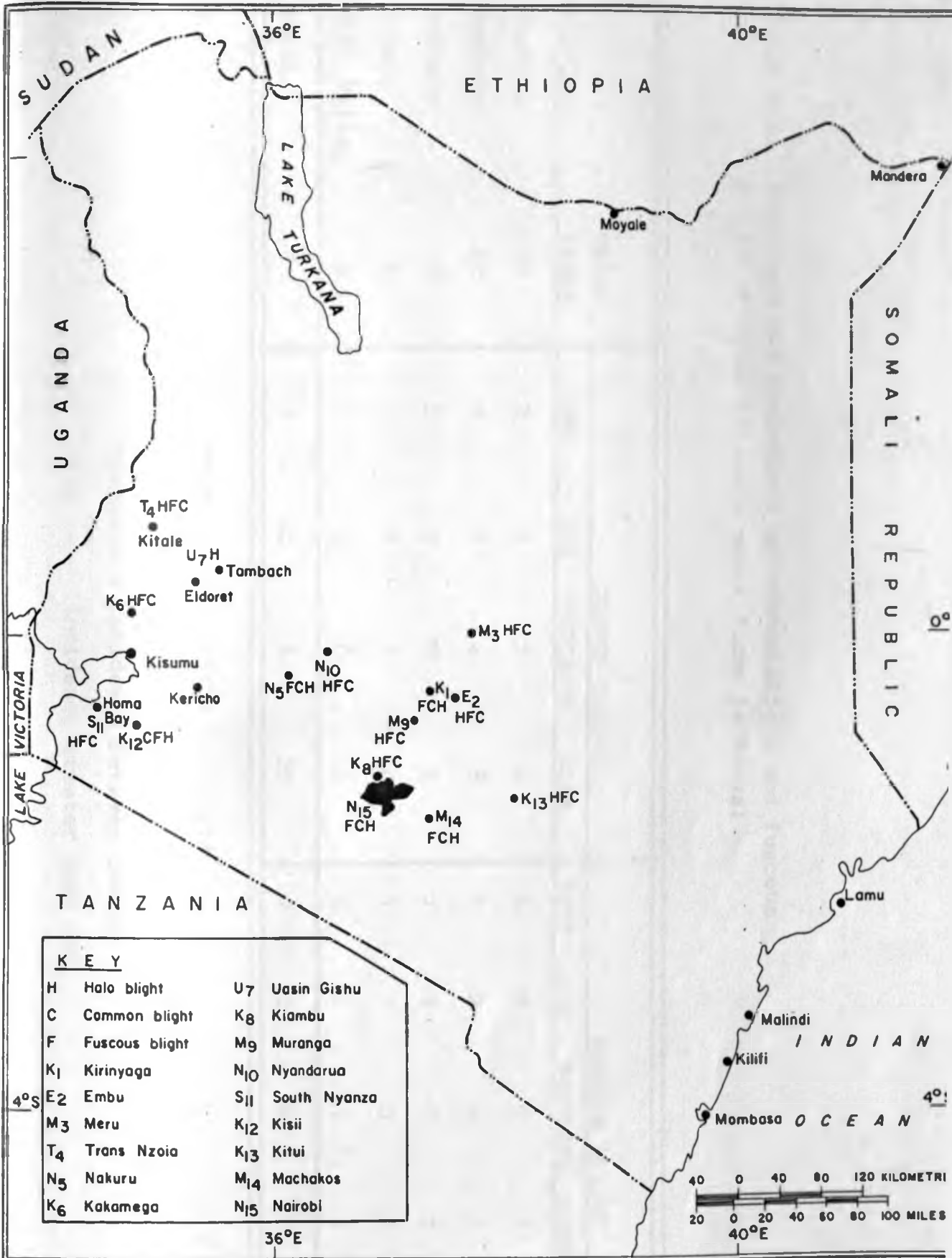


Table 4.2. Incidence and occurrence of common blight and fuscous blight of beans in small scale farms in Kenya:

PROVINCE	TOTAL FARMS	COMMON BLIGHT				FUSCOUS BLIGHT			
		NONE	LOW	MODERATE	HIGH	NONE	LOW	MODERATE	HIGH
Central	19	2	7	2	8	4	7	1	7
Eastern	22	3	8	2	9	0	9	4	9
Western	3	1	1	0	1	1	1	0	1
Nyanza	6	1	5	0	0	1	5	0	0
Rift Valley	4	2	0	0	2	1	0	1	2
<b>TOTAL</b>	<b>54</b>	<b>9</b>	<b>21</b>	<b>4</b>	<b>20</b>	<b>7</b>	<b>22</b>	<b>6</b>	<b>19</b>

None = No disease incidence

Low = Incidence less than 10%

Moderate = Incidence between 10-30%

High = Incidence greater than 30%

Table 4.3. Relative severity of common blight and fuscous blight of beans in 54 small scale farms in Kenya:

RATING	COMMON BLIGHT		FUSCOUS BLIGHT	
	FARMS SURVEYED	% SEVERITY	FARMS SURVEYED	% SEVERITY
NONE	9	16.7	7	12.9
LOW	21	38.9	22	40.7
MODERATE	4	7.4	6	11.1
HIGH	20	37.0	19	35.3
TOTAL	54	100.0	54	100.0

Young pods were shrivelled, and older ones had greasy watersoaked lesions. Beans at flowering and podding stages had more severe symptoms than those at any other stages of development.

Diagnosis of the diseases was confirmed by isolation of the respective pathogen in the laboratory. Xanthomonas phaseoli, the causal organism of common blight (Plate 1), produced yellow colonies on nutrient agar while X. phaseoli var. fuscans, the causal organism of fuscous blight (Plates 2 and 3), produced yellow colonies which browned the nutrient agar medium. The creamish white colonies on NA were produced by Pseudomonas phaseolicola, the causal organism of halo blight (Plate 4).

From the survey data (Table 4.2) disease severity differed from province to province. Of the 19 small scale farms surveyed in Central Province all except 2 had common blight and all except 4 had fuscous blight. In Eastern Province, of the 22 small scale farms surveyed, all except 3 had common blight, and all had fuscous blight. The blights had lower incidences in Western and Nyanza Provinces, but high incidences in fields in Rift Valley Province.

Fuscous blight of beans had a higher percentage distribution in Meru and Machakos districts than common blight. In these 2 districts, fuscous blight had 16.7% and 12.9% frequency respectively, while



Plate 1. Leaf symptoms of common blight of beans in the greenhouse.



Plate 2: Leaf symptoms of fuscous blight of beans in the greenhouse.



Plate 3: Fuscous blight of beans from a small scale farm  
in Kiambu district.





Plate 4: Leaf symptoms of halo blight of beans in the field.

common blight scored 12.9% and 11.0% respectively. Fuscous blight had a higher frequency (90.7%) than common blight (83.3%) in Kenya (Table 4.4).

Common blight and fuscous blight of beans, were negatively correlated to altitudes. At lower altitudes (1000-1500m) a higher frequency occurred for the 2 diseases. The frequency decreased as the altitudes increased, so that over 2500m a low frequency of 1.8% was recorded for both common and fuscous blight of beans (Table 4.5).

#### 4.2. Characterization and pathogenicity of the isolates:

The purified isolates were designated as (KBB) for the brown colonies, (KBY) for the yellow colonies, and (KBC) for the cream colonies produced on NA. Altogether 34 KBB, 26 KBY and 20 KBC isolates were handled during the characterization studies.

All the cultures were found to contain gram-negative rods. The bacteria were actively motile, each with a single polar flagellum. KBY cultures produced yellowish growth on NA and YDCA, due to a yellow pigment that was insoluble in water. There was a considerable variation in the intensity of the yellow colour produced by different samples of the KBY cultures. KBB cultures produced a yellow pigment first but it browned NA within 5 days. KBC cultures produced cream white growth on both NA and YDCA.

Table 4.4. Frequency percent distribution of common blight and fuscous blight of beans in Kenya:

DISTRICT	FREQUENCY			
	COMMON BLIGHT		FUSCOUS BLIGHT	
	FARMS SURVEYED	%	FARMS SURVEYED	%
Kirinyaga	3	5.6	3	5.6
Embu	3	5.6	3	5.6
Trans-nzoia	1	1.8	2	3.7
Meru	7	12.9	9	16.7
Nakuru	1	1.8	1	1.8
Kakamega	2	3.7	2	3.7
Uasin-Gishu	0	0.0	0	0.0
Kiambu	5	9.6	5	9.6
Muranga	5	9.6	5	9.6
Nyandarua	3	5.6	3	5.6
South Nyanza	3	5.6	3	5.6
Kisii	2	3.7	2	3.7
Kitui	3	5.6	3	5.6
Machakos	6	11.0	7	12.9
Nairobi	1	1.8	1	1.8
TOTAL	45	83.3	49	90.7
EXPECTED	54	100	54	100

Table 4.5. The correlation of altitude to distribution of common blight and fuscous blight of beans in Kenya.

ALTITUDE (METRES)  (X)	PERCENTAGE FREQUENCY			
	COMMON BLIGHT		FUSCOUS BLIGHT	
	FARMS SURVEYED	%	FARMS SURVEYED	%
1000-1500 (1250)	25	46.3	28	51.9
1500-2000 (1750)	13	24.1	13	24.1
2000-2500 (2250)	6	11.1	7	12.9
2500-3000 (2750)	1	1.8	1	1.8
TOTAL (8000)	45	83.3	49	90.7
Correlation coefficient	$r = - 0.981^{**}$		$r = - 0.969^{**}$	

(X) = Mid values of the altitude ranges

\*\* r is highly significant in both cases.

No obvious growth of the isolates occurred when cultured in NA slants covered with sterile liquid paraffin. The cultures were considered aerobic. Growth on NA plates produced circular, raised colonies with entire margins within 24 hours. The KBB cultures and some KBY ones produced butyrous growth in YDCA.

All cultures gave a positive catalase reaction, the oxidase reaction was negative. Indole was not produced and nitrate was not reduced. Asparagine was not utilized as a sole source of carbon and nitrogen. Hydrogen sulphide was produced. Starch was hydrolysed. The cultures liquified gelatin, producing saucers initially. Potato slants were digested producing honey coloured soft rot. Growth of KBB, KBY and KBC cultures was inhibited at 0.10% tetrazolium chloride salt and that of KBY and KBB at 0.02%. The growth was arrested within 10 days (Table 4.6).

Acid without gas was produced by the isolates in the sugars tested except in sorbitol, rhamnase, dulcitol, mannitol and salicin (Table 4.7).

Pathogenicity of the bacteria was established by artificial inoculations conducted in the greenhouse. The cultures were incubated for 24-48 hours, and the inoculum was standardized to  $1 \times 10^8$  cells per ml. The bean cultivar used for these tests was "Canadian wonder".

The artificial inoculations were conducted by pricking the suspension, at the primary leaf node, into 14 day old "Canadian wonder" seedlings, with a sterile needle. The disease symptoms produced were similar to those observed in the field. The initial water soaked lesions on the underside of the leaves appeared within 6 days. In some cases severe blighting and death of the seedlings occurred. Reisolations made from these artificially infected "Canadian wonder" beans yielded yellow colonies not browning NA, yellow colonies browning NA and cream white colonies which were characteristic of Xanthomonas phaseoli, X. phaseoli var. fuscans and Pseudomonas phaseolicola. Nicotiana tabaccum var. sumsun leaves reacted with the production of necrotic lesions 1 to 2 mm in diameter.

From the above tests, the cultures were identified as follows:

KBB cultures were X. phaseoli var. fuscans, KBY cultures X. phaseoli and KBC cultures Pseudomonas phaseolicola.

Table 4.6 Characterization of bean blight bacterial isolates from Kenya;

CHARACTER	REACTION		
	KBB Isolates	KBY Isolates	KBC Isolate
1. Colour on NA	Yellow, browns within 5 days	Yellow	Cream white
2. Motility			
-Hanging drop	Active	Active	Active
-Motility test media	Liquefied	Liquefied	Liquefied
-Flagellum	Polar	Polar	Polar
3. Gram reaction	-ve, rods	-ve, rods	-ve, rods
4. Anaerobic growth	-	-	-
5. Catalase test	+	+	+
6. Oxidase reaction	-	-	Variable
7. Pigment production			
-NA	Brown	Yellow	Cream
-YDCA	Brown	Yellow	Cream
-Potato slant	Honey coloured	Honey coloured	Honey coloured
8. Nitrate reduction	-	-	-
9. Indole production	-	-	-
10. Starch hydrolysis	+	+	+

Table 4.6 cont'd:

CHARACTER	REACTION		
	KBB Isolates	KBY Isolates	KBC Isolates
11. Levan production	+	+	+
12. Hydrogen sulphide production	+	+	+
13. Gelatin hydrolysis	+	+	+

- = Negative reaction for over 90% of the isolates
- + = Positive reaction for over 90% of the isolates
- ve = Negative reaction.



50(b)



**Plate 5: Soft rot on potato slants; from left to right:  
cultures 6 KBY, 12 KBY, 4 KBC and 22 KBB.**

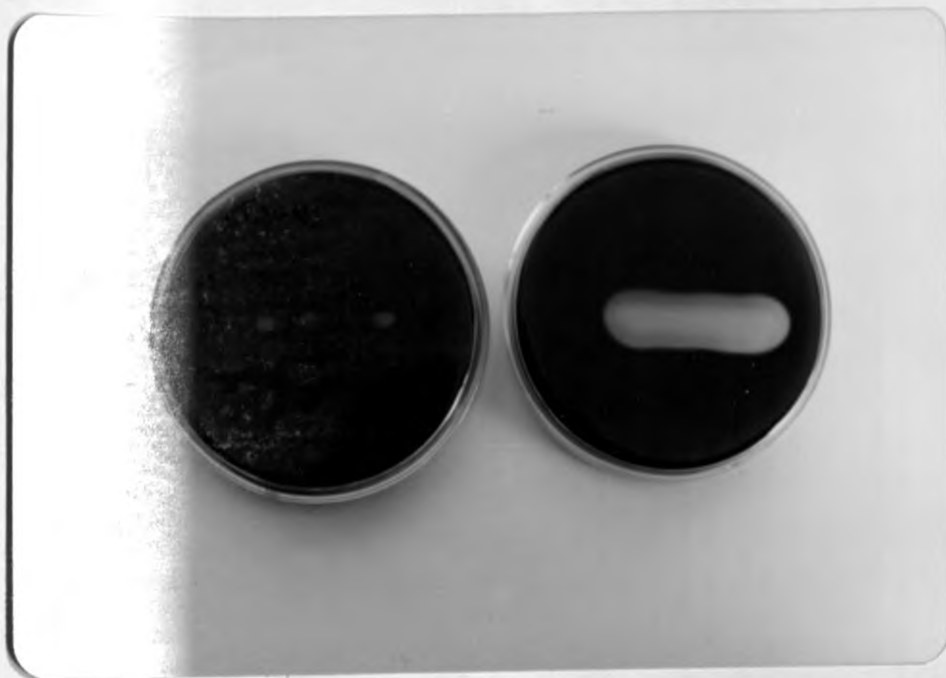


Plate 6: Starch hydrolysis by a culture of Xanthomonas phaseoli var. fuscans (right) versus medium without starch (left).

Table 4.7. Utilization of Carbohydrates by KBB, KBY, and KBC bacterial blight isolates from Kenya:

CARBOHYDRATE	REACTION					
	KBB Isolates		KBY Isolates		KBC Isolates	
	GAS	ACID	GAS	ACID	GAS	ACID
Lactose	-	+	-	+	-	+
Glucose	-	+	-	+	-	+
Sucrose	-	+	-	+	-	+
Mannose	-	+	-	+	-	+
Arabinose	-	+	-	+	-	+
Cellobiose	-	+	-	-	-	-
Xylose	-	+	-	+	-	+
Dulcitol	-	-	-	-	-	-
Rhamnose	-	-	-	-	-	-
Mannitol	-	-	-	-	-	-
Sorbitol	-	-	-	-	-	-
Salicin	-	-	-	-	-	-

+ = Positive reaction for over 90% samples tested.

- = Negative reaction for over 90% samples tested.

#### 4:3 Symptomatology in the greenhouse:

##### 4.3.1. Preparation and standardization of inocula:

The appropriate bacterial suspension was decided upon by counting viable bacterial cells in serial dilutions. At  $10^{-3}$  dilution, the viable cell counts were in the range  $7 - 8 \times 10^6$  cells, and at  $10^{-4}$  dilution they ranged between  $1 \times 10^6$  and  $2 \times 10^6$  cells per ml. For every dilution X. phaseoli var. fuscans gave more viable cell counts than X. phaseoli. It was decided to use  $10^{-3}$  dilution for X. phaseoli var. fuscans and  $10^{-2}$  dilution for X. phaseoli in the preparation of the standard inocula.

##### 4.3.2. Leaf inoculation techniques:

The leaf inoculation techniques compared, proved that injecting was superior to atomizing and rubbing. The atomization by a hand atomizer produced symptoms which were not severe enough to cause maximum disease intensity. Rubbing caused quite severe symptoms, to the extent of wilting. A lot of leaf tissue was damaged by abrasive activity during the rubbing, thus it was difficult to assess the actual damage due to the disease. Injecting caused systemic infections in most cases. The common symptoms produced were chlorosis and necrosis, followed by flagging of the foliage

Table 4.8: Reaction of KBB (Xanthomonas phaseoli var. fuscans), KBY (X. phaseoli) and KBC (Pseudomonas phaseolicola) isolates on "Red harricot" beans caused by three leaf inoculation techniques:

TECHNIQUE	I S O L A T E S		
	(KBB) <u>Xanthomonas phaseoli</u> var. <u>fuscans</u> .	(KBY) <u>X. phaseoli</u>	(KBC) <u>Pseudomonas phaseolicola</u>
ATOMIZING	Initial symptoms 6 days after inoculation. Major symptoms were flaccid, drooping leaves, water-soaked lesions, later necrotic lesions with yellow margins. The leaf was deformed. Severity at 17 days was moderate.	Initial symptoms 4 days. The leaves were water-soaked, later necrotic lesions with brown and yellow margins. Leaf was deformed. Severity at 17 days was low.	Initial symptoms 3 days. The leaves were water-soaked, later necrotic lesions with lemon margins. The leaves were deformed. Severity at 17 days was moderate.

Table 4.8 Cont'd:

TECHNIQUE	I S O L A T E S		
	(KBB) <u>Xanthomonas phaseoli</u> var. <u>fuscans</u>	(KBY) <u>X. phaseoli</u>	(KBC) <u>Pseudomonas phaseolicola</u>
INJECTING	Initial symptoms 6 days. The leaves were flaccid, with necrosis at the point of injection. The primary leaves were watersoaked. General chlorosis and necrosis, plants stunted and wilting. Severity at 17 days moderate.	Initial symptoms 4 days. The leaves were water- soaked. Necrotic lesions with brown to yellow margins. No browning at points of injection. Severity at 17 days was moderate.	Initial symptoms 6 days. The leaves were flaccid, yellow and chlorotic. Watersoaked lesions on leaves. Plant stunted. Severity at 17 days high, plants wilted.

Table 4.8 Cont'd:

TECHNIQUE	I S O L A T E S		
	(KBB) <u>Xanthomonas phaseoli</u> var. <u>fuscans</u> .	(KBY) <u>X. phaseoli</u>	(KBC) <u>Pseudomonas phaseolicola</u>
RUBBING	Initial symptoms 3 days. Water soaked lesions then necrotic lesions, with dry papery centres and lemon margins. Severe defoliation. Severity at 17 days quite high.	Initial symptoms 3 days. Water soaking very extensive. Necrosis extending over the whole leaf according to extent of rubbing. Defoliation severe. At 17 days, most plants were wilted.	Initial symptoms 6 days. The leaves were water-soaked with extensive necrosis. Bacterial ooze was evident, plants wilted by 17th day.

and wilting. Disease on the injected bean plants was quite severe.

The symptoms produced by KBB (Xanthomonas phaseoli var. fuscans), KBY (X. phaseoli) and KBC (Pseudomonas phaseolicola) cultures following inoculation by the techniques were quite similar on "Red harricot" beans (Table 4.8). They started as watersoaked lesions on the undersurfaces of the leaves. They extended into necrotic lesions with brown to yellow lemon coloured margins.

#### 4.3.3. Seed inoculation techniques:

The soaking of intact seeds for various periods produced varying disease intensity. The "Red harricot" bean seeds soaked for less than 6 hours produced no disease symptoms within 21 days. Those soaked for 12 hours showed disease symptoms in 14 days. The soaking of scratched seeds was quite successful. The seeds soaked for 2 hours showed infection in 14 days, and those soaked for more than 6 hours became wilted within 7 days. Soaking of scratched seeds for 2 hours was chosen for use in the susceptibility studies.

#### 4.3.4. Symptoms in the greenhouse:

Nine bean cultivars were used in the symptomatology study. The symptoms produced by KBB (Xanthomonas phaseoli var. fuscans) and KBY (X. phaseoli) cultures were



difficult to differentiate. The initial symptoms were small water soaked spots conspicuous on the undersides of the leaves. On the upper surfaces, yellow discolouration appeared above the soaked lesions. Drooping of leaves in severely infected plants was evident. This resulted in death of the affected plants by wilting. Longitudinal cracking of the stems, with brick-red colouration was noted in most of the inoculated plants. The pods of systemically infected plants showed large water soaked lesions.

The symptoms for the KBY cultures (X. phaseoli) on the cultivar "Contender", started as small translucent watersoaked spots on the leaves and the surrounding tissue quickly turned yellow and died leaving relatively large areas of dead leaf, of various shapes and sizes, with brown margins. The pods were shrivelled and water soaked. On the same cultivar, KBB cultures (X. phaseoli var. fuscans) produced necrotic lesions surrounded by yellow margins. On the pods, the necrotic lesions were raised and concentrated together to give a 'Pox-type' appearance, but they were not water soaked. On the cultivar "Kentucky Wonder" X. phaseoli produced longitudinal brick-red lesions on the stems, but X. phaseoli var. fuscans did not. On "Mwezi-moja" cultivar, the X. phaseoli produced large irregular water soaked lesions on the undersurfaces of the leaf, the top

showed chlorosis covering the whole leaf in most cases. On "Mwezi-moja" beans, X. phaseoli var. fuscans caused small pinhole lesions on the undersurfaces. The leaf was puckered and had chlorotic portions (Table 4.9).

To differentiate X. phaseoli and X. phaseoli var. fuscans a cultural assay was necessary. Table 4.10 shows how the 2 bacteria were differentiated from similar field symptoms.

#### 4.4. Host range of Xanthomonas phaseoli and X. phaseoli var. fuscans:

Plant species belonging to the genera Phaseolus and Vigna reacted to both Xanthomonas phaseoli and X. phaseoli var. fuscans by producing typical symptoms (Table 4:11). Initially water soaked spots were produced.

Vigna spp. reacted to the X. phaseoli var. fuscans isolates (Plate 7). Nicotiana tabaccum var. Sumsun, Zinnia elegans and Lathyrus sativum reacted by hypersensitive reaction.

Xanthomonas phaseoli var. fuscans infected the Phaseolus vulgaris cultivars tested. It also infected P. lunatus, Dolichos lablab and cultivars of Vigna unquiculata. X. phaseoli infected P. vulgaris, P. lunatus

Table 4.9. Symptomatology studies in the greenhouse:

BEAN CULTIVAR	I S O L A T E S	
	<u>Xanthomonas phaseoli</u> var. <u>fuscans</u> .	<u>X. phaseoli</u>
"Red harricot	WSL, TN, NLLYM C, W, LD.	WSL, TN, Y C, LD.
"Small Rosecoco"	LD, WSL, DL, W.	WSL, NLYM.
"Mwezi-moja"	WSL, DL, C, W.	WSL, C, W.
"Canadian wonder"	WSL, LD.	DL, WSL.
"Large Rosecoco	WSL, LD	DL, WSL, NLYM.
"Saxa"	WSL, DL, Y	DL, Y.
"Master piece"	WSL, NLBM	NLBM.
"Kentucky wonder"	DL, NLBM, PSW.	WSL, SBRL, PSW.
"Contender"	DL, WSL, NLYM, PNL.	LD, WSL, NLBM, PSW.

Abbreviations for descriptions of symptoms:

- WSL = Watersoaked lesions on leaves.  
 TN = Top necrosis.  
 NLLYM = Necrotic lesions surrounded by lemon yellow margins.  
 C = Chlorosis.  
 W = Plant wilted  
 LD = Leaf shape deformed.

- Y = Yellowing of leaves.
- DL = Drooping leaves.
- NLYM = Necrotic lesions surrounded by yellow margins.
- N = Necrosis of the whole plant.
- NLBM = Necrotic lesions surrounded by brown margins.
- SBRL = Stems with longitudinal brick-red lesions.
- PSW = Pods shrivelled and watersoaked.
- PNL = Pods with necrotic lesions.

Table 4.10 Field symptoms and colony colour production on nutrient agar (NA) and yeast-extract dextrose calcium carbonate agar (YDCA) of common and fuscous blight bacteria:

Field Symptoms	Colour in NA	Colour in YDCA	Remarks
<u>Pods</u> : Young pods shrivelled, with sunken watersoaked lesions, but some lesions pox-type and not water-soaked.	Brown  Yellow	Brown  Yellow	<u>Xanthomonas phaseoli</u> and <u>X. phaseoli</u> var. <u>fuscans</u> .
<u>Stems</u> : Watersoaked lesions brown to brick-red longitudinal lesions, not watersoaked.	Brown  Yellow	Brown  Yellow	<u>X. phaseoli</u> and <u>X. phaseoli</u> var. <u>fuscans</u> .
<u>Leaves</u> : Brittle, with necrotic lesions and slots surrounded by yellow margins.	Brown	Brown	<u>X. phaseoli</u> var. <u>fuscans</u> .
<u>Leaves</u> : Necrotic lesions of various sizes and with clear chlorotic regions.	Yellow	Yellow	<u>X. phaseoli</u>

Table 4:11 Reaction of some plant species to Xanthomonas phaseoli and X. phaseoli var. fuscans isolates from Kenya:

PLANT SPECIES	REACTION <sup>a</sup>	
	<u>Xanthomonas phaseoli</u>	<u>Xanthomonas phaseoli</u> var. <u>fuscans</u> .
1. <u>Phaseolus vulgaris</u>		
'Contender'	WSL, NL	WSL, NL
'Masterpiece'	NLBM	NLBM
'Kentucky-wonder'	NLBM	NLBM
'Great Northern		
Tara'	NLBM	WSL, NL
'Canadian wonder'	NLBM	NLBM
'Mwezi-moja'	NLBM	NLBM
'Small Rosecoco'	NLBM	NLBM
'Large Rosecoco'	NLBM	NLBM
'Red harricot'	NLBM	NLBM
'Monroe'	NLBM	NLBM
'Longtom'	NLBM	NLBM
2. <u>Phaseolus angularis</u>	-	-
3. <u>P. aureus</u>	0	0

Table 4:11 Cont'd:

PLANT SPECIES	REACTION <sup>a</sup>	
	<u>Xanthomonas phaseoli</u>	<u>Xanthomonas phaseoli</u> var. <u>fuscans.</u>
4. <u>P. mungo</u>	-	-
5. <u>P. coccineus</u>	0	0
6. <u>P. lunatus</u>	WSL, NL	WSL, NL
7. <u>Vicia faba</u>	0	0
8. <u>Dolichos lablab</u>	WSL, NL	WSL, NL
9. <u>Datura ferox</u>	0	0
10. <u>Vigna radiata</u>	0	0
11. <u>Vigna unguiculata</u>		
'California black-eye'	0	NLLM
'Mtwapa II'	0	WSL, C
'Vita 3'	0	WSL
'Machakos (74)'	0	WSL, C
'Maka. 1/39/B'	0	WSL, C
12. <u>Lucinus polyphyllus</u>	-	-
13. <u>Cicer arietinum</u>	0	0
14. <u>Glycine max</u>	0	0
15. <u>Strophostyles helrula</u>	0	0
16. <u>Stizolobium deeringianum</u>	0	0

Table 4:11 Cont'd:

PLANT SPECIES	REACTION <sup>a</sup>	
	<u>Xanthomonas phaseoli</u>	<u>Xanthomonas phaseoli</u> var. <u>fuscans</u> .
17. <u>Zea mays</u> (KCB)	0	0
18. <u>Cucurbita pepo</u>	0	0
19. <u>Nicotiana tabacum</u> var. <u>sumsun</u>	H	H
20. <u>Capsicum</u> spp.	0	0
21. <u>Brassica oleracea</u>	0	0
22. <u>Raphanus sativus</u> 'Long Red'	0	0
23. <u>Spinacia</u> sp.	0	0
24. <u>Brassica campestris</u>	0	0
25. <u>Zinnia elegans</u>	H	H
26. <u>Beta vulgaris</u>	0	0
27. <u>Lathyrus sativum</u>	H	H
28. <u>Lycopersicum</u> <u>esculentum</u> 'Rutgers'	0	0
'Marglobe'	0	0
29. <u>Lycopersicum</u> <u>esculentum</u> 'Money-maker'	0	0



Table 4:11 Cont'd:

PLANT SPECIES	REACTION <sup>a</sup>	
	<u>Xanthomonas phaseoli</u>	<u>Xanthomonas phaseoli</u> var. <u>fuscans</u> .
'Beauty'	0	0

Descriptions of abbreviations:

- a = observed 14 days after inoculation.
- WSL = watersoaked lesions.
- NL = Necrotic lesions.
- NLBM = Necrotic lesions surrounded by brown margins.
- NLLM = Necrotic lesions surrounded by lemon margins.
- NLYM = Necrotic lesion surrounded by yellow margins.
- 0 = No reaction.
- C = Chlorotic reaction.
- H = Hypersensitive type of reaction.
- = Plant species not tested.



Plate 7: Cowpea plant systemically infected by Xanthomonas phaseoli var. fuscans showing trifoliate leaves reduced in size and deformed. X. phaseoli var. fuscans was isolated from these leaves.

and D. lablab, P. aureus, P. coccineus, V. radiata and Vicia faba were not infected by the 2 bacterial isolates. The Zea mays and the vegetables tested were not infected, and therefore, not useful in the spread of the bacteria, even if used in mixed cropping.

#### 4.5. Susceptibility studies:

Using the disease scale of Yoshii et al (64), 96 beans entries were tested. Of these 9 were found resistant to the Kenyan isolates of Xanthomonas phaseoli and 11 to X. phaseoli var. fuscans (Table 4.12)• Isolations were made from leaves showing high degrees of resistance. Most of the entries were, however, moderate to extremely susceptible to the isolates. The resistant lines to X. phaseoli were NB 107, NB 116, NB 178, NB 207, NB 1362, NB 2306, GLP x 92, NB 1154 and NB 1208. Resistant entries to X. phaseoli var. fuscans were NB 107, NB 116, NB 134, NB 178, NB 207, NB 1154, NB 1208, NB 1362, NB 2234, NB 2306 and HLR.

Table 4.12 Mean disease reaction ratings of bean lines maintained at Kabete to Xanthomonas phaseoli and X. phaseoli var. fuscans.

E N T R Y			M E A N R E A C T I O N R A T I N G <sup>b</sup>	
NB NUMBER	ORIGIN	VARIETY/ LINE	<u>Xanthomonas</u> <u>phaseoli</u>	<u>X.phaseoli</u> var. <u>fuscans</u>
2	Columbia	Higuevillo	2.7 <sup>c</sup>	3.4
4	Turkey	White-harricot	4.4	5.0
11	U.S.A.	Kentucky wonder	3.5	3.5
13	Uganda	10/4/83 Diacolnima x. Cofinel	4.1	4.1
26	Kenya	Canadian wonder	4.2	3.8
48	Netherlands	Renka	3.9	4.5
62	Uganda	Calina	4.4	3.7
72	Uganda	K20	-	-
78	Kenya	Rosecoco	3.7	3.0
84	Kenya	Wairimu	4.3	3.8
89	Kenya	Rosecoco	5.0	4.25
107	Kenya	Canadian wonder	1.95	1.05
116	Kenya	-	1.5	1.3
120	Kenya	-	5.0	5.0

Table 4:12 Cont'd:

E N T R Y			MEAN REACTION RATING <sup>b</sup>	
NB NUMBER	ORIGIN	VARIETY/ LINE	<u>Xanthomonas</u> phaseoli	<u>X.phaseoli</u> var.fuscans
134	Kenya	Mwezi-moja	3.0	2.2
139	Kenya	Mwezi-moja	n	n
150	Kenya	Mwezi-moja	3.9	2.8
165	South Africa	Rosecoco	4.0	4.5
178	Kenya	Rosecoco	1.3	2.1
196	Kenya	Canadian wonder	4.5	4.6
206	Kenya	Beena	5.0	5.0
207	Kenya	Kabirum	1.1	1.0
211	Kenya	Saxa	4.15	4.05
213	Kenya	White harricot	5.0	3.8
223	Kenya	Mexican 142	4.5	4.6
249	Uganda	Kawanda' x Cofinel		
		Pauni x F <sub>6</sub> TK <sub>2</sub>	5.0	5.0
259	Uganda	5/4/1/5/2		
		Kampala leakey	n	n
273	Uganda	5/4/1/5/2		
		Rampala leakey	4.3	4.3
299	Uganda	5/22/3/1		
		leakey (F <sub>6</sub> TK <sub>3</sub> )	n	n

Table 4:12 Cont'd:

NB NUMBER	E N T R Y		MEAN REACTION RATING <sup>b</sup>	
	ORIGIN	VARIETY/ LINE	<u>Xanthomonas</u> <u>phaseoli</u>	<u>X.phaseoli</u> <u>var.fuscans</u>
315	Uganda	Diacol Nima X Verdon F <sub>6</sub> TK <sub>11</sub> Leakey	4.5	4.5
341	Uganda	19/3/2 Kampala Leakey 5'129x5' 68 F <sub>4</sub> (?) TK6	5.0	5.0
343	Uganda	"	n	n
347	Uganda	19/3/2 Kampala Leakey 5'129x 5'68 F <sub>4</sub> (?) TK16	5.0	5.0
359	Uganda	Cofinel x 5129F <sub>4</sub> (?) KT23 22/1/1/6 Kampala Leakey	4.6	4.4
377	Uganda	Kampala leakey No(23)34	3.7	4.4

Table 4:12 Cont'd:

E N T R Y			MEAN REACTION RATING <sup>b</sup>	
NB NUMBER	ORIGIN	VARIETY/ LINE	<u>Xanthomonas</u> <u>phaseoli</u>	<u>X.phaseoli</u> <u>var.fuscans</u>
400	Uganda	9/1/5/7 Kampala Leakey	n	n
413	Uganda	27/4/8 Kampala 540 x Cofinel F <sub>4</sub> TK20	4.6	4.5
427	Uganda	JI/2/21 Cofinel x 5153 F <sub>4</sub> TK23 Kampala	5.0	5.0
458	Holland	Red mexican U135	n	n
462	Holland	Great Northern UI 123	-	-
465	Holland	Light Red Kidney	4.5	4.3
495	Malawi	-	5.0	5.0
510	Kenya	LR/73/FB 31,32,33 (K20-BRP Sell1)	4.8	4.0
516	Kenya	-	3.55	3.0
517	Kenya	-	-	-
518	Kenya	Mwezi-moja	4.5	3.3
542	Italy	PI 2072 62	-	-
543	Italy	WBL 713938	-	-

Table 4:12 Cont'd:

E N T R Y			MEAN REACTION RATING <sup>b</sup>	
NB NUMBER	ORIGIN	VARIETY/ LINE	<u>Xanthomonas</u> <u>phaseoli</u>	<u>X.phaseoli</u> <u>var.fuscans</u>
556	Brazil	Calioca	3.9	3.8
566	Kenya	-	3.75	3.75
637	Kenya	-	2.5	2.9
658	Kenya	-	5.0	5.0
710	Kenya	-	3.9	2.5
739	Kenya	-	4.9	4.9
763	Kenya	-	4.0	2.5
782	E.A.C.	EAI 2722	3.8	3.8
797	E.A.C.	PI 165426	-	-
808	Kenya	Mwezi-moja	3.25	3.7
845	Kenya	Canadian wonder	4.55	4.05
861	Kenya	Gikara	2.7	2.6
890	Kenya	-	3.0	4.9
896	Kenya	-	5.0	5.0
966	Kenya	-	4.1	3.15
997	Kenya	-	-	-
1051	Kenya	-	5.0	5.0
1057	Kenya	-	3.45	4.1
1094	Kenya	-	5.0	5.0



Table 4:12 Cont'd:

E N T R Y			MEAN REACTION RATING <sup>b</sup>	
NB NUMBER	ORIGIN	VARIETY/ LINE	<u>Xanthomonas</u> <u>phaseoli</u>	<u>X.phaseoli</u> var. <u>fuscans</u>
1114	Kenya	-	4.6	4.8
1122	Kenya	-	3.85	3.65
1131	Kenya	-	4.1	4.3
1154	Kenya	-	2.5	1.3
1159	Kenya	-	3.8	5.0
1190	Kenya	-	5.0	5.0
1208	Kenya	-	2.65	1.65
1235	Kenya	EAI No.4075	5.0	5.0
1262	Columbia (CIAT)	EAI No.3946	4.6	4.6
1362	Kenya	Montio LRK 74-708	1.25	1.8
1371	Puerto Rico	Lavega	4.65	4.5
1390	Kenya	Rosecoco	5.0	5.0
1401	Kenya	Rosecoco	2.75	3.6
1421	Kenya	Rosecoco	3.8	4.1
1450	Kenya	Mwezi-moja	4.05	4.15

Table 4:12 Cont'd:

E N T R Y			M E A N R E A C T I O N R A T I N G <sup>b</sup>	
NB NUMBER	ORIGIN	VARIETY/ LINE	<u>Xanthomonas</u> <u>phaseoli</u>	<u>X.phaseoli</u> <u>var.fuscans</u>
1530	Kenya	Mwezi-moja	3.3	4.0
1692	Kenya	Canadian wonder	5.0	5.0
1722	Kenya	Canadian wonder	5.0	5.0
1790	Kenya	Canadian wonder	4.6	4.35
1801	Kenya	Canadian wonder	5.0	5.0
2144	Kenya	SR/72/LKY19	-	-
2191	Kenya	Sanilac	5.0	4.2
2234	Kenya	Pueblo 152	3.2	1.8
2296	France/ Maryland	PR-4	4.15	3.35
2306	"	Castario 1031	1.2	1.5
GLP2 <sup>o</sup>	Kenya	-	4.4	4.0
GLP16 <sup>o</sup>	Kenya	-	4.1	4.4
GLPx				
92 <sup>o</sup>	Kenya	-	1.2	3.1
HLR <sup>o</sup>	U.S.A.	-	4.4	2.4

- b = mean reading of four replicates of 10 plants each.
- c = ratings:
  - 1 = Highly resistant variety.
  - 2 = Resistant variety.
  - 3 = Intermediate variety.
  - 4 = Susceptible variety.
  - 5 = Highly susceptible variety.
- = entry not tested.
- n = no germination noted.
- = Seeds from Thika National Horticultural Research Station, Ministry of Agriculture, Kenya.

#### 4.6 : Seedborne transmission test:

The seed samples collected from farms with bacterial blights and planted in pots in the greenhouse were observed for disease symptom development. Seeds of "Mwezi-moja" scored an incidence of 72% typical bean blight symptoms. "Longtom" had an incidence of 86%. Isolations done in the laboratory indicated that 26% of the seedlings were infected with xanthomonas phaseoli, 18% with X. phaseoli var. fuscans and the rest with Pseudomonas phaseolicola of the "Mwezi-moja" lot. For "Longtom", 28% were infected with X. phaseoli, 38% with X. phaseoli var. fuscans and the rest with P. phaseolicola.

Seedborne transmission from infected bean plants was confirmed. Xanthomonas bacteria were effectively transmitted through infected seeds of "Mwezi-moja", "Red-harricot" and "Canadian wonder" from greenhouse inoculated beans. X. phaseoli var. fuscans recorded a higher transmission rate (26-34%) than X. phaseoli (18-22%).

## 5. DISCUSSION:

Bean is an important staple food crop in Kenya (35). The main varieties of beans grown by most farmers are "Rosecoco", "Canadian wonder", "Red harricot", "Mwezi-moja" and "Mexican 142". Most farmers however, grow mixed varieties.

In Kenya, bacterial blights of beans were found to be widely distributed. Common blight and fuscous blight of beans were prevalent in all areas, but reached epidemic proportions in a few areas such as Meru, Kitui, Machakos, Kakamega, Embu, Trans-nzoia and Muranga districts. Previous surveys by Schonherr and Mbugua (52) had shown that bacterial diseases of beans were widely distributed in Eastern province. Meru and Kitui districts had incidences of 59% and 19% respectively. Results of the present study are in agreement with those of Schonherr and Mbugua.

Several other districts which indicated high incidences were Meru, 12.9%; Kiambu and Muranga, 9.6% each, Machakos, 11.0% for common blight; and Meru 16.7%; Machakos, 12.9%; Kiambu and Muranga 9.6% each for fuscous blight. In the 54 small scale farms surveyed the incidence of fuscous blight was 90.7% compared to 83.3% for common blight. In many farms where common

blight and fuscous blight of beans occurred, their interaction resulted in complete leaf necrosis, severe defoliation and wilting. Zaumeyer, et al (67) reported that common and fuscous blight bacteria are found frequently in association in plant tissues. This was proved true by the laboratory isolations.

Disease severity differed from province to province. In Eastern province more cases of high incidences were recorded than in the other provinces. Common and fuscous blights of beans were in high incidences in Kenya. Farmers used seeds acquired from the local markets, neighbours or their own harvested seeds. Most of these seeds were infected as shown by a seed survey conducted in Central and Eastern provinces (37). This survey disclosed that Xanthomonas bacteria had a high incidence in seeds. Since seed is the major source of bacterial inoculum, (60,67), it was suggestive that the high incidences of bacterial blight in the farmers fields in Kenya were associated with the practice of farmers using infected seeds. Bean seed certification procedures, and the availability of the certified seed are part and parcel of increased bean production in Kenya. The use of disease free seed has been found to increase bean yields considerably (36).

The symptoms noted in the field were as described for common blight, fuscous blight and bacterial blight diseases of beans (8, 41, 67). The similarity of field symptoms of fuscous and common blight made their identification difficult and differentiation of the 2 diseases was determined by cultural characteristics in the laboratory. Xanthomonas phaseoli produced a yellow pigment on NA and X. phaseoli var. fuscans a yellow pigment that browned the NA medium.

Disease severity increased with maturity of beans. Beans at flowering to podding stages were more severely infected than those at other stages of development. No attempts were made to estimate the losses caused by the pathogens, but in some cases, sections of farms were completely destroyed with no hope of any harvests.

Common and fuscous blight of beans were more prevalent at the lower altitudes (1000 to 2000m) than at the higher altitudes (2000 to over 2500m). Kaiser (25) described both common blight and fuscous blight of beans as warm temperature diseases. Low rainfall retards the spread of common and fuscous blight, but fog and heavy dew are ideal for blight development (60). Heavy rain in the bean growing areas of Kenya decreases bean yields due to increased disease incidences and severity (35).

From the morphological, cultural, physiological and biochemical characters determined, the isolates were identified to belong to the genera Xanthomonas and Pseudomonas (7, 20, 22, 41). The KBY cultures were characterised as Xanthomonas phaseoli, the KBB cultures as X. phaseoli var. fuscans, and the KBC cultures as Pseudomonas phaseolicola. The criteria used in the study, that were effective, were the colony growth on NA and the symptoms produced by the isolates on the bean cultivars tested.

Colonial variation was noted in YDCA medium. Correy and Starr (14) found that colony types and virulence do vary in Xanthomonas phaseoli. They correlated the amount of polysaccharide and the ability to produce symptoms on the host. In this study, X. phaseoli var. fuscans produced more mucoid types than X. phaseoli, and caused more severe symptoms. The 4 colony types in X. phaseoli were normal mucoid, semi mucoid, rough types and smooth types (14).

Xanthomonads are not salt tolerant. There was no growth at 6% sodium chloride (NaCl) and very little at 5% NaCl. Dye (20) had noted similar results. At these rates of salt concentration Pseudomonads were growing normally.



The hypersensitive reaction induced by KBB, KBY and KBC cultures on tobacco leaves in 3 days was as described by Klement, et al (29). They found that the intensity of the reaction depended on the viable inocula.

The isolates produced symptoms within 6 days on bean seedlings. The symptoms produced by KBB and KBY isolates were very similar, but those produced by KBC isolates indicated a clear halo around the necrotic lesions. Hence the KBC cultures were found to be Pseudomonas phaseolicola, the causal bacterium of halo blight of beans.

Inoculation methods influence disease reaction. A method which produces reliable results is preferred to any other. Injection was superior to hand atomization, and rubbing; and soaking scratched seeds better than soaking intact seeds. Leaf water soaking by an atomization device fitted to a pressure system was found more superior in other studies (3). Saettler (49) found injection of bean seedling quite useful in differentiating bean blight bacteria. Rubbing is useful because the bacteria have a good chance of initiating disease through the wound avenues created.

The initial symptoms of common blight and fuscous blight of beans were small, watersoaked spots on the leaves. Areas between the lesions turned yellow and died. The

spots coalesced to form large brown lesions of irregular shape with lemon yellow margins. On stems the symptoms occurred as longitudinal lesions. The symptoms of common blight and fuscous blight differed slightly on "Mwezi-moja" and "Contender" beans. Isolates of Xanthomonas phaseoli var. fuscans caused small pinhole lesions on the undersurfaces of the leaves of "Mwezi-moja". The leaf was distorted and had chlorotic lesions. On "Mwezi-moja" X. phaseoli caused large irregular water-soaked lesions on the undersurfaces of the leaf and showed chlorosis covering the whole leaf on the upper side. On "Contender" the symptoms differed on the pods. X. phaseoli var. fuscans produced small raised necrotic lesions, concentrated in small areas with no watersoaking, but X. phaseoli produced dark, green watersoaked flat lesions. Jindal and Patel (24) used this criterion in differentiating common blight from fuscous blight of beans. Zaumeyer (66) had noted that the symptoms of bean blights were difficult to differentiate. Saettler (49) found no symptom differences between X. phaseoli and X. phaseoli var. fuscans. Logan (30) reported that X. phaseoli var. fuscans caused more intense symptoms than X. phaseoli but the symptoms were similar. Jensen and Livingston (23) suggested that the difficulty in

differentiating the bean blight symptoms was due to the variation in the symptoms. The symptoms of bacterial blights of beans vary due to temperatures, varietal differences and pathogenic variations of the bacterial isolates.

Xanthomonas spp are host specific and therefore restricted in host range (19, 30, 51). X. phaseoli infected Phaseolus vulgaris lines, P. lunatus and Dolichos lablab; and X. phaseoli var. fuscans infected P. vulgaris, P. lunatus, D. lablab and Vigna unguiculata. This was used in confirming the identity of the isolates. Since X. phaseoli and X. phaseoli var. fuscans are restricted in host range, crop rotation was recommended as a cultural control measure. Cowpeas should be avoided in crop mixtures with beans and in the rotations.

The development of disease resistant varieties of beans has been recognized as the most promising line of investigation towards the prevention of large crop losses (45). In this study attention was directed towards the development of new varieties possessing resistance to X. phaseoli and X. phaseoli var. fuscans. The screening was done under greenhouse conditions. The lines identified as resistant were recommended for use in the bean programme at the University of Nairobi and the National bean

improvement programme of the Ministry of Agriculture, Kenya. From the study 9 lines were found to possess resistance to Xanthomonas phaseoli and 11 to X. phaseoli var. fuscans. The use of the resistant germplasm identified in this study will be of great help in controlling bacterial blight diseases of beans in Kenya. The surveys indicated that bean blights are of economic importance in bean production in Kenya and controlling them is essential.

Xanthomonas phaseoli and X. phaseoli var. fuscans were found to be transmitted in bean seeds. Mukunya, et al (37) found that Xanthomonas spp had high incidences of 9-24% in farmers seeds sampled in Central and Eastern provinces of Kenya. High incidences of bacterial blights in farmers fields in Kenya are associated with the practice of farmers using infected seeds. The use of certified seed was recommended. This is possible since Kenya has an organized bean seed certification and distribution system through the Kenya Hortiseed Company.

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## 7. APPENDICES:

Appendix 1: Bean production in Kenya according to provinces for the 1974/75 crop season showing area planted to pure and mixed stands.

PROVINCE	PURE STAND	MIXED STAND	TOTAL
Central	8.4 <sup>*</sup>	224.0	232.4
Coast	0.3	17.2	17.5
Eastern	25.3	259.3	284.6
Nyanza	3.5	70.1	73.6
Rift Valley	0.9	6.1	7.0
Western	11.5	136.9	148.4
Total	49.9	713.6	763.5

\* Area in hactares  $\times 10^3$

Adapted from: Republic of Kenya  
Statistical Abstract (1979)  
Central Bureau of Statistics.



Appendix 2: Bean growing districts in Kenya in  
1969/70 Crop Year.

PROVINCE	DISTRICT	AREA UNDER BEAN CROP <sup>a</sup>
CENTRAL	• Muranga	48.4
	• Kirinyaga	34.0
	• Kiambu	17.7
	• Nyeri	13.4
	• Nyandarua	0.2
COAST	Taita	6.4
	Kilifi	1.9
	Kwale	-
EASTERN	• Machakos	61.4
	• Kitui	33.1
	• Meru	27.0
	• Embu	9.4
NYANZA	• South Nyanza	14.3
	Siaya	12.4
	Kisumu	9.1
	• Kisii	4.2

PROVINCE	DISTRICT	AREA UNDER BEAN CROP <sup>a</sup>
RIFT VALLEY	Elgeyo Marakwet	7.9
	Nandi	1.2
	Kericho	0.2
WESTERN	• Kakamega	10.8
	Busia	5.0
	Bungoma	4.6

• Districts surveyed

a Area in hectares  $\times 10^3$

Adapted from: Republic of Kenya  
Statistical Abstract (1978)  
Central Bureau of Statistics.

Appendix 3: Bean production in Kenya for 1978 and 1979.

PROVINCE	LAND PLANTED (ha)		PRODUCTION (kg)	
	1978	1979	1978	1979
Central	48181	49894	31236	31635
Eastern	95923	78669	89821	31741
Western	37062	30416	-	-
Nyanza	33810	31896	17546	19270
Rift Valley	23526	37629	23901	35097

- = Estimates not available

Adapted from:

Annual reports for 1979

Ministry of Agriculture, Kenya.