"DIFFERENTIATION OF ISOLATES OF *Uromyces appendiculatus* (Per. ex. per.) Unger var. *appendiculatus* AND EVALUATION OF BEAN CULTIVARS FOR RESISTANCE TO BEAN RUST IN KENYA".

BY

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A THESIS SUBMITTED IN PARTIAL FULFILMENT FOR THE DEGREE OF MASTER OF SCIENCE IN PLANT PATHOLOGY IN THE UNIVERSITY OF NAIROBI.

1989

DECLARATION

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

AKihurani.

AGNES W. KIHURANI

DATE 18/4/89

(b) This thesis has been submitted for examination with our approval as University Supervisors.

PROFESSOR D.M. MUKUNYA

DATE 18/4/89

DR. R.A. BURUCHARA

DATE 18/4/89

DEDICATION

This Thesis is dedicated to my mother MRS. SOPHIA W. GACHIGUA, and my husband, DR. DAVID O. KIHURANI, whose love and encouragement continue to be a great inspiration in my life.

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ABSTRACT

Bean rust caused by Uromyces appendiculatus (Per. ex. per) Unger var. appendiculatus is an important disease of beans in Kenya. Work was carried out on this disease to investigate the variability of the pathogen in Kenyan rust isolates and to evaluate bean cultivars for resistance to rust.

Rust isolates from four different sites in Kenya were differentiated using 20 differential cultivars. Inoculation was done by spraying a urediospore suspension onto the primary leaves of nine-day old seedlings, which were then incubated for 24 hours in the greenhouse. Host reactions were recorded 14 days after inoculation.

The host-pathogen interactions indicated variability of Uromyces appendiculatus among the isolates from the different sites. Seventy-seven cultivars were evaluated for resistance to a mixture of specific isolates from different sites. The cultivars NB 2405 and NB 132 were immune and NB 123, NB 14, NB 127,NB 225 and NB 63 were highly resistant.

It was found that rust isolates from different sites may cause different reactions on the same host due to variability of the rust fungus, and the cultivars identified as either immune or highly

resistant were recommended as possible sources of resistance in the development of resistant bean varieties in Kenya.

ACKNOWLEDGEMENTS

My most sincere appreciation goes to my supervisors, Professor D.M. Mukunya and Dr. R.A. Buruchara for their encouragement, guidance and help throughout the course of this project.

I am deeply indebted to M.A. Pastor-Corrales who assisted me in obtaining seeds of the differential cultivars, the bean rust grading card and related literature from Centro Internacional de Agricultura Tropical (CIAT).

I am also grateful to the technical staff of the Department of Crop Science, and the greenhouse attendants, for their cooperation and help.

This project was made possible by financial support of a scholarship awarded by Deutscher Akademischer Austrauschdienst (DAAD) (German Academic Exchange Service) through the University of Nairobi, to which I am extremely thankful.

Lastly my thanks go to Mrs. Musonye for tirelessly typing this manuscript.

CHAPTER ONE

INTRODUCTION

Beans (*Phaseolus vulgaris L.*) are very important in the diets of the majority of Kenyans. They are a cheap source of protein, rich in the essential amino acid lysine, and therefore form a tasty and highly nutritious additive to starchy components like potato, maize and other grains. Several English names have been given to beans such as food beans, dry beans, common beans and field beans.

The consumption of beans in Kenya is 20 kg per head per year (Zoebl, 1983) about the same amount as in Mexico which is a major bean consuming nation. The bean crop was introduced in Kenya in the 17th century, and it has since overtaken, in importance, older indigenous pulses of Kenya such as cowpeas, *Dolichos lablab*, pigeon peas and others of less importance. The total area under beans in Kenya in 1983 was 500,000 ha., whereas the second most important pulse, cowpea, covered about 150,000 ha . (Zoebl, 1983).

Beans are grown over a wide range of ecological zones mainly in the medium altitude areas. They are one of the most important crops grown in the semi-arid areas of Kenya. Most beans are grown by small-holders, mainly for subsistence purposes and are intercropped with other food crops especially maize.

About 13 pests and 20 diseases cause widespread damage to beans in Kenya, but only a small number of these are of economic importance. Bean rust has been reported as one of the major bean diseases in Kenya (Bock, 1970; Mukunya, 1974; Anonymous, 1976; Mukunya and Keya, 1978; Anonymous, 1981; Anonymous, 1982; Smit et al, 1983; Stoetzer and Omunyin, 1983), and there is therefore great need to develop or identify cultivars that are resistant to the pathogen.

In the light of the above, work was carried out with the following objectives:

1. To investigate the variability of Uromyces appendiculatus in isolates from different sites in Kenya using the recommended differential cultivars.
2. To evaluate bean cultivars for resistance to isolates of bean rust using the standard system for the evaluation of bean germplasm.

CHAPTER TWO

LITERATURE REVIEW

Rusts comprise a large group of fungi that are parasitic on plants causing great losses to many cultivated plants. They are so called because they produce rusty brown symptoms on the surface of the host plant.

Bean rust is caused by Uromyces appendiculatus (Per. ex. per.) Unger var. appendiculatus (= U. phaseoli (Reben) Wint.) The pathogen is an obligate parasite and belongs to the subdivision Basidiomycotina, the class Basidiomycetes and the order Uredinales (Alexopoulos and Mims, 1979).

The disease is of worldwide occurrence being found practically wherever its hosts are distributed throughout the world (Fromme and Wingard, 1921; Zaumeyer and Meiners, 1975).

Economic Importance

Rust is a major disease problem on beans (Phaseolus vulgaris L.) worldwide (Stavely et al, 1983; Baker et al, 1985). In many areas it is the most important foliar disease commonly causing 25 - 100% losses (Stavely et al, 1983; Venette and Jones, 1982). Most dry and green beans are susceptible to rust and when environmental conditions are

favourable, the disease may develop rapidly causing leaves to drop before the pods are mature resulting in substantial yield reduction (Hart and Saettler, 1981). In the republic of South Africa, for example, all dry bean cultivars grown, except one, are susceptible to rust and they must be sprayed regularly with fungicides to avoid serious yield losses (Nieuwoudt, 1984).

In East Africa, bean rust is an important pathogen of beans (Bock, 1970) and of economic importance (Howland and Macartney, 1966). Heavy attacks may destroy a crop almost completely or severely degrade any seed produced. The disease frustrated several attempts to produce white haricot beans for the canning trade and it can reach epidemic level at any time during the vegetative growth of the plant (Howland and Macartney, 1966).

Etiology

U. appendiculatus has an autoecious macrocyclic life cycle typically exhibiting all five reproductive stages. The life cycle is completed entirely on the bean host (Andrus, 1931; Hart and Saettler, 1981). Although all the five spore stages have successfully been developed on the bean plant (Groth and Mogen, 1978), the most commonly observed spores are the urediospores (summer or vegetative spores) and telio-

spores (winter or resting spores). The former have a short hyaline pedicel and are light brown in colour, one celled, spiny and thin-walled and globoid to ellipsoid in shape, whereas the latter have a short hyaline pedicel and are dark brown, smooth and thick-walled, and globoid to broadly ellipsoid in shape.

Being an obligate parasite, U. appendiculatus does not grow in culture, but viable spores can be successfully preserved in the laboratory. Urediospores can stay viable for as long as two years if the leaves bearing them are dried for a few days at room temperature before storage at -20°C (Harter and Zaumeyer, 1941). Germination is higher in spores from young leaves and pustules than from old leaves and pustules (Imhoff et al, 1981). Abundant infection can be obtained after urediospores have been stored in vials for over 600 days at -18°C provided that excess moisture is removed before storage (Davison and Vaughan, 1963b). In liquid nitrogen (-150°C) spores retain viability without loss of infectivity (Cunningham, 1973).

Temperature can modify rust response. Seven days at 32°C induced a locally necrotic response in a host normally fully susceptible (Schein, 1961a). It also affects spore germination (Shands and Schein, 1962), and symptom expression and development (Schein, 1961b).

Epidemiology

More than 10 hrs of moisture and temperatures between 18-27°C are required for infection by urediospores of U. appendiculatus (Shands and Shein, 1962; Hart and Saettler, 1981). Optimal range of temperatures for urediospore germination has been reported as 12.5 - 22.5°C (Shands and Shein, 1962, Bell and Daly, 1962, Imhoff et al, 1981). At 15 - 22.5°C, 90% of all germination occurred within the first six to eight hours of wetness (Imhoff et al, 1981).

Spore production is affected by humidity and photosynthesis. Sporulation increased when infected bean plants were exposed to high humidity (Yarwood, 1961) while Cohen and Roten (1970) reported that highest sporulation occurred following the longest photoperiods to which the host plants were exposed. Spore release is greatest following a long dew period or rain (Hart and Saettler, 1981).

Under field conditions, urediospores tend to remain viable for only a short time, but where climatic variation enable bean growing throughout the year, a reservoir of inoculum may be present always (Howland and Macartney, 1966). Urediospores are mainly disseminated by wind or air currents and sometimes carried many miles away (Howland and Macartney, 1966; Hart and Saettler, 1981).

Infection

When the rust spore lands on the host, a germ tube grows and develops an appressorium when it comes into contact with stoma (Wynn, 1976). An infection peg develops from the appressorium and pushes between the guard cells until it reaches the substomatal chamber. A substomatal vesicle is formed which gathers the fungal cytoplasm and then elongates to form infection hyphae. Haustoria develop at the tip of the infection hyphae in contact with the host cell (Mendgen, 1979). The fungus thus penetrates leaf cells and proceeds intercellularly throughout the host tissue, eventually forming a young lesion.

Host physiology and biochemistry are affected during the infection and sporulation processes. Mendgen (1979) found that a flow of labelled metabolites from the host cell to the haustoria and then to the intercellular hypha occurred, and that hyphae, haustoria and spores of the fungus took up and accumulated metabolites from the host cells.

Symptomatology

U. appendiculatus may affect leaves, pods, and rarely stems and branches (Howland and Macartney, 1966; Hart and Saettler, 1981).

In severe cases leaves may shed early (Howland and Macartney, 1966; Hart and Saettler, 1981; Stoetzer and Omunyin, 1983).

Infection may occur on both the lower and upper surface of the leaf but symptoms first appear on the lower surface as minute, white, slightly raised spots, five to six days after inoculation (Howland and Macartney, 1966; Hart and Saettler, 1981; Stoetzer and Omunyin, 1983). The spots enlarge gradually to form mature rusty-brown pustules which rupture the leaf epidermis to expose rust-coloured spores (urediospores). Sometimes a yellow halo may be present (Stoetzer and Omunyin, 1983; Nieuwoudt, 1984). By 10 days after inoculation, abundant sporulation has taken place (Pastor-Corrales 1985). The pustules may reach a diameter of one to two millimeters within 10 - 12 days after inoculation (Howland and Macartney, 1966). Secondary and tertiary pustules may develop around the margin of the primary pustule (Howland and Macartney, 1966; Hart and Saettler, 1981). The entire infection cycle occurs within 10 - 15 days (Hart and Saettler, 1981).

Control

Some species of bacteria in the genus Bacillus especially B. subtilis gave efficient control of bean rust when applied in liquid culture to greenhouse

plants prior to inoculation with urediospores of U. appendiculatus (Baker et al, 1983). In tests, B. subtilis has been 99 percent effective in the greenhouse (Stavely and Baker, 1985), and in the field it has reduced severity by 75 percent when applied 3 times per week (Baker et al, 1985). It must be noted, however, that to obtain such a high level of control, spraying was done every few days and this is expensive in practical terms.

Crop rotation, removal of plant debris (Mostade, 1977; Hart and Saettler, 1981; Stoetzer and Omunyin, 1983;), and adjustment of planting time (Hart and Saettler, 1981) are other ways of controlling bean rust.

Many fungicides have been evaluated for effectiveness against rust. Infections that occur early affect yields most and thus spraying done at this time will become most effective (Hart and Saettler, 1981). Proper timing of application is therefore an important factor in the fight against the disease. Four applications with Propineb (Polymeric zinc propylenebis) beginning four weeks after planting, and then at an interval of 10 days, gave significantly good control (Anonymous, 1966). Zineb (Zinc ethylenebis) 65% WP has been recommended for control of rust when applied at seven-day intervals three weeks after planting (Mostade,

1977). Better control has been achieved by spraying Mancozeb (Manganese ethylenebis complex with zinc salt) at seven-day intervals than at 14-day interval, although not always with yield advantages (Lindgren and Steadman, 1985). Languidey and Aguilera (1983) found Mancozeb, Benomyl[a], Metiran (Zinc ammoniate ethylenebis) and Captafol[b] effective against rust in that order when applied at 15-day interval. In the same experiment, Maneb (Manganese ethylenebis) and Copper Oxychloride (Dicopper chloride trihydroxide) were inefficient at this interval. In other evaluations, however, Maneb has shown effectiveness when applied at seven-day intervals (Mullins and Hilty, 1985) and at two-day interval (Anonymous, 1967). Bitertanol[c] is commercially used with higher recommended rates for aerial than ground application (Nieuwoudt, 1984). It is more effective than Benomyl and Mancozeb (Anonymous, 1979b) but the three fungicides are recommended for bean rust control in the production of french beans in Kenya (Stoetzer and Omunyin,

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a = (methyl 1-butylcarbamoyl) benzimidazol-2-ylcarbamate

b = N-(1,1,2,2-tetrachloroethylthio) cyclohex-4-ene-1,2-dicarboximide

c = all-rac-1-(biphenyl-4-yloxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)

1983). Chemical application is not considered feasible in the production of dry beans (Howland and Macartney, 1966; Stoetzer and Omunyin, 1983).

Breeding for resistance in dry and french bean varieties is the most effective control method of bean rust (Stavely and Baker, 1985). Many cultivars and lines have already been bred for resistance with some contributing significantly in reduction of losses from the disease (Anonymous, 1979a). The problem that breeders face is the great variability of U. appendiculatus, and the presence of a narrow genetic base and vertical resistance to rust races in bean varieties that provide only temporary production (Coyne and Schuster, 1975; Stavely and Baker, 1985). It is however hoped that a combination of horizontal resistance and different major genes controlling vertical resistance can provide a more useful and effective genetic control measure.

Work to identify cultivars that are resistant to the prevalent rust races in East Africa was reported in 1963 (Howland and Storey, 1963). Some group of Central American white haricot beans showed high resistance. In later work, breeding for resistance has been recommended as the only practical method of bean rust control (Howland and Macartney, 1966; Macartney, 1966). In Kenya, germplasm evaluation for

resistance has been carried out on indigenous and introduced dry bean lines, and on french beans under field and greenhouse conditions at the University of Nairobi, and at the National Horticultural Research Station in Thika (Mukunya, 1975; Mukunya and Keya, 1978; Anonymous, 1982; Omunyin et al, 1984). The investigations have however been based on other systems other than the standard system for the evaluation of bean germplasm (Anonymous, 1987), and the cultivars tested for response to a general host population and not to specific host isolates.

Work done on race identification

The variability of U. appendiculatus has been reported by many authors since 1935 (Harter et al, 1935). Harter and Zaumeyer (1941) were the first to report physiological races in the United States. They identified 20 races using a set of seven differential cultivars. Fisher (1952) with an addition of two cultivars identified another ten races, and other races have subsequently been identified and reported (Sappenfield, 1954; Zaumeyer, 1960; Hikida, 1961; Groth and Shrum, 1977; Stavely, 1984). Races have also been identified in other parts of the world. They include such places as Australia (Ogle and Johnson, 1974; Ballantyne, 1975),

Brazil (Dias and Da Costa, 1968; Netto et al, 1969; Augustin and Da Costa, 1971a; Carrijo et al, 1980), Colombia (Zuniga de Rodriguez and Victoria, 1975), East Africa (Howland and Macartney, 1966), Jamaica (Shaik, 1985), Mexico (Crispin and Dongo, 1962), New Zealand (Yen and Brien, 1960), and a few other countries (Christen and Echandi, 1967; Guerra and Dongo, 1973). The ones that have been identified in the United States and Colombia have been given successive numbers from race 1 to race 57 (Harter and Zumeyer, 1941; Fisher, 1952; Sappenfield, 1954; Zaumeyer, 1960; Hikida, 1961; Zuniga de Rodriquez and Victoria, 1975; Stavely, 1984). In Brazil, the races have been assigned numbers that are preceded by capital letter prefixes to indicate the area from which they were identified (Netto et al, 1969; Augustin and Da Costa, 1971a; Coelho and Chaves, 1975). The Australian races have been assigned small letters to correspond to virulence genes theoretically possessed by the pathogen for each of the differential cultivars used (Ballantyne, 1978).

Sometimes, small samples of rust collections are sufficient to reveal the variability of U. appendiculatus (Carrijo et al, 1980; Groth and Roelfs, 1982). It has also been found that at any one area, some races become more or less prevalent in

relation to the others or their prevalence may remain constant from time to time (Augustin and Da Costa, 1971a). Some isolates when tested on the differential cultivars have shown different reactions from those observed for previously described races in an area, designating the occurrence of a new physiological race (Sappenfield, 1954; Zaumeyer, 1960; Augustin and Da Costa, 1971b).

The first race identification was done using the scale developed by Harter and Zaumeyer (1941). The scale is based on the relative size of pustules developed on the differential cultivars by the pathogen, and it consists of 11 grades. Grade 0 denotes immunity, and grade 1a necrotic flecking reaction. Grades 2 - 10 are determined by the relative size of the spore-bearing pustule. This scale was later simplified by Davison and Vaughan (1963a) to five grades, with Grade 1 denoting immunity and Grade 2 necrotic flecking. Grade 3 is pustules 300 micrometers or smaller, Grade 4 pustules 301 - 499 micrometers and Grade 5 pustules 500 micrometers or larger. They also published a grading card with dots about 300 and 500 micrometers with which pustules on the leaves of the host can be

compared visually by placing them side by side to help determine the respective grade. This is the scale that more recent investigators have made use of.

The differential cultivars used in the identification of physiological races have mostly included the seven used by Harter and Zaumeyer (1941) with additional ones, but sometimes different cultivars altogether have been utilized (Crispin and Dongo, 1962).

In East Africa, the first scale used was based on that of Harter and Zaumeyer (1941) but with modification to suit the inoculation technique (Howland and Macartney, 1966). It was however found too complicated for useful application (Howland, 1963) and was dropped. The Mexican system (Crispin and Dongo, 1962), with a slight modification on nomenclature was adopted (Howland and Storey, 1964; Howland and Macartney, 1966;). It was found to correspond closely with the symptom expression of rust in East Africa (Howland, 1963; Howland and Storey, 1964; Howland and Macartney, 1966). The differential cultivars used included six of the ones used by Harter and Zaumeyer (1941) with an addition of a highly resistant selection (Tengeru Selection No. 8) (Howland and Macartney, 1966).

The differences in the differential cultivars and the grading scales used meant that comparison of research findings was not possible. It is for this reason that in an International Bean Rust Workshop held in Puerto Rico in 1983, (Stavely et al, 1983), a base set of 20 homozygous differential cultivars (Table 1) was selected to be used for race identification. A standard grading scale was also adopted for rating host reaction (Table 2). It included the scale developed by Davison and Vaughan (1963a) with an addition of Grade 6 denoting pustules larger than 800 micrometers, and limiting Grade 5 to pustules 500 - 800 micrometers in diameter (Stavely et al, 1983).

CHAPTER THREE

MATERIALS AND METHODS

Bean cultivars

Seeds of the cultivars selected at the 1983 International Bean Rust Workshop (Stavely et al, 1983) as standard differentials for defining races of Uromyces appendiculatus (Table 1) were received from Dr. Pastor-Corrales of Centro Internacional de Agricultura Tropical (CIAT). The cultivars were multiplied by planting one set of seeds consisting of all the differential cultivars in the greenhouse and another set in the field (University of Nairobi, Field Station, Kabete). Seeds of all the other bean cultivars used were obtained from the University of Nairobi bean germplasm store in Kabete.

Urediospore collections

Rust samples were collected in bean fields from four sites in Kenya. They included Kabete, Thika, Athi River and Naivasha. All the collections with the exception of the that from Kabete, were made from French bean fields, where irrigation water was applied. This was because at the time of collecting there were no beans growing in the rain fed fields where dry beans are grown.

Table 1: Standard differentials for defining races of *Uromyces appendiculatus*.

1.	United States (U.S) 3
2.	California Small White (C.S.W) 643
3.	Pinto 650
4.	Kentucky Wonder (K.W.) 765
5.	Kentucky Wonder (K.W.) 780
6.	Kentucky Wonder (K.W.) 814
7.	Golden Gate Wax
8.	Early Gallatin
9.	Mountaineer White Half Runner (M.W.H.R)
10.	Redlands Pioneer
11.	Ecuador 299
12.	Mexico 235
13.	Mexico 309
14.	Brown Beauty
15.	Olathe
16.	Actopan x Sanilac Selection (AxS) 37
17.	NEP - 2
18.	Aurora
19.	51051
20.	Compuesto Negro Chimaltenango (C.N.C)

Fungicides are used for disease control in French bean production (Stoetzer and Omunyin, 1983) and so even in the few cases where pustules and urediospores were present, they were not abundant. For this reason, it was not possible to collect enough urediospores from the field and so whole leaves with many pustules were collected and put in paper bags bearing the name of the collection site. In Kabete, rust-infected leaves were collected from an experimental plot where a local susceptible cultivar (GLP X92) was growing. This cultivar was found to be most suitable for increasing rust inoculum and was used throughout the investigations.

By using a camel hair brush, the urediospores from leaves collected from the field were inoculated onto the upper surface of the leaves of nine day old seedlings of GLP X92 in the greenhouse. Inoculum from each site was kept under isolation until single pustule isolates were made.

Single pustule isolation

One pustule was selected in each collection and urediospores from it were transferred onto the primary leaves of nine day old seedlings of GLP X92. This was done by first moistening the brush by dipping it in sterile distilled water and then

repeatedly touching the pustule, then painting on primary leaves of the seedlings. The seedlings which were kept in rust free isolation chambers were returned there after inoculation.

Culturing of Single Pustule Isolates

Isolation chambers were wooden frames five feet tall with sides measuring three feet by two feet and covered on all sides with a transparent polythene sheet (Fig. 1). The polythene chamber could be opened on one side to facilitate putting plants inside or outside of the chamber. High relative humidity was achieved inside the chambers by covering individual pots with polythene paper after inoculation. Temperatures were recorded daily.

Preservation of inoculum

Inoculum from the single-pustule isolates was increased by repeated inoculations onto nine-day old seedlings of GLP X92. Later mature urediospores were collected by tapping the leaves bearing the pustules over flimsy paper. After removing any debris present, the urediospores were placed in vials that were then plugged with cotton-wool and placed in a dessicator (Fig.2) for a few days. They were then closed tightly and stored in a freezer. (-5°C to -10°C).

Figure 1: Rust Isolation Chamber in which Inoculum of different rust isolates was multiplied

Figure 2: A desiccator showing Urediospores in vials.

Note: Vials have been plugged with cotton-wool and placed in a dessicator to allow the spores to dry for a few days before storage in a freezer.

Plant propagation

Seeds of each cultivar were germinated in moist soil in plastic pots of 12.5cm diameter in a shaded greenhouse (Fig.3). The soil mixture consisted of one part of each of sand and cow-dung manure and two parts of soil. To every 20 litre bucket of this mixture, 20gms of Diammonium phosphate (DAP) fertilizer was added. The pots were covered with a black polythene sheet and they were not uncovered or watered until the plants began to emerge from the soil, usually about four days after planting. All the pots were kept in a rust-free greenhouse until after inoculation and incubation.

Inoculation technique

Inoculum was prepared by placing urediospores in sterile distilled water and adding a few drops of tween 80, then shaking to mix. The concentration of the resulting suspension was determined with a haemacytometer and adjusted to approximately 3×10^4 urediospores per millilitre.

Inoculation was done just before sunset by spraying the inoculum as evenly as possible to both the lower and upper surface of leaves with a manually operated atomizer (Fig. 4). Plants were inoculated nine to ten days after planting.

Figure 3: A greenhouse showing the shaded sides and roof where the experimental plants were grown.

Note: The shading ensured that the environment inside the greenhouse remained cool even in a hot weather condition

Figure 4: An atomizer used to inoculate leaves

The inoculated plants were incubated for 24 hours in the greenhouse by covering them with moistened transparent polythene bags placed upside down over the pot (Fig. 5) to create a humid environment around the leaves.

For the next five days, the leaves were sprayed with water five times a day at a 2-hour interval as recommended by Pastor-Corrales (1985) using a hand-sprayer (Fig. 6). Fourteen days after inoculation, the leaves were detached from the plants and put in plastic bags. The bags were placed in an upright position and left open to allow air circulation inside them, and this ensured that the leaves remained fresh until host reactions were recorded.

Pathogenic Variation System

Reaction grades were recorded using the Standard Scale (Table 2) adopted at the International Bean Rust Workshop (Stavely et al., 1983). The pustule size was estimated using a card similar to that of Davison and Vaughan (1963a) with printed dots approximately 300,500 and 800 micrometers in diameter (Fig. 7). Urediospores protruding from the pustules were removed from the leaf surface by brushing, blowing or tapping the leaf and then the actual pustule diameter was estimated and recorded. This

Figure 5: Inoculated bean plants during incubation. Moisture condensation on the inner side of the polythene bag indicated a humid environment around the inoculated leaves

Figure 6: A hand-sprayer used for wetting the inoculated leaves

Table 2: The International uniform bean rust grading scale

Grade	Definition
1	Immune, no visible symptoms
2	Necrotic spots, without sporulation, and less than 300 µm in diameter.
2+	Necrotic spots, without sporulation, 300 - 1000 µm (1 mm) in diameter.
2++	Necrotic spots, without sporulation, 1-3 mm in diameter.
2+++	Necrotic spots, without sporulation, larger than 3 mm in diameter.
3	Sporulating pustules less than 300 µm in diameter.
4	Sporulating pustules 300 - 500 µm in diameter.
5	Sporulating pustules 500 - 800 µm in diameter.
6	Sporulating pustules larger than 800 µm in diamter.

When several pustule grades are present, they are recorded in order of predominance, the most prevalent type being listed first, the least prevalent type last. Both leaf surfaces should be examined and if the grades differ, either both or the highest grade should be recorded (Anonymous, 1987).

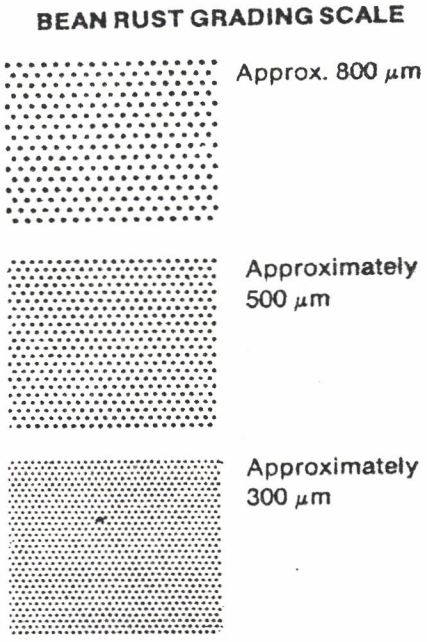


Figure 7: The bean rust grading scale used for estimating pustule size

was done by placing the host leaf and card side by side on a table and comparing the size of the pustules with that of the printed dots with the aid of a hand lens, and thus determining the pustule size.

Both leaf surfaces were examined and where several pustule grades were present on the same leaf surface, they were recorded in order of predominance, the most prevalent type being listed first and the least last.

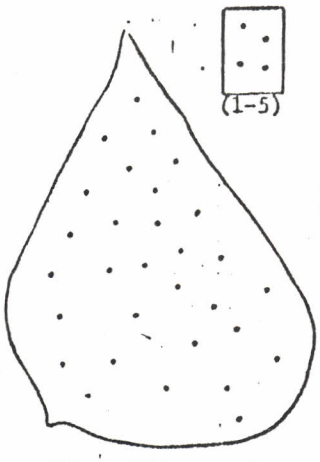
The infection grade and the infection intensity recorded on the leaf were both considered to give the final host reaction. The infection grade was recorded as explained above, and then the infection intensity was estimated by using the modified Cobb Scale (Fig. 8) as the percentage of leaf area covered by necrotic spots or sporulating pustules (Stavely, 1985).

The combination of the pustule type and the infection intensity gave a final host reaction classification of five categories 1, 3, 5, 7 and 9 respectively, for Immune, Resistant, Intermediate, Susceptible and Very Susceptible (Fig. 9). It was on the basis of the different reaction categories that the response of the differential cultivars to the inoculated isolates was determined.

Figure 8: THE MODIFIED COBB SCALE FOR ESTIMATING BEAN RUST INTENSITY.

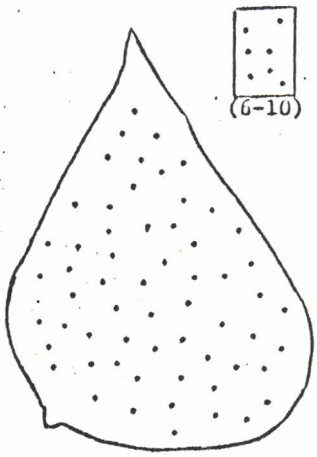
Notes: The diagram shows six degrees of rustiness, which were used in estimating the percentage of rust infection on the leaf. The shaded spots represent rust, and the figures represent approximately the rust percentage computed on the basis of the maximum amount of surface covered by rust as shown in the 99% figure. This figure (No.6 in the diagram) represents 37% of actual surface and is arbitrarily selected as 99%. Other percentages are in terms of No.6.

1



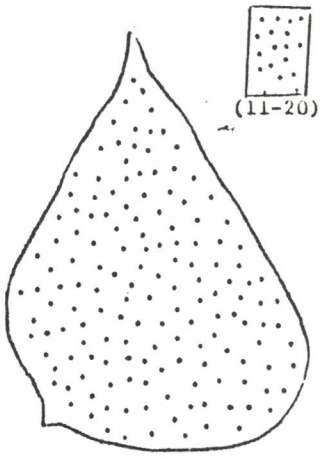
5% (± 30)

2



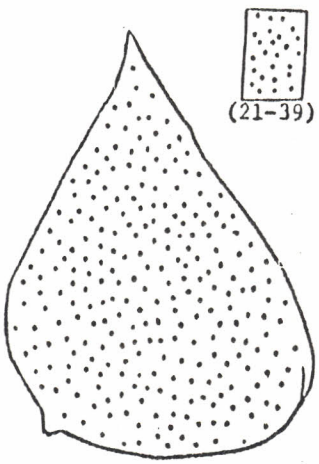
10% (± 60)

3



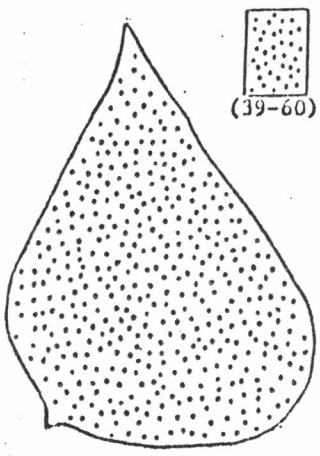
25% (± 150)

4



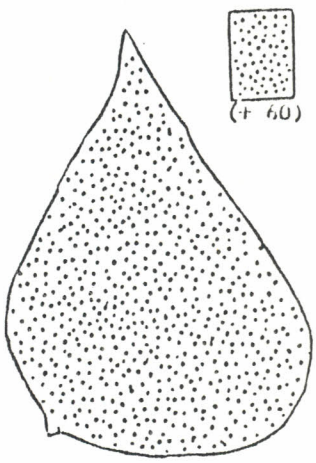
40% (± 250)

5



65% (± 400)

6



99% (+ 600)

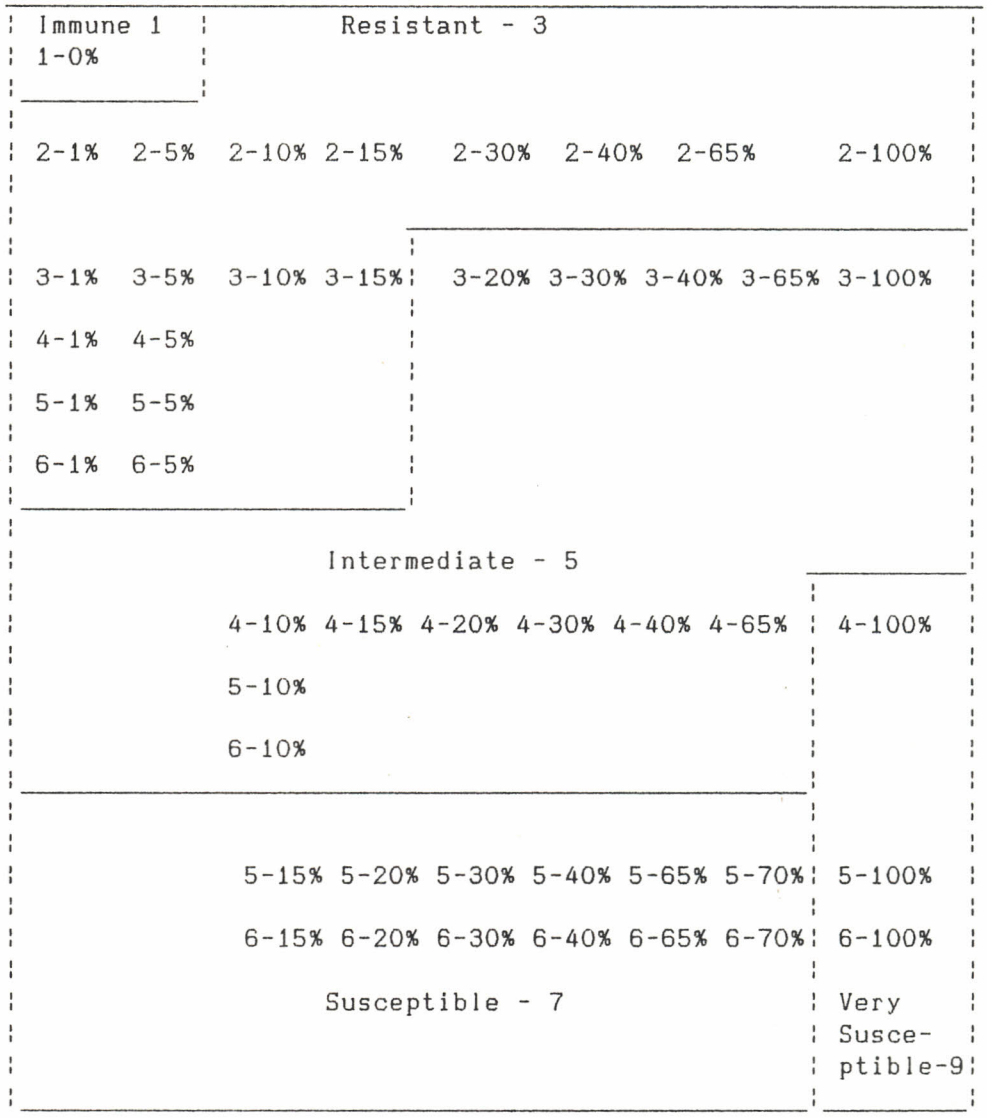


Figure 9: Plant reaction classification determined by the combination of pustule type, and the infection intensity.

Notes: The pustule type is based on a 1-6 scale, and the infection intensity is given as a percentage. For example, a pustule type 4 (the number on the left) with rust intensity of 30% (number on the right) corresponds to a plant reaction of 5, or the intermediate category (Anonymous, 1987).

Evaluation for Resistance to Rust

Rust evaluation was done according to scale 2 of the standard system for evaluation of bean germplasm for resistance or susceptibility to rust (Anonymous, 1987). Both leaf surfaces were examined and where the grades differed, the highest one was recorded. When several pustule grades were observed on the same leaf surface, only the highest grade was recorded. The final host reaction recorded was as a result of the combination of pustule type and infection intensity as shown in figure 9.

The Kenyan cultivars (GLP 1004, GLP 24 and GLPx92) were evaluated for resistance or susceptibility to single pustule inoculum from the different sites, and 77 other cultivars to a mixture of rust isolates.

CHAPTER FOUR

RESULTS

Multiplication of differential cultivars

The germination of seeds of the differential cultivars was satisfactory both in the field and in the greenhouse. Most of the cultivars had climbing tendency. Aphids and mites were found to affect the plants in the greenhouse but they were effectively controlled by using Diazinon 60% and Dicofol* respectively. In the field, the pests encountered included rodents and bean flies. By regularly spraying the plants with Cypermethrin** and Diazinon 60%, and using rat poison, the pests were kept under control. Benomyl was used to protect the plants from disease infection. The differential cultivars were found to require top-dressing with nitrogen unlike a local cultivar that was grown side by side with them.

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* Dicofol - used as Kelthane

** Cypermethrin - used as Ambush

Urediospore collections

Paper bags that do not allow vapour condensation on the inside were found to be more suitable for transporting urediospore-bearing leaves from the field. Water drops that develop as a result of condensation were found to be undesirable as they can wash off urediospores from the surfaces of the leaves.

Single pustule isolation

The pustules that developed on the susceptible cultivar after the first inoculation were widely spaced. This was desirable because it enabled removal of urediospores from the chosen pustules without risk of contamination from the neighbouring pustules.

Culturing of single pustule isolates

Plants kept in the isolation chambers germinated and developed in the same way as those kept in the greenhouse. When inoculation was done, no difference in disease development was observed. The chambers were also effective in maintaining a rust-free environment where plants were grown in isolation and free from contamination from the outside. Contamination from the chambers to the outside also did

not occur. The average minimum and maximum temperatures recorded were 16°C and 30°C respectively.

Multiplication of inoculum from single pustules

Abundant sporulation occurred ten days after inoculation. The urediospores were harvested and more were produced from the same pustules. This together with repeated inoculations using the same inoculum ensured that as much inoculum as required was available.

Preservation of inoculum

The inoculum was kept in a freezer for varying periods of time, the longest being four months. Both freshly produced and stored urediospores were used. In each case symptoms developed on the inoculated susceptible bean cultivars.

Inoculation technique

When drops of tween 80 were added, the urediospores were uniformly dispersed forming a good suspension whose urediospore concentration was conveniently determined using a haemacytometer.

The atomizer was found to be very convenient for easy and even wetting of the leaves. It also ensured that spillage of the inoculum did not occur. From a few minutes after the polythene bags were

inverted over the plants, up to the end of the 24-hour incubation period, abundant vapour condensation occurred on the inner surface of the bags provided the latter fitted tightly onto the pot, and had no punctures. The plants remained moist most of the time during incubation.

Symptoms began to appear on the leaf surface of susceptible plants at the end of the water-spraying period which was about 5-6 days after inoculation. About 4-5 days later (10 days after inoculation), abundant sporulation had occurred on these plants.

Pathogenic variation

The reactions exhibited by the 20 differential cultivars to the four isolates are shown in Table 3. Some of the host-pathogen interactions observed gave an indication of pathogenic variation among the tested isolates. For example the cultivar Kentucky Wonder 814 gave a reaction of category 3 with isolate A but was immune with Isolate N, whereas cultivar 51051 was immune with Isolate A but gave a reaction of category 3 with Isolate N. On the basis of these two cultivars the two Isolates, A and N, were found to vary pathogenically. In the same way, Isolate T was found to vary pathogenically from Isolate K in that the cultivar Kentucky Wonder 780 gave a reaction of category 7 with Isolate T and one of category 5

Table 3: Reactions of differential cultivars to bean rust isolates

Differential Cultivar[a]	Reactions[b] to Isolate[c]			
	A	N	T	K
1. U.S.3	5	3	5	5
2. C.S.W. 643	3	3	-[d]	3/5
3. Pinto 650	5	5	5	3
4. K.W. 765	5	3	5	3
5. K.W. 780	5	5/7	7	5
6. K.W. 814	3	1	1	3
7. Golden Gate Wax	5	3	3	3
8. Early Gallatin	5,3/5	3	3	3
9. M.W.H.R.	3	-	5	5
10. Redlands Pioneer	3	3	3	3
11. Ecuador 299	5	3	5	3/5,3
12. Mexico 235	3/5	3	3	3
13. Mexico 309	1	1	-	1
14. Brown Beauty	3	3	3	3
15. Olathe	-	3	5	5,3/5
16. AXS 37	3	3	5	3
17. NEP-2	3	3	3	3
18. Aurora	5	3	5	5
19. 51051	1	3	1	1
20. C.N.C	3	3	3	3

[a] U.S.= United States, C.S.W.= California Small White, K.W. = Kentucky Wonder, M.W.H.R. = Mountaineer White Half Runner, AxS 37 = Actopan x Sanilac Selection 37, C.N.C. = Compuesto Negro Chimaltenango

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Table 3 Cont'd

[b] Reaction classification as given in Fig. 9. 1 = Immune; 3 = Resistant; 5 = Intermediate; 7 = Susceptible; 9 = Very Susceptible. Where a slash is given, the numerator designates reaction on upper leaf surface and denominator on lower leaf surface. Where several figures are given, they are listed in order of predominance from most to least

[c] Isolates A, N, T and K are respectively from Athi River, Naivasha, Thika and Kabete

[d] A dash denotes that the figure was not obtained

with Isolate K, but cultivar Kentucky Wonder 814 was immune to Isolate T whereas it gave a reaction of category 3 with Isolate K. In this way variation between the different isolates was shown by the reactions exhibited by specific differential cultivars. Some of the differential cultivars, however, had similar reactions to all the isolates. No cultivar was immune to all the isolates.

The reactions observed on some of the differential cultivars to the isolates are shown in figure 10.

Evaluation of cultivars for resistance or susceptibility to rust

As mentioned earlier, cultivar evaluation for resistance or susceptibility to rust was based on the same scale as that utilized by the International Bean Rust Nursery (IBRN) which considers two criteria namely pustule type and infection intensity. The two are combined to obtain a plant reaction classification of five categories as shown in Fig. 9. Cultivars falling under classifications 1, 3, 5, 7 or 9 were considered to be Immune, Resistant, Intermediate, Susceptible or Very Susceptible respectively. The evaluation results for the three Kenyan cultivars, GLP 1004, GLP 24 and GLP x92 are given in Table 4 and for the other cultivars in Table 5.

Figure 10: Reactions of differential cultivars to different Kenyan isolates of Uromyces appendiculatus

(A) Lower leaf surface of Mountaineer White Half Runner showing grade 4, and some grade 3, pustules induced by isolate A

(B) Lower leaf surface of Kentucky Wonder 780 showing large pustules of grade 5 induced by isolate N

(C) Upper leaf surface of U.S.3 showing grade 5 pustules induced by isolate T.

(D) Upper leaf surface of C.W.S. 643 showing barely visible grade 3 pustules induced by isolate N.

GLP 1004 was resistant to all the isolates, GLP 24 to three and GLP x92 to two. None of the three cultivars was immune or susceptible to any of the isolates. They were all either resistant or intermediate.

Two of the cultivars evaluated for resistance to a mixture of specific isolates were immune. They were NB 132 and NB 2405. Sixty-five of the cultivars were resistant with NB 127, NB 63, NB 14, NB 225 and NB 123 showing a very high degree of resistance. Nine of the cultivars were intermediate and one, NB 139, was susceptible.

Table 4: Reactions of three Kenyan cultivars to bean rust isolates. (1 = immune; 3 = resistant; 5 = intermediate; 7 = susceptible; 9 = very susceptible).

Cultivar identification	Variety/line	Reaction to isolate[a]			
		A	N	T	K
1. GLP 1004	"Mwezi Moja"	3	3	3	3
2. GLP 24	Canadian Wonder	5	3	3	3
3. GLP x 92	"Mwitemania"	5	3	5	3

[a] Isolates: A from Athi-River; N from Naivasha; T from Thika and K from Kabete.

Table 5: Reactions of bean cultivars to a mixture of bean rust isolates. (1 = Immune; 3 = Resistant; 5 = Intermediate; 7 = Susceptible; 9 = Very susceptible).

Cultivar Identification	Variety/Line	Reaction
GLP x 1127 (a)	New "Mwezi Moja"	3
GLP 288	"Tongmire - Kabumbu"	3
NB 569	[a]	3
NB 40	"Banja" 2	3
NB 70	"Kabanima"	3
NB 164	Rose Coco	3
NB 189	Canadian Wonder	3
NB 1181	-	5
NB 404	12/1/2/1 Kampala, Leaky	3
NB 2437	-	5
NB 461	Red Mexican UI 3	5
NB 46	"Renka"	3
NB 36	"Diacolnima"	3
NB 288	5/4/1/5/2 Kampala, Leaky	3
NB 340	19/3/2 Kampala, Leaky	3
NB 2409	-	3
NB 127	"Karapul Dichol"	3
NB 1122	-	3
NB 440	51/2/21 Kampala, Leaky	5
NB 171	Bush B1 Lake Tendercrop	3

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Table 5 cont'd

Cultivar Identification	Variety/Line	Reaction
NB 68	"Kabanima"	3
NB 460	Red Mexican UI 34	5
NB 241	5/4/1/5/2	3
NB 63	"Calima"	3
NB 1340	SR-73/74-118	3
NB 227	Early Harvest	3
NB 2425	-	3
NB 2406	-	3
NB 77	Rose Coco	3
NB 66	"Calima"	3
NB 272	5/4/1/5/2 Kampala, Leaky	3
NB 31	-	3
NB 14	50/1/2/1	3
NB 138	"Mwezi Moja"	3
NB 74	K20	3
NB 194	Canadian Wonder	3
NB 144	"Mwezi Moja"	3
NB 280	5/4/1/5/2 Kampala, Leaky	3
NB 2196	Eagle	3
NB 79	Rose Coco	3
NB 175	Rose Coco	3
NB 96	Canadian Wonder	3
NB 38	"Kabanima"	3

Table 5 cont'd

Cultivar Identification	Variety/Line	Reaction
NB 132	"Karapul Dichol"	1
NB 76	K20	3
NB 489	Navy pea bean	3
NB 26	Canadian Wonder	3
NB 86	"Wairimu"	5
NB 1287	75-N2B-50-R29-2-3-1BK	3
NB 168	Rose Coco	3
NB 225	White Haricot	3
NB 393	5/4/1/5/2 Kampala, Leaky	3
NB 1372	Santa Ana	3
NB 457	Black Rose Coco	3
NB 1339	SR-73/74-118	3
NB 170	Rose Coco	3
NB 121	-	3
NB 114	Canadian Wonder	3
NB 142	"Mwezi Moja"	3
NB 130	"Karapul Dichol"	5
NB 55	Rose Coco	3
NB 131	"Karapul Dichol"	5
NB 139	"Mwezi Moja"	7
NB 140	"Mwezi Moja"	3
NB 2146	SR-172/LKY 33	3
NB 2416	Zebra bean	3

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Table 5 cont'd

Cultivar Identification	Variety/Line	Reaction
NB 29	-	3
NB 123	-	3
NB 2350	-	3
NB 2405	-	1
NB 2371	-	5
NB 2317	Rose Coco	3
NB 2369	-	3
NB 2348	-	3
NB 2127	-	3
NB 2363	-	3
NB 2426	-	3

[a]A dash indicates that information was not available.

CHAPTER FIVE

DISCUSSION AND CONCLUSIONS

DISCUSSION

When growing the differential cultivars for multiplication or for other purposes, it will be necessary to have wider spacing than the one used for non-climbing varieties. Use of supports may also be helpful. Unlike the local cultivar, GLP2, that was planted side by side with them, the differential cultivars were found to require top-dressing with nitrogen and they were more seriously attacked by bean flies in the field. This was probably because they were not adapted to the local environment.

Commercial french bean farms where fungicides are used for disease control are not ideal for rust collections to be carried out. They nevertheless ensure availability of rust samples at any time of the year. Abundant rust may be found if samples are collected at a time when there are dry beans in the fields. Once the plants with rust have been identified, manual collection of spores may proceed using a sieve no. 200 through which the spores pass and the plant debris and leaves are left behind (Pastor-Corrales, 1985). The spores may be used immediately or kept for use at a later date.

Widely separated pustules were the most ideal in the transfer of urediospores for single-pustule isolation. They ensure ease of the actual transfer and low risk of contamination from neighbouring pustules.

Inoculum of single-pustule isolates must be increased in isolated conditions. This is because during sporulation, urediospores escape easily from the pustules producing them and float about in the air currents and may in this way bring about considerable contamination (Harter and Zaumeyer, 1941). The use of wooden frames covered with polythene sheeting was found to be very effective under Kenyan conditions. The chambers are cheap and easy to construct and therefore as many as required are obtainable. This ensures that many different rust isolates can be handled at once.

The inoculum of the individual races may then be kept available by inoculating susceptible plants at frequent intervals during the year or by storage of the spores in liquid nitrogen (Pastor-corrales, 1985).

The recommended basic set of 20 rust differential cultivars and the standard rust grading scale have not been utilized before in Kenya. Howland and Macartney (1966) had however used the

cultivars U.S.3, C.S.W. 643, K.W. 765, K.W. 780, K.W. 814 and Golden Gate Wax to classify rust samples in East Africa. It was not possible to compare their results with those obtained in this investigation because different grading systems were utilized. They had used a scale (see appendix III) which is a modification of the Mexican System (Crispin and Dongo, 1962).

In this investigation, the pathogenic variation system used was based on qualitative reactions of the host plants. The reactions ranged from immunity through various consistent types of non-sporulating or sporulating necrotic reactions to very small or large pustules (Table 2) (anonymous, 1987). The infection intensity was expressed in percent and was estimated using a modified Cobb Scale (Stavely, 1985) similar to the one used for bean varieties in the International Bean Rust Nursery (IBRN). The plant reaction classification of five categories as in figure 9 was then assigned as a result of combining the pustule type and the infection intensity. This means that within each reaction classification, except for category 1, there are variations of pustule type and infection intensity. This ensured that cultivars with the most similar reactions were grouped together in one category.

All the isolates were found to be pathogenic to all the tested differential cultivars, but they were found to differ in their reactions (Table 3). The gene-for-gene relationship has been shown to occur in the U. appendiculatus - P. vulgaris host-pathogen interaction (Christ and Groth, 1982) and this would therefore explain the differences observed in the host pathogen interactions. The reaction expressed by a cultivar was dependent on whether or not the isolate involved possessed genes for pathogenicity which overcame the matching genes for resistance present in the host. Zaumeyer and Harter (1941) have also shown that cultivar resistance to U. appendiculatus may be governed by a single factor or by more than one factor and that resistance may be dominant or incompletely dominant.

The noted variability among the isolates from different sites means that a cultivar may be resistant to one isolate of bean rust but be susceptible to another. There is therefore great need to determine and identify the physiological races of U. appendiculatus present in this country and to develop or identify bean cultivars with broad resistance to the most prevalent races. Some work done in the sixties showed that by 1963, four races existed in East Africa and by 1966, before the work was brought

to a close, up to 8 races had been identified. The races were designated A to H. Races A - F was found to occur in all the three territories (Kenya, Uganda, Tanzania), but the distribution of races G and H was not established due to inadequate data (Howland, 1963; Howland and Macarteney, 1966). The revival of this work in Kenya will be of great importance. Once this is done, bean cultivars known to be resistant to specific races may be recommended for growing in particular areas to reduce loss of yield and quality that occurs as a result of infection by rust.

The evaluation results for three Kenyan cultvars, GLP 1004, GLP 24, and GLP x92, were comparable to those obtained in a previous field evaluation done in Kenya. The scale used then was ranging from zero for immunity to 10 for highest susceptibility. GLP 1004 gave an average score of 2.0, GLP 24 of 3.3 and GLP x92 of 10.0 (Anonymous, 1984a). There is an indication, therefore, that GLP 1004 is the most resistant and GLP x92 the least resistant of the three cultivars. GLP 24 and GLP 1004 have also been described as tolerant to rust in Kenya (anonymous, 1984b).

Among the 65 cultivars that had a plant reaction classification of 3, which stands for resistance, five of them showed a very high degree of resistance

as indicated both by the pustule type and by the infection intensity (see appendix II). NB 127 and NB 123 gave very small necrotic spots (grade 2) with a low infection intensity of 1% and 5% respectively, and cultivars NB 63, NB 225 and NB 14 exhibited small sporulating pustules of grade 3 with low infection intensity. The other 60 cultivars had either large pustules with low intensity or small pustules with a high infection intensity.

From this investigation, it was found that there is variability in U. appendiculatus in rust isolates in the country, and that there exists resistance to the pathogen in many cultivars. In view of this, the following suggestions are made:

1. That a survey should be conducted to establish the distribution and prevalence of races of U. appendiculatus in the bean growing areas of the country.

2. That more evaluation of bean germplasm for resistance to rust should be done to identify more cultivars with broad resistance to the most prevalent races of the pathogen in the country.

3. That breeding strategies should be designed to make use of the cultivars identified as possible sources of resistance to rust in the development of cultivars that are resistant to the pathogen.

CONCLUSIONS

The identification of the races of U. appendiculatus indigenous to a region is a prerequisite in a bean breeding program (Davison and Vaughan, 1963a). In this investigation, the diverse pathogenic potential possessed by the rust fungus in the isolates tested was brought out by using the recommended basic set of 20 differential cultivars. It was found that different cultivars brought out the variability among the different isolates, thus making differentiation of the isolates easier than if fewer differential cultivars were used.

By using the standard system for the evaluation of bean germplasm, bean cultivars were evaluated for resistance to rust and possible sources of resistance were identified. With the use of the two criteria, pustule type and infection intensity, it was possible to distinguish not only between cultivars that are immune, resistant, intermediate, susceptible and very susceptible, but also between cultivars with various

degrees of resistance. The cultivars identified as immune or highly resistant to the rust isolates were recommended for consideration as possible sources of rust resistance after further field testing. They include the cultivars NB 2405, NB 132, NB 123, NB 14, NB 127, NB 225 and NB 63.

CHAPTER SIX

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APPENDIX I

Highest infection grades and average infection intensity (%) exhibited by Kenyan cultivars GLP1004, GLP24 and GLP X92 to specific rust isolates.

Cultivar identification	Variety/Line	Rust isolate	Infection grade	Infection intensity
GLP 1004	" Mwezi Moja"	A	4	5
		N	4	1
		T	4	5
		K	4	5
GLP 24	Canadian Wonder	A	4	25
		N	5	5
		T	4	5
		K	4	5
GLP X92	"Mwitemania"	A	6	10
		N	6	5
		T	6	10
		K	5	5

APPENDIX II

Highest infection grades and average infection intensity (%) produced on cultivars by a mixture of specific isolates of bean rust.

Cultivar identification	Variety/Line	Infection grade	Infection intensity
GLP x1127(a)	New "Mwezi Moja"	4	5
GLP 288	"Tongmire-Kabumbu"	4	5
NB 569	[a]-	5	5
NB 40	"Banja" 2	4	5
NB 70	"Kabanima"	4	5
NB 164	Rose Coco	5	5
NB 189	Canadian Wonder	4	5
NB 1181	-	4	10
NB 404	12/1/2/1 Kampala, Leaky	4	5
NB 2437	-	4	5
NB 461	Red Mexican UI3	5	10
NB 46	"Renka"	4	5
NB 36	"Diacolnima"	4	5
NB 288	5/4/1/5/2 Kampala, Leaky	4	5
NB 340	19/3/2 Kampala, Leaky	4	5
NB 2409	-	4	5
NB 127	"Karapul Dichol"	2	1
NB 1122	-	4	5
NB 440	51/2/21 Kampala, Leaky	3	25
NB 171	Bush BI Lake Tendercrop	4	5
NB 68	"Kabanima"	4	5

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APPENDIX II Cont'd.

Cultivar identification	Variety/Line	Infection grade	Infection intensity
NB 460	Red Mexican UI34	6	10
NB 241	5/4/1/5/2	5	5
NB 63	"Calima"	3	5
NB 1340	SR-73/74-118	4	5
NB 227	Early Harvest	4	5
NB 2425	-	6	5
NB 2406	-	4	5
NB 77	Rose Coco	4	5
NB 66	"Calima"	4	5
NB 272	5/4/1/5/2 Kampala, Leaky	4	5
NB 31	-	5	5
NB 14	50/1/2/1	3	10
NB 138	"Mwezi Moja"	4	5
NB 74	K20	5	5
NB 194	Canadian Wonder	4	5
NB 144	"Mwezi Moja"	4	5
NB 280	5/4/5/2 Kampala, Leaky	4	5
NB 2196	Eagle	4	5
NB 79	Rose Coco	4	5
NB 175	Rose Coco	5	5
NB 96	Canadian Wonder	4	5
NB 38	"Kabanima"	5	5
NB 132	"Karapul Dichol"	1	0

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APPENDIX II Cont'd.

Cultivar identification	Variety/Line	Infection grade	Infection intensity
NB 76	K20	5	5
NB 489	Navy pea bean	4	5
NB 26	Canadian Wonder	4	5
NB 86	"Wairimu"	4	10
NB 1287	75-N2B-50-R29-2-3-1BK	4	5
NB 168	Rose Coco	5	5
NB 225	White Haricot	3	5
NB 393	5/4/1/5/2 Kampala, Leaky	4	5
NB 1372	Santa Ana	5	5
NB 457	Black Rose Coco	4	5
NB 1339	SR-73/74-118	4	5
NB 170	Rose Coco	4	5
NB 121	-	5	5
NB 114	Canadian Wonder	4	5
NB 142	"Mwezi Moja"	4	5
NB 130	"Karapul Dichol"	5	10
NB 55	Rose Coco	4	5
NB 131	"Karapul Dichol"	6	10
NB 139	"Mwezi Moja"	5	25
NB 140	"Mwezi Moja"	4	5
NB 2146	SR-172/LKY 33	5	5
NB 2416	Zebra bean	5	5
NB 29	-	4	5

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APPENDIX II Cont'd.

Cultivar identification	Variety/Line	Infection grade	Infection intensity
NB 123	-	2	5
NB 2350	-	5	10
NB 2405	-	1	0
NB 2371	-	6	10
NB 2317	Rose Coco	4	5
NB 2369	-	5	5
NB 2348	-	4	5
NB 2127	-	4	5
NB 2363	-	4	5
NB 2426	-	5	5

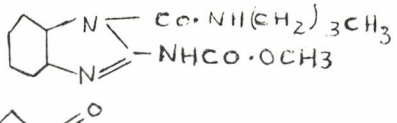
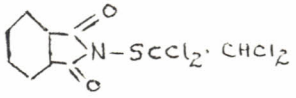
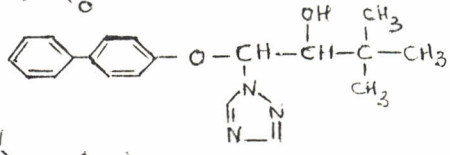
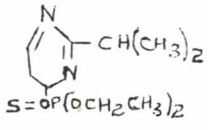
[a]A dash denotes that information was not available.

APPENDIX III: Comparison of E.A.A.F.R.O. and Mexican Classification of Infection Types

SYMPTOM	E.A.A.F.R.O*	MEXICAN
No visible symptoms	0	0
Chlorotic flecks only, no necrosis, no sori	01	not used
Necrotic Lesions, no sori	1-	1
Necrotic Lesions, surrounding small sori	1+	2
Mixture of class 1 + Lesions and sori on green, non-necrotic areas	x	not used
Small open sori without necrosis, hardly visible on lower surface	2-3	3
Full susceptibility. Fairly good size sori on both surfaces	4	4
Large sori on both surfaces, margins of leaves dead or entire surface becomes chlorotic	not used	5

* E.A.A.F.R.O. = East African Agricultural and Forestry Research Organization. In this table it stands for the scale used previously in East Africa for differentiation of rust races. Howland and Storey, 1964.

APPENDIX IV: Formulae for chemicals mentioned in the text

Common name	Chemical name*	Emperical formula
Zineb	Zinc ethylenebis (dithiocarbamate) (polymeric)	$(C_4H_6N_2S_4Zn)$
Mancozeb	Manganese ethylenebis (dithiocarbamate) (polymeric) complex with zinc salt	$(SCS.NHCH_2CH_2NHCS.S.Mn)_x(Zn)_y$
Propineb	Polymeric Zinc Propylenebis (dithiocarbamate)	$(C_5H_8N_2S_4Zn)$
Maneb	Manganese ethylenebis (dithiocarbamate) (Polymeric)	$C_4H_6N_2S_4Mn$
Metiram	Zinc ammoniate ethylenebis (dithiocarbamate) poly (ethylenebis (thiuram) disulphide	$\left[(C\text{-}S.CS.NHCH_2CH_2NH.CS.S.Zn(NH)_3^{++}\right.$ $\left.(C\text{-}SCS.NH.CH_2CH_2NH.CS.S\right]_x$
Copper Oxychloride	Dicopper chloride trihydoxide	$Cu_2Cl(OH)_3$
Benomyl	Methyl 1-(butylcarbamoyl) benzimidazol-2-y lcarmamate	
Captafol	N-(1,1,2,2-tetrachloroethylthio) Cyclohex-4-ene-1,2-dicarboximide	
Biternol	all-rac-1-(biphenyl-1-(1H-1,2,4-triazol-1-yl)	
Diazinon	0,0-di-ethyl 0-2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate	

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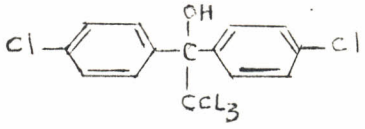
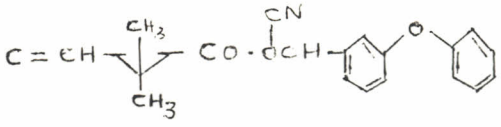
Appendix IV Cont'd

Dicofol	2,2,2-trichloro-1,1-bis (4-chlorophenyl) ethanol; 2,2,2, trichloro-1,1 di (4-chlorophenyl)	
Cypermethrin	(RS)-α-cyno-3-phenoxybenzyl (IRS,3RS; IRS, 3SR)-3-(2,2-dichlorovinyl)-2,2- = dimethylcyclopropane carboxylate	

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* IUPAC - International Union of Pure and Applied Chemistry

Source: The Pesticide Manual, A World Compendium. 8th Ed. Edited by Charles R. Worthing. 1081 pp.