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Races of Bean Anthracnose (*Colletotrichum Lindemuthianum*)
From Malawi, Germplasm Screening and the Genetics of
Resistance in Selected Bean (*Phaseolus Vulgaris* L.)
Genotypes

presented by

James Morris Bokosi

has been accepted towards fulfillment
of the requirements for

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RACES OF BEAN ANTHRACNOSE (COLLETOTRICHUM
LINDEMUTHIANUM) FROM MALAWI, GERmplasm
SCREENING AND THE GENETICS OF RESISTANCE IN
SELECTED BEAN (PHASEOLUS VULGARIS L.)
GENOTYPES

By

James Morris Bokosi

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ABSTRACT

RACES OF BEAN ANTHRACNOSE (COLLETOTRICHUM
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Bean anthracnose caused by Colletotrichum lindemuthianum is a major fungal disease of beans in Malawi and is found in all bean growing areas. Studies of 16 isolates showed more pathogenic variation. Alpha-Brazil and lambda were the only known races identified. Many of the Malawian bean landraces were susceptible to a mixture of two Malawian isolates. Screening of other bean germplasm identified additional sources of resistance. Studies on genetics of resistance in selected bean genotypes to two Malawian isolates, BA3 and BA4, of C. lindemuthianum indicated that resistance to BA3 was mainly governed by a single pair of genes while that of BA4 was by two pairs of interacting genes. No linkage was detected in factors conditioning resistance to the two isolates.



In memory of my father



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INTRODUCTION

Beans (Phaseolus vulgaris L.) play a very important nutritional role in the diets of people in Malawi. Compared to other legumes, beans are second only to groundnuts in total production. But groundnuts are generally grown for cash and beans are grown essentially for food. Beans are high in protein content and provide a balanced diet when eaten with maize, rice, or cassava, which is quite common in Malawi. Thus, they serve as an economic source of protein for the majority of the population, who cannot afford expensive protein sources like beef, fish or poultry. When immature pods or green tender leaves are eaten, as vegetables, they provide vitamins. This practice is quite common.

Despite the importance, bean production has lagged behind demand and yields are consistently low. The main constraints are many but diseases are probably the single most important single factor limiting yields. Among these diseases, bean anthracnose, caused by Colletotrichum lindemuthianum (Sacc and Magn.) Scrib. is one of the most widespread and destructive. Efforts to control this disease are complicated by the pathogenic variability that exists for this organism.

This study was conducted with the following objectives:

1. To identify pathogenic variation in Malawian isolates of bean anthracnose.
2. To identify sources of resistance in the Malawian bean germplasm pool.
3. To identify sources of resistance in foreign bean germplasm pool.
4. To determine the inheritance of genetic resistance involving a Malawian cultivar and Malawian isolates of the disease.

LITERATURE REVIEW

The Pathogen

Bean anthracnose is incited by the fungus Colletotrichum lindemuthianum (Sacc. and Magn.) Scribner. It has been common for most workers to cite the fungus to Colletotrichum lindemuthianum (Sacc. and Magn.) Briosi and Cavara. Earlier publications classified it as Gleosysorum lindemuthianum (Sacc. and Magn.).

The pathogen is a member of the Fungi Imperfecti. The perfect stage of the fungus has been reported, and was originally called Glomerella lindemuthianum. Kimati and Gali (40), renamed it G. cingullata.

C. lindemuthianum produces a septate, branched mycelium which changes color from hyaline to nearly black upon maturity. It produces hyaline unicellular conidia (18) measuring 4 to 5 by 22 μ which usually contain a clear vacuole-like body near the center. The shape of the conidia may be oblong, cylindrical, kidney-like with rounded or slightly pointed ends. The conidia are born in acervuli on unbranched conidiophores.

In cultures optimum growth of the fungus occurs at 22-25°C (18) while optimum conidial production takes place from 14-18°C. It has been reported by Edgerton (25) that temperatures above 30°C limit or prevent conidial production. Sporulation is best at pH 5.2-6.5 and virtually stops below pH 3 but is unaffected by aeration or ultraviolet light (48).

Cultures of C. lindemuthianum are frequently grown on bean pod agar (BPA) medium; sterilized pods, potato-dextrose agar (PDA), and Czapek medium (67). But Mather et al. (48) found excellent sporulation on a medium containing glucose, mineral salts and Neopeptone. Chaves (18) reported that isolates may lose viability and pathogenicity when repeatedly transferred in culture, unless occasionally re-isolated from inoculated plants or stored under low temperatures. In evaluating the cryo-preservation and pathogenicity of some fungi, Hwang et al. (38) found that neither freezing nor ultra-low temperature storage (in liquid nitrogen-- -150°C to -196°C) altered the pathogenicity or damaged the viability of the C. lindemuthianum isolates, and no morphological changes were observed in subcultures after four years of storage in the liquid nitrogen.

The Host

In most cases, C. lindemuthianum occurs on common bean (Phaseolus vulgaris L.). But, while the disease is more severe on P. vulgaris, it has also been reported on other hosts including lima beans (P. limensis Macf.), scarlet runner bean (P. coccineus Willd), tepary bean (P. acutifolius Gray var. latifolius Freeman), Cowpea (Vigna unguiculata Walp), kudzu bean (Dolichos biflorus L.) and broad bean (Vicia faba L.).

The Disease

Bean anthracnose is known wherever P. vulgaris is cultivated. The disease has been reported in Africa, Asia, Australia and Oceania, Europe, North America, Central America, West Indies, and South America. The wide geographic distribution has been well illustrated by the Commonwealth Mycological Institute (22).

Although the pathogen was actually collected by mycologists as early as 1843 (65) the first description of bean anthracnose in 1875 was based on specimens originating in Bonn, Germany. The disease is of high economic importance. Crop losses can approach 100% when infected seed is planted under conditions favorable for disease development (67).



From about 1912 to 1920 bean anthracnose was considered the most serious bean disease in the United States, losses in Michigan in 1914 were estimated (67) at about \$1.5 million and double that amount in 1915. However, since about 1920 the importance of the disease in the United States has declined because of use of disease-free seed and development of resistant varieties. But the disease remains one of the most destructive bean diseases in other parts of the world, especially in East Africa and Latin America.

Compared to other bean diseases, C. lindemuthianum is ranked first as a priority disease in practically all of Eastern Africa (the major bean production area in Africa) where it causes substantial yield losses.

In Malawi, where the common bean P. vulgaris L. is one of the most important grain legumes, second only to groundnuts (27) diseases are probably the most important single factor limiting bean yields. Bean anthracnose occurs in practically all bean growing districts and most bean varieties are susceptible. Because of its widespread and destructive nature, it is ranked as the number one fungal bean disease and the objective is to breed for resistance to it.

Epidemiology

Humidity and Temperature

Bean anthracnose is favored by humid or rainy weather combined with comparatively low temperatures. Lauritzen (43) found that high humidity, greater than 92% or free moisture, and temperatures between 13° to 26°C were required for infection. The lowest limit for infection during a 24 hour period of incubation was 14°C. No infection occurred above 27°C and was prevented or inhibited at 13°C. Abundant infection occurred at 17°C.

Rahe and Kuč (53) have reported limited infection and development of the fungus when temperatures are greater than 30°C. However, the pathogen is capable of tolerating temperatures as low as -15° to -20°C, for several days.

Spread

The build up of an epidemic requires moderate rainfall at frequent intervals (18). This condition is essential for local dissemination of conidia. Cultivation of bean fields while rain or dew is present on the foliage provides for dissemination of the disease through contact of spore masses with man, insects, animals and implements (65, 67).



Work done in Canada (Southern Ontario) on Fleetwood, a white bean cultivar, demonstrated that periods of heavy rain are responsible for the spread of C. lindemuthianum in the field (62). The direction of spread from an initial infection focus followed the direction of the prevailing wind. Field temperature of Southern Ontario did not appear to be a decisive factor in the infection and spread of the disease. The spread was limited to the dispersal distance of splashing raindrops. Disease severity was highest at or near the axis of the sector nearest the initial infection focus and decreased gradually toward the periphery of the sector.

For long distance spread, the seed is the principle means of dissemination. C. lindemuthianum is a seed borne disease and will survive from one season to the next as dormant mycelium within the seedcoat or the cells of the cotyledon or as spores between cotyledon or elsewhere in the seed.

Longevity

The survivability of C. lindemuthianum varies greatly depending on environmental conditions. Tu (63) found that moisture had a profound effect. The fungus survived at least 5 years in infected pods and seeds that were air dried and kept in storage at 4°C. Dry



infected plant material had similar longevity when left in the field in sealed polyethylene envelopes and having no contact with water. An alternating wet-dry cycle was detrimental to the survival of the fungus. Infected pod segments, under laboratory conditions, lost viability after three cycles of 72 hour wet and 72 hour dry periods.

Control

Cultural Practices

One method of limiting the spread of bean anthracnose is by the use of disease-free seed. This has been done in various regions of the world (67).

Heat treatment has been used in some cases but leads to reduced seed viability.

Walker (65) and Zaumeyer (67) recommended the practice of crop rotation of three years duration. In Kenya, Van Rheenen et al. (64) reported that beans grown in association with maize showed less incidence of bean anthracnose. Mixed cropping effected a kind of cultural control of the pathogen especially in the wetter areas but yield was considerably less under mixed cropping than in monoculture.

It is highly recommended that people refrain from working in the field when conditions favor the spread of the disease. Above all, quarantine and legal

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requirements for bean seed exchange and/or marketing between and among countries need to be adhered to.

Chemical Control

Various chemicals have been evaluated and used to control bean anthracnose (18, 67) either through seed treatment or foliage sprays. An effective seed treatment must be able to destroy the mycelium and spores in the seed without greatly impairing germination.

In Tanzania, Shao (59) reported that benomyl (Benlate, 0.55 g/l) gave effective control of bean anthracnose as well as angular leaf spot. Yield losses by anthracnose in unsprayed treatments were 46%.

In Malawi, Edje and Mughogho (26) evaluated six fungicides and found that triphenyltin hydroxide (Du-Ter) was the most effective in controlling the disease. Sprays with Daconil 75% w p at 3.5 g/liter or Zineb 70% w p at 3.7 g/liter at bi-weekly intervals have been found effective (27).

Pyregrine (51) recommended the use of fentin hydroxide at the rate of 1.5 oz. a.i./150 gallons of water per acre for good quality seed production.

Use of Resistant Varieties

The observation that bean varieties differ in their reaction to anthracnose came as early as 1883 (65)

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but the first organized screening for resistance and susceptibility of different varieties to different isolates of the fungus was by Barrus (10). A bean selection from Red Kidney showed resistance to the alpha and beta races of C. lindemuthianum and was named Wells' Red Kidney. A few years later, Burkholder (14) produced a resistant white marrow bean variety resulting from a cross between Well's Red Kidney and a white marrow bean. McRostie (46) utilized the same Well's Red Kidney in a cross with a selection (p.B. No. 1986) of Michigan Robust to develop a navy bean resistant to the disease. Search for resistance to bean anthracnose has been expanded into many bean producing areas and regions (18, 67). Goth and Zaumeyer (33) tested fifty-four bean varieties for their reaction to four races of anthracnose and found that snap bean varieties resistant to alpha, beta, and gamma races could be developed by recombining resistance in existing snap bean varieties while resistance to the delta race could be derived from the dry bean, PI304110.

In Malawi, varietal improvement is aimed at producing disease resistant varieties (27). Earlier screening identified some sources of resistance from the land races. In addition to identifying resistance in the land races, attempts are being made to use exotic



cultivars in crosses with Malawian gemplasm, to develop new resistance sources.

Plant Infection and Symptomology

Under favorable conditions conidia of C. lindemuthianum may germinate in six to nine hours (23) to form a germ tube and appressorium which are attached to the host cuticle by a gelatinous layer. Penetration occurs by means of an infection peg which forms on the side of the appressorium in contact with the host and penetrates the cuticle and epidermis by mechanical pressure applied by the appressorium and infective hyphae which develop from it (23, 65). The infective hyphae (18, 67) continue to grow and enlarge between the cell wall and protoplast for two to four days without apparent damage to the host cells. Later, the cells are degraded and the protoplast dies and water soaked lesions appear.

The symptoms appear on all above-ground parts of the plant. The term 'anthracnose' is descriptive of the appearance of the symptoms in the form of 'burnt black' lesions (16). The fungus primarily attacks young organs of the plant (stems, leaf petioles, leaf veins, and the fruit). The lesions are characteristically dark brown to black. In moist weather, pinkish spore masses may be present on the surface. Lesions on leaves appear mostly on the veins and on the lower side of the leaf (Figure



1). Petioles and stems may also bear lesions. In the hypocotyl they often extend to deep cankers. The most striking symptoms (65) appear on the immature pod. Lesions develop from small brown spots which enlarge quite rapidly. They become dark brown to black in the center and light to pinkish at the border. As the pod matures, the colors lighten and the edges of the lesions are raised. When the pods are opened the lesions are often found to have penetrated through the seed coat into the seed.

Affected seeds bear lesions colored in various shades of brown. Plants which grow from such seeds show blackened cankers on the cotyledon. Badly infected plants may form lesions several inches long from coalesced spots especially on the stem and young stems may collapse due to cracking and rotting of the tissues.

Host Pathogen Interactions

Disease resistance of higher plants usually depend on metabolic responses of host cells to the pathogen, and successful pathogens overcome all of the defense mechanisms of their hosts.

Cell Wall Degradation

Plant infection by microbial pathogens usually involves degradation of the cell walls of the plant.



Figure 1. Lower side of a bean leaf showing typical symptoms of Colletotrichum lindemuthianum.



The importance of pectic degrading enzymes is indicated by the ability of these enzymes to macerate plant tissue and to cause cell death. Anderson and Albersheim (2) indicated that the pectic degrading enzymes secreted by the pathogens and the proteins present in plants which inhibit them can play a critical role in the infection process. Among the enzymes secreted by plant pathogens to degrade the polysaccharides of plant cells, polygalacturonases are considered of great importance in the infection process (28).

Phytoalexins

Phytoalexins are "low molecular weight antimicrobial compounds that are both synthesized by and accumulated in plants after exposure to microorganisms" (24). Many plants respond to invasion by a pathogen or non-pathogenic microorganism by accumulating phytoalexins (1, 7, 8, 34, 35, 55, 66). Considerable evidence support the view that accumulation of phytoalexins at the sight of infection is one mechanism by which plants resist disease (21). It cannot be said that all plants have the ability to synthesize phytoalexins but the ability to produce them is a characteristic of plants that is widespread (1). Indeed several types of resistance responses to attempted infection apart from phytoalexins have been reported.

The Impact

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Plants can entrap invading microbes by secreting extra-cellular materials composed, in different host-microbe interactions, of lignin-like compounds, callose, silicon, cellulose or unidentified materials.

Many pathogens are capable of detoxifying the phytoalexins produced by their hosts. There often appears to be competition between plants' ability to accumulate inhibitory concentration of phytoalexins and the ability of the microbes to detoxify phytoalexins. Successful pathogens are able to tip the balance in their favor. They are able to evolve a mechanism of avoiding toxic effects of phytoalexins.

One mechanism might be the ability of an infective strain to grow away from the areas in which the plant is accumulating toxic levels of phytoalexins. A pathogen might secrete a toxin which kills the plant cells in the region of the pathogen before those cells are capable of synthesizing enzymes needed for production of phytoalexins. Some pathogens can become tolerant to high concentration of phytoalexins. Pathogens may secrete molecules that specifically inhibit synthesis of phytoalexins such as secretion of an effector molecule that activates a repressor of a gene encoding an enzyme required to synthesize the phytoalexins or by secretion of molecules that inhibit a required enzyme (21).

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Further still, some pathogens may penetrate their hosts so efficiently that they invade the host tissue before the tissue can accumulate enough quantities of phytoalexins to stop the pathogen. Other pathogens can be so well adapted to their hosts that they grow through the plant tissue biotrophically, without causing apparent damage to host cells and without triggering host defenses (9).

The amount of phytoalexins accumulated in challenged plants is highly variable (21) and is likely to depend on such things as the physiological state of the plant (temperature, water potential, nutrition), time of day, eliciting microbe, and the genetic background of the cultivar. The mechanisms by which phytoalexins accumulate in plant tissues are not well understood.

In studying the relationship between symptom expression and phytoalexin concentration in P. vulgaris infected with C. lindemuthianum, Bailey (6) demonstrated that four antifungal compounds--phaseollin, phaseollidin, phaseollenoflavin and kieveton--accumulated in all infected tissues. Skipp et al. (61) showed that phaseollin caused drastic changes in a range of fungal species. Spores of the bean pathogen C. lindemuthianum were killed within two minutes of exposure to a solution containing 10 mg phaseollin per ml. Phaseollin was also

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shown to kill plant cells. But Darvill and Albersheim (21) have reported that phytoalexins are ineffective antibiotics, requiring millimolar concentration to inhibit microbes. Thus they are less effective, on a molar basis, as compared to commercial pesticides but have the advantage of being concentrated where they would be most effective. They are also indiscriminate in that they affect procaryotic and eucaryotic cells at similar concentration and appear to be toxic to higher plant cells and mammalian cells in addition to microbes.

Elicitors

The molecules that signal plants to begin the process of phytoalexin synthesis are called elicitors. Elicitors can be solubilized from plant cell walls both by enzymes secreted by infecting microbes and by enzymes in the plant that are thought to be activated during infection. Many biotic and abiotic elicitors have been identified (21). Most work with elicitors has centered on two experimental systems: Colletotrichum lindemuthianum, Phaseolus vulgaris, and Phytophthora megasperma var. Sojae--Glycine max soybean. In each case, researchers were investigating race--specific monogenic resistance. Evidence indicates that these elicitors do not induce differential phytoalexin accumulation in host varieties to explain the striking

1870

(1870)

race--variety specificity characteristic of natural systems (1). An elicitor was extracted from P. vulgaris hypocotyl sections and cell suspension cultures following a treatment with denatured ribonuclease A, a known abiotic elicitor (24). The elicitor was capable of passing through a dialysis membrane and inducing phytoalexin accumulation in healthy tissue on the other side of the membrane.

Induced Resistance

Inoculations with a non-pathogenic race of C. lindemuthianum has been shown (12, 29, 52, 60) to induce resistance against a pathogenic race. The recognition of the organism by the host and by the organism early in the development of the interaction appears to be essential for the activation of a disease resistance mechanism.

Induced resistance has also been reported with heat treatment of infected plants (54). Andebrhan et al. (3) was able to induce resistance by irradiating susceptible bean hypocotyls with ultraviolet light. Resistance was accompanied by accumulation of phytoalexins.

There is also gradual acquisition of resistance, apparently with age. As the bean plant ages, tissue

1947-1948

1949-1950

1951-1952

that is susceptible when young gradually acquires resistance to C. lindemuthianum.

Pathogenic Variation

Physiological specialization or races of C. lindemuthianum were first observed by Barrus (10), who noted that varieties resistant to repeated inoculation made with an isolate obtained in one locality became seriously infected when inoculated with an isolate from another locality. The results demonstrated that at least two physiological races were involved. The races were later designated alpha and beta.

In 1922, Burkholder (15) isolated a third race and designated it gamma. Many more races have since been reported. These include delta (4), epsilon (13), kappa (42) which was originally called ebnet (57), alpha-brazil (30), lambda (36), which was originally called alpha SN, jota (37), and lambda-mutant (32). C-236 is another race added to this list.

The race nomenclature system used by most workers is based upon Greek letters but in other instances (18) researchers have used different codes such as Groups I-III in Mexico, 1-8 in Australia, A-X in Germany and Races PV6, D10, E8b, I4, and L5 in France.

In Kenya, the occurrence of alpha race has been reported (41). Leakey and Simbwa Bunnya (44) have

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indicated the existence of a number of races in Uganda, and reported that some isolates with affinities to the alpha and beta managed partially to overcome the immunity of the cultivar Cornell 49-242. Shao (59) has reported the existence of alpha, beta, gamma, epsilon and kappa races in Tanzania. In Malawi, Ayonoadu (5) reported the presence of beta, gamma, and delta and three new races.

Sources of Resistance

The detection of varietal resistance to C. lindemuthianum can be traced back to Barrus (10) who later released a resistant selection known as Wells Red Kidney (11). Since then, considerable effort has been devoted to screening for broader and more stable resistance to the disease.

Sources of resistance to alpha race has been found in Charlevoix, Manitou, Montcalm and Mecosta red kidney varieties (56). Sanilac and Fleetwood varieties are resistant to alpha, beta, and gamma races but susceptible to delta. Tuscola confers resistance to alpha and beta but is susceptible to gamma and delta races. The Venezuelan accession PI326418 (Cornell 49-242), which contains the ARE gene, is resistant to alpha, beta, gamma, delta, epsilon and lambda races (18), but is susceptible to C-236, kappa, and jota races.

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Resistance to alpha, beta, and kappa has been identified in Coco ala creme, Kievit Koekork, Bo-22 and Evolute, while PI1501414, Titan and Metorex confer moderate resistance to kappa (42). A black seeded Mexican bean, PI165422 and a small light tan Colombian bean, PI207264 (37), show resistance to kappa and jota races. A variety Nep-2 has resistance to delta race and breeding lines with favorable plant architecture as well as delta race resistance are now available (39). Fouilloux (31) reported Mexico 222 and Mexico 227 as sources of resistance to kappa and Brazilian-alpha races. Bo-22, Kaboon, To (Mexique 2 gene) and Tu (Mexico 3 gene) are reported to give resistance to the same two races (18). Other varieties, AB136, BAT841, and IVT7420, appear to be effective against most races of C. lindemuthianum. Ayonoadu (5) showed that some cultivars from Malawi gave differential reactions to alpha, beta, gamma, and delta races. Two cultivars (Acc. Nos. 2 and 118/2) were able to resist all four races.

Workers at Centro Internacional de Agricultura Tropical (CIAT), in screening for sources of resistance to bean anthracnose have obtained some important results. More than 13,000 bean accessions from the CIAT germplasm bank were screened during 1978-1981 (19). Among these, 162 were resistant or intermediate to the Popayan isolates of C. lindemuthianum, 27 accessions

were resistant to all known and available races from Europe, Colombia and other parts of Latin America. Many of the resistant sources originated from Mexico and Central America and showed great diversity for geographic origin, plant type, seed color and seed size. Thus, it is considered possible to identify genotypes with resistance effective against a wide range of pathogen races from within and across different bean production regions. The stability and longevity of anthracnose resistant varieties is dependent upon the pathogenic variation displayed by C. lindemuthianum (58).

Genetics of Resistance

The genetic control of resistance to different races of bean anthracnose has been a subject of considerable study. Burkholder (14) crossed Well's Red Kidney with a white marrow bean and showed almost an exact 3:1 ratio in the F₂ segregating population, indicating a single factor difference between the resistant and susceptible plants with respect to one race of bean anthracnose involved in the cross. Resistance was found to be dominant. McRostie (46) obtained similar results. In another cross by McRostie (47) between selection B, resistant to alpha and beta races, and Germany wax, susceptible to both races, a segregation ratio of 9R:7S

in the F₂ was obtained with a mixed inoculum indicating that a single dominant gene governed resistance to each race.

The first evidence of greater complexity in the genetic reaction to a particular race of C. linde-muthianum, race beta, was by Andrus and Wade in 1942 (4).

Mastenbroek (45) described the existence of the dominant ARE gene found in Cornell 49-242 and used it in a breeding program. There were no undesirable linkages noted in the back crossing program that was used.

Cardenas et al. (17) evaluated nine varieties of beans and their crosses for their genetic resistance to alpha, beta and gamma races. The study showed that genetic resistance to alpha was by dominant alleles at each of two loci, unlinked but behaving as duplicate factor loci. A similar system governed reaction to beta and gamma races with evidence favoring multiple allelism and dominance of alleles conferring susceptibility in some cases for beta. Distinct systems of complementary factors and genetic linkages were also observed but genes belonging to members of the set(s) affecting any one race alone showed independence with regard to linkage.

Another study by Muhalet et al. (50), involving five cultivars and one breeding line of P. vulgaris,



showed that independent and complementary gene action at 1 or 2 loci accounted for a resistant reaction to beta, gamma, and delta races. Resistance to the beta race was further controlled by an alleomorphic series of three alleles.



GENERAL MATERIALS AND METHODS

Media Preparation

Potato dextrose agar (PDA) and bean pod-agar bean (BPAB) were the two media used in this study. PDA plates were prepared by dissolving 39 g of commercial PDA (Difco) in 1 litre (1,000 ml) of glass-distilled water. The suspension was steamed for about 20 minutes, transferred (in 250 ml lots) to prescription bottles and autoclaved for 20 minutes at 250°F and 15 psi. The contents were allowed to cool and then poured in small lots of 25 ml to petri dishes. The plates were checked for contamination after two days and any contaminated plates were discarded.

BPAB plates were prepared by steaming 10 g of navy bean seeds in 100 ml of glass distilled water for 60 minutes; the beans were then ground in a mortar and pestle. 20 g grams of agar (Difco) and 20 g of ground bean pods were then dissolved in 900 ml of distilled water and steamed for 30 minutes. The previously prepared 100 ml + 10 g ground navy bean suspension was then added to the 900 ml agar/ground pod mixture by sieving it through a double layer cheese cloth, and thoroughly stirred. The contents were autoclaved for 20



minutes at 250°F and 15 PSI, allowed to cool, and poured in 25 ml lots to petri dishes, and checked for contamination after 2 days.

Isolation of the Pathogen

For infected plant tissue that contained sporulating lesions a drop or two of sterilized distilled water was put on the lesion and a fine needle, previously flamed, was used to dislodge the spores into a suspension. A sterile pipette was then used to suck the spore masses and transfer them to 1 ml sterile distilled water in a test tube. The suspension was diluted three or four times to reduce the spore concentration to facilitate single spore isolations later.

The spore suspensions from the 1 ml sterile distilled water were then poured onto the surface of petri dishes containing potato dextrose agar (PDA) made from 39 g of Difco PDA, and 1,000 ml distilled water. Inoculated plates were incubated at 19-21°C, for 24 to 48 hours, during which time germinating spores could be seen under the stereo-microscope. When the germinating spores became visible, a fine needle was used to transfer 10 to 12 of the spores plus a small amount of media to two fresh PDA plates. These spores developed into colonies and the colony showing maximum sporulation and

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freedom from contamination under the stereo-microscope was selected, transferred and maintained on PDA.

For materials that showed little or no sporulating lesions, the diseased tissue was excised from the infected tissue and sterilized with 10% sodium hypochlorite for two minutes and rinsed twice with sterile glass-distilled water. The pieces were then transferred to PDA plates and incubated at 19-21°C. During incubation, spores were produced and the same procedure as described for sporulating lesions was used to obtain single spore isolates.

Maintenance of Isolates and Inoculation Preparation

Cultures were maintained on PDA and transferred at 8-10 day intervals. Small discs of PDA from an aging culture (8-10 days) were transferred to new PDA plates. When greater sporulation was needed for inoculum preparation, BPAB was the medium used. Spores for inoculation were obtained by scraping them from plates that had been incubated at 21°C for 5 to 7 days. Glass sterile-distilled water of 25 ml containing 0.5 v/v Tween 80 (polyoxythelene sorbitan monooleate), a wetting agent, was added to the spore-containing plates and spores were dislodged with a sterile microscope slide. The suspension was filtered through a double layer of cheese

cloth. Spore concentration was adjusted to about 10^6 spores/ml by the use of a haemocytometer.

For a preparation of mixed race inoculum the same procedure was used except that the spore concentration of each race before the mixing was 2.0×10^6 instead of 1.0×10^6 spores/ml to take care of the dilution effect when mixing. This ensured a concentration of 1.0×10^6 spores/ml for each of the two isolates in the mixture.

Disease Assessment

Plants were rated at "+" for susceptible and "-" for resistant, with no visible symptoms of infection. A 1-5 scale was adapted where resistance corresponded to 1-2 while susceptibility corresponded to 3-5, rating as follows:

1. Clean, no visible symptoms of infection.
2. A few scattered, small lesions on the midrib and occasionally on the main veins. Lesions corky in appearance.
3. Many small lesions scattered on the midrib and veins with collapse of the tissue.
4. Few large lesions scattered over the leaf blade or many large lesions over the leaf blade.

5. Many large coalesced lesions accompanied by tissue breakdown and sometimes leaves became chlorotic and abscised.



RACE IDENTIFICATION

The pathogenic variation of C. lindemuthianum has been well documented (4, 10, 13, 15, 30, 32, 36, 37, 42). Ayonoadu (5) has reported this variation in bean anthracnose from Malawi and identified the presence of beta, gamma, delta and three new races. But the number of race groups appeared to depend on number of differential varieties used and the system of classification was unsatisfactory because of lack of standardization. This study was aimed at using a proposed standard set of differentials.

Materials and Methods

Collection of Isolates

Sixteen bean anthracnose isolates (Table 1 and 2) were collected for race identification. Nine of these came from Malawi and were brought by Dr. M. W. Adams in 1984 and 1985. Six came from Tanzania and one from Kenya collected by Dr. J. D. Kelly during a trip to Tanzania and Kenya in 1985. All isolates came from bean plants that were naturally infected in the field. The infected tissues were preserved by air drying upon their collection and put under refrigeration (6°C) when they arrived in Michigan.



TABLE 1. Isolates of bean anthracnose (Colletotrichum lindemuthianum) from Malawi

Isolate ^a	Part Isolated From	Site	Altitude (m)	Temperature (°C) ^b (Range)	Rainfall (mm) ^c (Oct-Apr)
BA1	Leaf	Dowa	1350	-	941.2
BA2	Leaf	Dedza	1467	16-20	1616.5
BA3	Leaf	Dowa	1350	-	941.2
BA4	Pod	Thyolo	870	20-24	848.0
BA5	Leaf	Dedza	1467	16-20	1016.5
BA6	Leaf	Lilongwe	1020	21-25	819.9
BA7	Leaf	Dedza	1467	16-20	1016.5
BA8	Leaf	Dedza	1467	16-20	1016.5
BA9	Leaf	Rumphi	1200	-	1308.4

^aIsolates are abbreviated (BA) for Bean Anthracnose.

^bTemperature range taken in four growing seasons 1970/80, 1980/81, 1981/82, and 1982/83.

^cAverage seasonal rainfall taken from four growing seasons 1979/80, 1980/81, 1981/82 and 1982/83.



TABLE 2. Isolates of bean anthracnose (Colletotrichum lindemuthianum) from Tanzania and Kenya.^a

Isolate ^b	Part Isolated From	Site
BA10	Leaf	Magamba
BA11	Leaf	Same
BA12	Leaf	Lyamungu
BA13	Pod	Kabete
BA14	Leaf	Mlingano
BA15	Leaf	Same
BA16	Leaf	Magamba

^aIsolate BA13 came from Kenya.

^bIsolates are abbreviated (BA) for Bean Anthracnose.



Seed Source

The bean cultivars used as standard differentials (Table 3) for race identification were from stocks maintained by Dr. A. W. Saettler at Michigan State University.

Plant Management

The twelve differential varieties were grown in plastic flats containing a pasteurized 3:1 mixture of soil and vermiculite. Water was provided as needed. Overwatering was avoided especially during the first week of planting which led to better germination. Each flat had twelve inserts and each insert was planted to six seeds, one differential variety for each insert. The greenhouse temperature fluctuated between 24 and 30°C.

Inoculation of Plants

Plants were inoculated using a spraying technique. In this method the inoculum was sprayed as a fine mist onto upper and lower leaf surfaces and the entire stem. An aerosol containing can (Spra-tool No. 8011 power pak, Crown Industrial Products Co.) was used as a sprayer (Figure 2). Care was taken to avoid contamination between isolates more than one isolate was used. Physical barriers were put in the chamber to separate inoculations with different isolates. Inoculations were

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TABLE 3. Proposed standard range of differentials for the identification of races of Colletotrichum lindemuthianum, according to E. Drijfhout, IWT, Holland.

[illegible]





Figure 2. An aerosol containing can (Spra-tool No. 8011 Soil Power Pak; Crown, Industrial Products Co.).



done 12 to 14 days after planting. Plants were put in mist chambers maintained at near 100% relative humidity and ambient greenhouse temperature. The high humidity inside the chambers was maintained by using humidifiers (Model 500 Hermidifier, Hermidifier Co. Inc.). The humidifiers were supplied with tap water and made to run continuously. Plants were kept in the chambers for about a week. Symptoms usually appeared between 6 to 8 days after inoculations. Disease counts were done twice for each inoculation, and every inoculation was repeated.

Results

Isolates BA1, BA2, and BA3 (Table 4) were the same as Alpha-Brazil. BA4, BA5, BA6 were unique isolates. BA7 behaved as BA8 and BA9 and was different from all known races. The Tanzanian isolates, (Table 5) BA10, BA12, and BA14 differed from all known races. BA16 was the same as lambda. BA11 and BA15 were the same and represented a unique isolate. The Kenyan isolate, BA13, behaved differently from all known races.

All the Malawian isolates infected Auguille Vert. Michelite was another poor source of resistance to these isolates showing resistance only to BA6. Cornell 49-242, Evlutie and AB136 were the only differential varieties that were resistant to all the isolates.

TABLE 4. Reactiona of standard anthracnose differentials to isolates of *Colletotrichum lindemuthianum* from Malawi.

Differential Variety	Isolatesb								
	BA1	BA2	BA3	BA4	BA5	BA6	BA7	BA8	BA9
Michelite	+	+	+	+	+	-	+	+	+
Auguille Vert.	+	+	+	+	+	+	+	+	+
Michigan D.R.K.	-	-	-	+	-	+	+	+	+
Sanilac	-	-	-	+	-	-	-	-	-
Perry Marrow	-	-	-	+	-	+	+	+	+
Coco ala creme	-	-	-	+	+	+	+	+	+
PI167399	+	+	+	-	+	-	-	-	-
PI165426	+	+	+	+	+	-	-	-	-
Cornell 49-242	-	-	-	-	-	-	-	-	-
Evolutie	-	-	-	-	-	-	-	-	-
Mexico 222	+	+	+	-	-	-	-	-	-
AB 136	-	-	-	-	-	-	-	-	-

a+ = Susceptible; - = Resistant.
 Isolates are abbreviated BA (Bean Anthracnose).

TABLE 5. Reaction^a of standard anthracnose differentials to six isolates of Colletotrichum lindemuthianum from Tanzania and one from Kenya.

Differential Variety	Isolates ^c					
	BA10	BA11	BA12	BA13b	BA14	BA15 BA16
Michelite	+	-	-	-	-	- +
Auguille Vert.	+	+	+	+	+	+
Michigan D.R.K.	+	+	+	+	-	+
Sanilac	-	-	-	-	+	+
Perry Marrow	+	+	+	-	+	+
Coco ala creme	+	+	+	+	+	+
PI167399	-	-	+	-	+	+
PI165426	+	-	+	+	-	-
Cornell 49-242	-	-	-	-	-	-
Evolutie	-	-	-	-	-	-
Mexico 222	-	-	-	-	-	-
AB 136	-	-	-	-	-	-

^a + = Susceptible; - = Resistant.
^b isolate BA13 is from Kenya, the rest from Tanzania.
^c isolates are abbreviated BA (Bean Anthracnose).

All Tanzanian (non-Malawian) isolates attacked the differential varieties Auguille vert. and Coco ala creme, but Cornell 49-242, Evolutie, Mexico 222 and AB 136 showed resistance to all seven isolates.

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CROSS PROTECTION VERIFICATION

Before the screening of the Malawi bean land races was conducted a small study was done to evaluate the independence of reaction of the two individual isolates, BA3 and BA4, that were to be used as a mixture. Various researchers, Berald et al. (12), Elliston et al. (29), Rahe et al. (52), Skipp and Daverall (60) have reported induced resistance by a non-pathogenic race against a pathogenic one. This study was done to verify any interference or non-interference by the two isolates when used in the screening program.

Materials and Methods

Eight varieties (Table 6) from the standard differentials were chosen based on their reaction to each of the two individual Malawian isolates, BA3 and BA4. These varieties were planted in plastic flats and all treatments remained the same as those used for race identification. In the preparation of the inoculum, each isolate was prepared separately, adjusted to a concentration of 2.0×10^6 and then mixed in equal volumes. Inoculation procedures were the same as for race identification.



TABLE 6. Selected varieties and their reaction^a to two isolates.

Variety	Individual	Reaction	Mixture Reaction
	BA3	BA4	
Auguille Vert.	S	S	S
Michigan D.R.K.	R	S	S
Coco ala creme	R	S	S
PI167399	S	R	S
PI165425	S	S	S
Cornell 49-242	R	R	R
Mexico 222	S	R	S
AB 136	R	R	R

^aS = Susceptible, R = Resistant.

Results

Assuming no interference between the two isolates the expected susceptible reaction for all S + S, R + S, and S + R combinations and resistance only to the R + R combination. Any deviation from these, for instance, getting a resistant from S + S, R + S, or S + R combinations, would be indicative of some cross protection or induced resistance.

The results presented in Table 6 show no evidence of cross protection.

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GERMPLASM SCREENING

Breeding for disease resistance to C. lindemuthianum depends on identifying bean genotypes that show resistance to the races of the pathogen. Malawi has one of the most diverse bean germplasm pools in Eastern Africa. The germplasm collection at Bunda College probably contains the largest collection of African bean landraces (27). The genetic variability of this germplasm pool should inevitably be reflected in disease resistance. This study was conducted in order to identify sources of resistance to two of the isolates of C. lindemuthianum from Malawi. Some of the bean germplasm pool was also tested against a Tanzanian isolate to assess its potential as a source of resistance to other C. lindemuthianum races.

Materials and Methods

Seed Source

The Malawian bean landraces were obtained through the Bean Cowpea Collaborative Research Support Program (CRSP), and supplied by Dr. M. W. Adams. These landraces were collected from the central and northern regions of Malawi and were quite diverse (Figure 3).

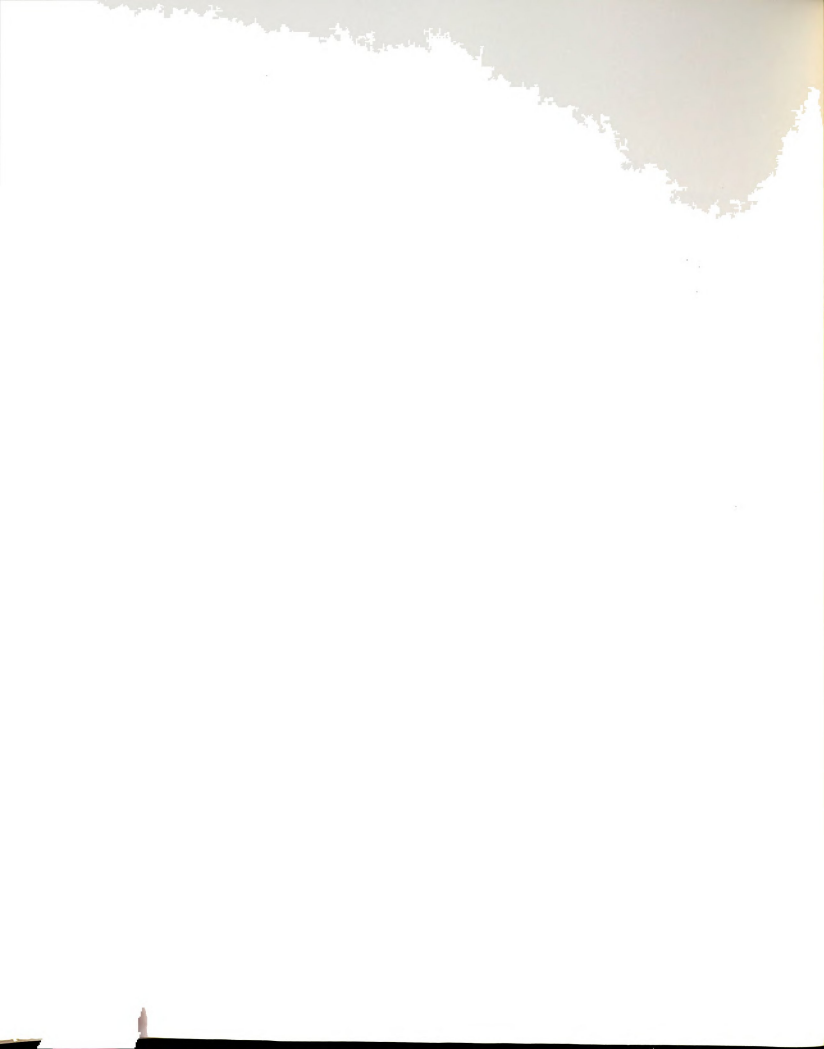




Figure 3. A Sample of Malawian bean landraces used in screening for sources of resistance.



The Malawian bean varieties were from the stocks of the Bean Research Program of Bunda College of Agriculture in Malawi. The rest of the bean varieties came from stocks maintained by Dr. Adams and Dr. A. W. Saettler at Michigan State University.

Plant Management

The landrace lines were grown in plastic flats containing a pasteurized 3:1 mixture of soil and vermiculite, water was provided as needed and all management remained the same as those used for differentials in race identification. The same plant management was applied to varietal screening.

Inoculation of Plants

Plants were inoculated using a spraying method. All the procedures were as those used for race identification. The landraces were screened using the mixture of BA3 and BA4 while varieties were screened with the same isolates separately.

Results

When more than 220 bean landrace lines were screened with a mixture of BA3 and BA4 isolates, 19 landraces (Table 7) were resistant to both isolates. Only about 8.6 percent of the lines tested were

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TABLE 7. Bean landrace germplasm identified as resistant to a mixture of two isolates of Colletotrichum lindemuthianum from Malawi.

Accession ^a	Seed		Isolates Resistant to
	g/100 seeds	Color	
MB6	35.0	Black	BA3 + BA4
MB17	21.3	Red	"
MB25	36.7	Brown	"
MB28	39.5	Brown	"
MB70	32.7	Brown	"
MB90	34.3	Tan	"
MB92	44.5	Red Tan	"
MB97	28.5	White	"
MB99	28.9	White	"
MB108	23.9	Mottled	"
MB112	29.2	White	"
MB113	25.9	White	"
MB114	27.4	White	"
MB119	42.0	Red	"
MB124	40.0	Brown	"
MB157	41.7	Red	"
MB191	30.8	Black	"
MB198	34.5	Red	"
MB205	28.2	Red	"

^aMB = Malawian Bean.

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identified as sources of resistance to both isolates. Although resistance to each isolate individually may be higher than 8.6 percent, the results show a remarkably low level of resistance to bean anthracnose in the Malawian bean landrace germplasm. However, it is unlikely that the 8.6 percent could have been much lower had all the 9 Malawian isolates been used for screening, since the two isolates chosen for the mixture (BA3 and BA4) represented almost all the total possible combinations of reaction of the 9 isolates to the differentials used for race identification.

Screening using mixtures proved to be an efficient way of searching for resistance when dealing with large populations or limited seed where fewer repeats are necessary. The system saves time and space.

There were some lines that showed resistance to more than the two Malawian isolates when they were inoculated with a third isolate, BA16 from Tanzania. Out of 70 Malawian bean landrace lines screened to all three isolates, six (Table 8) were shown to confer multiple resistance. This represents about 8.5% of the lines tested.

Reaction of Malawian Bean Varieties to Three Anthracnose Isolates

Some of the Malawian bean varieties are resistant to the individual isolates BA3, BA4, and BA16. Six

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TABLE 8. Malawian bean landraces identified as resistant to three isolates^a of Colletotrichum lindemuthianum.

Accession ^b	Seed		Isolates Resistant to
	g/100 seeds	Color	
MB99	28.9	White	BA3+BA4+BA16
MB108	23.9	Mottled	"
MB112	29.2	White	"
MB113	25.9	White	"
MB198	34.5	Red	"
MB205	28.2	Red	"

^aIsolates are abbreviated BA (Bean Anthracnose). BA3 and BA4 are from Malawi and BA16 is from Tanzania.

^bMB = Malawian Bean.

of the varieties conferred resistance to isolate BA3 (Table 9). However, there was very low frequency of resistance to isolates BA4 and BA16. Only two varieties gave resistance to isolate BA4 and one to BA16. Although no variety was able to give resistance to all three isolates, the Malawian variety 1212 D gave an immune reaction to the two Malawian isolates (BA3 and BA4). Variety 336 was also resistant to the Malawian isolate BA4 and the Tanzanian isolate BA16. These two isolates attacked many of the differentials and represented the most virulent isolates. Figure 4 shows the reaction of some Malawian bean germplasm to BA4. Only one variety, 1768 D, succumbed to all three isolates.

There was an appearance of complementarity between the two Malawian isolates, BA3 and BA4. Except for the reaction of 1768 D, all the varieties attacked by one isolate gave resistance to the other. The Tanzanian isolate BA16 behaved similarly to the BA4 isolate, although its reaction to 1212 D was not tested.

Reaction of Other Bean Varieties of
P. vulgaris to Two Isolates of
C. lindemuthianum From Malawi

When other bean varieties were inoculated with the two Malawian isolates, BA3 and BA4, some resistance to either isolate was identified. Six varieties (Table

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TABLE 9. Reaction^a of some Malawian bean varieties of Phaseolus vulgaris L. to three isolates of Colletotrichum lindemuthianum.

Variety	Seed		Isolate ^b		
	g/100 seeds	Color	BA ₃	BA ₄	BA ₁₆
336	26.9	Red	S	R	R
1039D	46.8	Mottled	R	S	S
1212D	15.7	White	R	R	NT
1251D	48.2	Mottled	R	S	NT
1768D	41.3	Red	S	S	S
2482D	36.6	Yellow	R	S	S
2539D	41.2	Mottled	R	S	S
Nasaka	44.9	Tan	R	S	NT

^aS = Susceptible; R = Resistant; NT = Not Tested.

^bIsolates are abbreviated BA (Bean Anthracnose).
BA₃ and BA₄ are from Malawi and BA₁₆ is from
Tanzania.





Figure 4. Reaction of some Malawian bean germplasm to isolate BA4.



10) gave resistance to isolate BA3 and 17 gave a susceptible reaction. Approximately 1/3 of the varieties tested were resistant to this isolate. Isolate BA4, which represents the most virulent of the Malawian isolates, managed to infect only 8 of the 24 varieties tested. This indicates a higher frequency of resistant varieties to susceptibles for this isolate. But this was just the opposite for isolate BA3 which showed lower frequency for resistance to susceptibility in the same varieties. Although resistance to either isolate was identified in quite a number of varieties, only three varieties, Charlevoix, Montcalm, and Seafarer, gave resistance to both isolates. Five varieties, 70-700 (an experimental line), Isabella, Pompadour-Checa, Redcloud, and Ruddy showed susceptibility to both isolates.

From the 70 bean landrace components tested against the Tanzanian isolate, BA16, only 13 lines (Table 11) showed a resistant reaction.

This indicates the potential role the Malawi bean landraces have as sources of resistance to foreign isolates of C. lindemuthianum. And since the BA16 isolate represented the more virulent, in terms of number of differentials it attacked, it is entirely possible that more resistant lines could be identified for less virulent isolates that come from Tanzania.

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TABLE 10. Reaction^a of other bean varieties of *Phaseolus vulgaris* L. to two isolates of *Colletotrichum lindemuthianum*.

Variety	Seed		Isolates ^b	
	g/100 seeds	Color	BA3	BA4
Aurora	14.7	White	S	R
Bat 41	20.0	Dark Red	S	R
Bat 332	16.0	Cream	S	R
C-15	18.9	White	S	R
C-20	19.7	White	S	R
70-700	52.9	Light Red	S	S
Charlevoix	53.5	Dark Red	R	R
Fleetwood	18.9	White	NT	R
G-04534	62.4	Mottled	R	S
Isabella	53.5	Light Red	S	S
Jamapa	21.4	Black	S	R
Manitou	53.5	Light Red	R	S
Mecosta	53.5	Light Red	R	S
Midnight	16.8	Black	S	R
Montcalm	53.5	Dark Red	R	R
Pinto VI-111	40.7	Pinto	S	R
Pompadour (Checa)	43.6	Mottled	S	S
Porrillo	22.0	Black	S	R
Puebla	13.0	Black	S	R
Redcloud	53.5	Light Red	S	S
Ruddy	50.5	Light Red	S	S
Seafarer	18.9	White	R	R
Swan Valley	18.2	White	S	R
T 39	16.8	Black	S	R

^aS = Susceptible, R = Resistant, NT = Not Tested.^bIsolate are abbreviated BA (Bean Anthracnose). BA3 and BA4 are from Malawi.



TABLE 11. Malawian bean landraces identified as resistant to a Tanzanian isolate of Colletotrichum lindemuthianum.

Accession	g/100 seeds	Seed		Isolate Resistant to
		Color		
MB 12	23.5	Brown	BA16	
MB 14	18.2	Red	"	
MB 22	34.3	Black	"	
MB 23	24.9	Black	"	
MB 23	24.9	Black	"	
MB 99	28.9	White	"	
MB 108	23.9	Mottled	"	
MB 112	29.2	White	"	
MB 113	25.9	White	"	
MB 169	18.5	Dark Brown	"	
MB 198	34.5	Red	"	
MB 205	28.2	Red	"	
MB 210	26.3	Cream	"	

5A6 Isolates are abbreviated BA (Bean Anthracnose).



GENETIC STUDY

The genetics of resistance to C. lindemuthianum has been studied in various bean genotypes using different races (4, 14, 17, 45, 46, 47, 50). However, no such study is documented for Malawi. Many of the bean anthracnose studies have concentrated on screening for resistance and not on genetics of resistance. Knowledge of the genetics of resistance enables a plant breeder to anticipate possible interactions and linkages in selected crosses and devise the type of breeding strategy and selection procedures to use. This study was formulated to determine the inheritance of resistance in five selected genotypes one of which was from Malawi, and in which two bean anthracnose isolates from Malawi were used.

The joint segregation of the F₂ populations to simultaneous reaction to two isolates of the pathogen was tested against the theoretical joint segregation ratios for the two isolates. This was obtained by multiplying together the segregational ratios to each of the isolates. Plants were classified as resistant to both isolates, resistant to the first isolate but susceptible to the second, susceptible to the first



isolate but resistant to the second isolate and susceptible to both races, and denoted as R R, R S, S R, and S S, respectively. The observed and expected numbers of plants in their respective categories were compared by using a χ^2 (Chi-Square) test.

Materials and Methods

Seed Source

Parental and F₂ seeds used in the genetic study were supplied by Dr. J. D. Kelly. The choice of the parents of the selected crosses was based on their reaction to two bean anthracnose isolates from Malawi (Table 6).

Plant Management

Plants were planted in 10 cm clay pots (1 plant per pot). Soil/vermiculite mixture, water requirements and greenhouse temperature remained the same as the growth conditions for differentials used in race identification.

Inoculation of Plants

Parental plants were screened using a spraying technique for inoculation. The F₂ plants were inoculated with BA3 and BA4 using a brushing technique. Under this method, a brush (No. 4 camel's hair brush) was used to apply the inoculum. The inoculum was

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deposited on the under side of each of the lateral leaflets of the first trifoliate leaf.

Each of the lateral leaflets was inoculated with one of the two isolates; two people using two separate brushes, one for each isolate, performed the inoculations. Plants were usually inoculated 14 to 18 days after planting when the first trifoliate leaf was fully expanded. Chamber and greenhouse environments remained as for differentials.

Data Analysis

The segregating plants in the F₂ populations for resistance and susceptibility were tested against the theoretical ratios of resistance to susceptible plants by using a X² (Chi-Square) test.

Results

Reaction of Parent Bean Cultivars to Each of the Two Malawian Isolates

The Malawian navy bean variety 1212 D showed an immune reaction to both isolates. This variety was the only white seeded type used in the study. The rest were all red seeded. BAT 41 gave a resistant reaction when inoculated with BA⁴ isolate but was susceptible to BA³ isolate. Isabella showed a susceptible reaction to BA³ and was moderately susceptible to BA⁴. Montcalm gave resistance to both isolates, however, this resistance

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differed from that of 1212 D. Very tiny flecks occasionally appeared on Montcalm as opposed to a complete immunity shown in 1212 D. Ruddy was the only variety that showed complete susceptibility to both isolates. The reaction results and seed characteristics of these bean varieties are presented in Table 6.

Reactions of F_2 plants were observed in susceptible leaflets (Figure 5).

BA3 Isolate

Four F_2 populations were tested against the BA3 isolate. The observed segregation of resistant and susceptible plants to this isolate is presented in Table 12. In crosses between Ruddy X 1212 D the proportion of resistant to susceptible plants fit a 3:1 ratio. This indicates that a single dominant gene conferred resistance to this isolate. This gene came from the resistant parent 1212 D. The same segregation ratio was observed in the cross between Isabella and 1212 D, indicating a similar gene system. In crosses between Montcalm X 1212 D the F_2 plants showed no segregation. All plants gave a resistant reaction to BA3. This may mean that the genes conferring resistance from both parents are identical or are closely linked. It would seem unlikely that the two parents have the same gene or genes for resistance because of the previous differences observed



Figure 5. Trifoliate leaf of F₂ plant from a Ruddy x 1212 D cross showing a resistant reaction to BA₃ (right) susceptible to BA₄ (left).



TABLE 12. F₂ reactions to two isolates of *Colletotrichum lindemuthianum* and expected ratios of resistant (R), and susceptible (S) plants.^a

Cross	Parental Reaction	N	Observed		Expected		Expected Ratio		Probability between
			R	S	R	S	R	S	
Ruddy x 1212 D	S x R	184	134	50	138.0	46.0	3	1	.25 - .50
Ruddy x BAT 41	S x S	81	24	57	15.2	65.8	3	13	.01 - .025
Montcalm x 1212 D	R x R	199	199	0	199.0	0	AR		-
Isabella x 1212 D	S x R	78	56	22	58.5	19.5	3	1	.5 - .75
Ruddy x 1212 D	S x R	184	150	34	149.5	34.5	13	3	.90 - .95
Ruddy x BAT 41	S x R	81	48	33	45.6	35.4	9	7	.5 - .75
Montcalm x 1212 D	R x R	199	199	0	199.0	0	AR		-
Isabella x 1212 D	S x R	78	74	4	73.1	4.9	15	1	.5 - .75

^aThe upper part of the table gives the results for reaction to BA3 and the lower part for reaction to BA4 isolates.

BAR = All Resistant.

*Total number of F₂ plant population tested.



in the degree of resistance, as 1212 D showed complete immunity, though the difference may be due to different genetic backgrounds or modifying genes.

In the cross Ruddy x BAT 41 a 3:13 ratio was observed although both parents are susceptible. I postulate that one parental variety has a resistant gene pair but is unable to express it, because of an inhibiting factor. If the inhibiting factor is denoted by I and the resistant factor by R, one variety has proposed genotype IIRR and the other has genotype iirr. In the F₂ population resulting from a cross between the two susceptible parents, 3/16 of the progeny would show resistance and 13/16 susceptibility. Although this ratio is not like the other two-factor ratios, it may be explained in the same way with the additional assumption that the inhibiting factor I is epistatic to the effect of the resistant factor R.

In determining the appearance of the F₂ plants, it should be remembered that, wherever I is present, resistance is inhibited, so that plants which inherit I are susceptible whatever other factors they may receive such that IIRR, IIRr, IiRR, IiRr, IIrr, Iirr and iirr genotypes would show a susceptible reaction while iirr and iiRr would be resistant.

The above postulation fit the 3:13 ratio at a very low probability (P between 0.01-0.025) and gives a

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weak support to the inhibitory hypothesis. A ratio of 1:3 would have given a better fit (P between 0.25-0.5) and this would mean that a single recessive gene confers resistance to BA3. But both parents, Ruddy and Bat 41, are susceptible to BA3 and this cannot be explained by a single gene hypothesis. It would, therefore, seem appropriate not to discard the 3:13 ratio on account of its poor fit.

The results for reaction to the BA4 isolate show a completely different mode of inheritance as compared to the BA3 isolate. In crosses between Ruddy and 1212 D the observed segregation of resistant and susceptible plants fit a 13:3 ratio. Apparently two independent segregating loci are involved in conferring resistance to the BA4 isolate in this particular cross. One of these gene pairs segregates into a 3:1 and the other into a 1:3 ratio. Both genes would have come from variety 1212 D which was resistant to both isolates. The single dominant gene responsible for the 3:1 segregation may have been the same one that conferred resistance to the BA3 isolate. The two genes segregate independently and either one is capable of producing a resistant reaction. Denoting the Ruddy x 1212 D cross as $aaBB \times AAbb$, the small b is epistatic resistant over small a and large A epistatic resistant over large B such that $AABB$, $AABb$, $AaBB$, $AaBb$, $AAbb$ and $aabb$

genotypes would show a resistant reaction but aaBB and aaBb genotypes would be susceptible.

In crosses between Ruddy x BAT 41 the observed segregation of resistant to susceptible F₂ plants fit a 9:7 ratio. This segregation pattern suggests that there were two pairs of complementary genes conferring resistance to this isolate. These genes may have come from BAT 41 which was the resistant parent in the cross.

The F₂ plants from the Montcalm x 1212 D cross did not segregate for susceptibility. All the plants gave a resistant reaction. This may mean that the genes conferring resistance in the two parents are closely linked or possibly the same genes since the difference in the resistance reaction may be a result of differences in genetic background or results of effects of modifying genes. The same findings were true for the BA3 isolate. The segregation of resistant and susceptible F₂ plants in the cross between Isabella x 1212 D showed a good fit to a theoretical ratio of 15:1. The data indicate that two dominant duplicate gene pairs confer resistance. One pair may have come from 1212 D and the other from Isabella. However, Isabella is moderately susceptible to the BA4 isolate. It is therefore more conceivable that both pairs of duplicate genes could have come from 1212 D.

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Evaluation of the four F₂ populations with regard to their reaction to the BA4 isolate indicates that resistance is controlled by more than one pair of genes compared to that of BA3 which showed simple genetic control--mostly by single dominant genes.

Joint Segregation in F₂ Populations

There were three F₂ populations that were evaluated for joint segregation for reaction to the BA3 and BA4 isolates. This was done in order to ascertain a degree of association in factors conditioning reaction to the two isolates. The results are shown in Table 13.

In the cross Ruddy x 1212 D the segregation ratio of resistant and susceptible F₂ plants was 3:1 for the BA3 isolate and 13:3 for BA4. The theoretical joint segregation computed as the product of the two ratios was 39:9:13:3. The observed number of plants classified in the reaction classes fit the theoretical ratio. This means that there was independent association in factors conditioning reaction to the BA3 and BA4 isolates.

The observed joint segregation of F₂ plants in the cross between Ruddy x BAT 41 was computed but gave inconclusive results. The joint segregation observed in the cross between Isabella x 1212 D did not deviate from the theoretical ratio of 45:3:15:1. It fit the expected ratio at the probability level of 0.5-0.75.

TABLE 13. Observed and expected frequencies in reaction of F₂ populations of two crosses involving *Colletotrichum* for reaction to two isolates of *Colletotrichum lindemuthianum* from Malawi.

Cross and F ₂	Reaction Class		Theoretical Ratios	Number of Plants		χ ²	P
	BAJ	BAV		Obs.	Exp.		
Ruddy	R	R	39	104	112.13	0.59	
	R	S	9	30	25.88	0.66	
	S	R	13	46	37.38	1.99	
	S	S	3	4	8.63	2.48	
Totals			64	184	184.0	5.72	.1-.25

Isabella	R	R	45	54	54.84	0.01	
	R	S	3	2	3.66	0.75	
	S	R	15	20	18.28	0.16	
	S	S	1	2	1.22	0.50	
Totals			64	78	78.0	1.42	.5-.75

In all, the three F_2 populations studied for joint segregation for reaction to the BA3 and BA4 isolates showed independent association.

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DISCUSSION

Based on the reaction of the nine Malawian Colletotrichum lindemuthianum isolates to the standard set of differentials for the identification of races of C. lindemuthianum, according to T. Drijfhout, IVT, Holland, an alpha Brazil race has been identified. Although previous studies have reported the existence in Malawi of beta, gamma, and delta races (5) this is the first time that the existence of alpha Brazil is revealed in Malawi. The other isolates tested show some differences to the known races. The lack of correspondence between Malawian isolates and the identified races on the standard differentials is not surprising. Leahey and Simbwa-Bunnya, (44), reported similar findings with Ugandan and European isolates. It was reported from CIAT (18) that when several isolates from different areas of Colombia were tested against the European differentials (the same as those used in this study) they appeared to be different from all races of C. lindemuthianum previously reported. All this reinforces the view of the previously known world diversity of races and sub-races of this organism.

A similar lack of conformity is evident for the other isolates (six from Tanzania and one from Kenya). Only one isolate, BA16, gave a reaction that clearly identified it as lambda.

It is worth noting that as the number of standard differentials used increases, so does the likelihood of separating isolates that might have been previously lumped together in one group, based on their reaction to fewer differentials.

A comparison between and among the Malawian and the other isolates show some notable similarities and differences. None of the Malawian isolates broke down the resistance of Cornell 49-242, Evolutie and ABL36. Auguille Vert. was the most vulnerable differential, being attacked by all the isolates. Another similarity is evident when the most virulent Malawian isolate (BA4) is compared to BA16, another most virulent isolate from Tanzania. Both isolates attacked seven differentials and gave a similar pattern of reaction except that BA4 attacked PII65426 instead of PII67399.

Some isolates found both in Malawi and Tanzania were identical. For instance, isolate BA6 from Lilongwe-Malawi, was identical to BA11 and BA15 from Tanzania. Some of the Malawian bean anthracnose isolates occurred in more than one location. Isolate BA1 and BA3, both of which were from Dowa, in the

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central region, were identical to BA2 from Dedza, in the southern region. These isolates were the same as alpha-Brazil. Dedza was one location that showed more variation of isolates. This may be due to the greater number of isolates collected from the district, which was far more than from any other location/site.

When the results on race identification in this study are compared to those of Ayonoadu (5), some notable differences arise. This comparison, which is based on five differentials, Michelite, Dark Red Kidney, Perry Marrow, Sanilac and Cornell 49-242, show that the 16 isolates (BA1 to BA16) used in the current study would have been classified into alpha, delta, gamma and one unidentified group. BA1, BA2, BA3, and BA5 would have been identified as alpha. Isolate BA4 would have been classified as delta. BA6 would have been called gamma while BA7, BA8 and BA9 would have been determined as a new race. This means that more known races and few unique ones would have been identified. The current study has identified only one known race, alpha-Brazil, and many unique types from the Malawian isolates. It is important to note that in the previous study, gamma was found to be the most abundant race, comprising more than half of the isolates. The present study found no such domination by any single isolate although alpha-Brazil (BA1, BA2, and BA3) and the unique isolate represented

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by BA7, BA8, and BA9, were both found three times. The lack of domination by any single race can be explained by the genetic diversity found in both host and pathogen. Plants affect their own environments and thus the environments of their pathogen. The nature, dispersion, and genetics of crops affect not only disease development in local populations but the spread of disease among populations, from field to field, and region to region, and the genetic adaptation of fungal parasites.

Traditional landraces and cultivars have advantages to the limited resource farmer. They represent centuries of continuous selection for local performance. The farmers' strategy was not so much for greater performance in the best years as for adequate performance in the worst. These landraces lag behind in yield but may excel in adaptability, in resistance to diseases, and other extreme conditions. Such qualities are enhanced by genetic diversity. Modern agriculture demands uniformity much to the detriment of yield stability.

The co-evolution of host and parasite has a strong equilibrium component, laid down in the genetic make-up of both host and pathogen. The result is a balance between the amount of host and the amount of pathogen. The pathogen needs the host and is influenced

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by it, where as the host does not need the parasite but is nevertheless influenced by it. The balance results from a dynamic equilibrium. Actual amounts of host and pathogen vary between and within years. The more diverse the genotypes, the more diverse the pathogen. It is, therefore, less surprising that more unique isolates were identified.

Another notable finding is that all the isolates classified as 'new races' in the previous study by Ayonoadu (5) had been from Bunda. It may not be surprising to find that the new races could have been either alpha-Brazil or a unique isolate corresponding to BA6. This isolate was found both at Bunda and in Tanzania by the current study. It may have been transmitted through bean seed exchange between Bunda-Malawi and Tanzania.

The importance of using a standardized set of differentials for race identification is further supported in this study. Proper race identification is crucial for breeding for bean anthracnose and seems to depend on the number and type of differentials. A plant breeder may identify alpha, beta and gamma as the existing races in an area and may request seed, from elsewhere, that is resistant to the three races. However, if some unique races exist but were not revealed by the differentials used, the requested "supposedly resistant" variety may show susceptibility. Plant breeders need to

test any given variety against indigenous known races. This would act as a check for any unique races that may have been incorrectly identified on account of the number and type of differentials used.

Among the differences was the reaction of Mexico 222 to the Malawian and the other isolates. The three Malawian isolates belonging to alpha-Brazil overcame the resistance of Mexico 222 while none of the non-Malawian isolates could do so. Michelite was resistant to many of the other isolates but succumbed to all but one isolate from Malawi. Coco ala creme gave an opposite reaction to that of Michelite, showing susceptibility to all other isolates but resistance to some of the Malawian isolates.

The screening of the Malawian bean landrace lines revealed a low level of resistance to the Malawian C. lindemuthianum isolates. Only 8.6 percent of the more than 220 bean landrace lines gave resistance to the two isolates used in the mixed-isolate screening program. This is rather puzzling because one would expect that the diverse nature and persistence of these landraces over the years would be reflected in their ability to maintain adequate gene frequencies for adverse environmental factors, disease resistance being one of them, especially so for resistance to bean anthracnose, a disease found in practically all bean

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growing areas of Malawi and ranked as the most destructive. The only plausible explanation may be that under natural conditions these environmental factors exert varying selective pressures from season to season, field to field and year to year. Because of this inconsistent selective pressure, gene frequencies for various traits shift from time to time depending on what pressures are present. In a season when bean anthracnose becomes more prevalent gene frequencies for resistance to the disease may be selected for but if the disease becomes less prominent in the next season, there may be selection towards another trait. This kind of natural selection, although assuring stable performance over numerous adverse environmental conditions, reduces gene frequency levels in the landrace populations for any single trait, and this could be true not only for disease resistance factors but for other characters as well.

The screening of the existing Malawi bean varieties, although based upon far less numbers (only 8) in comparison to the landraces (over 220), showed the presence of some good sources for resistance.

With respect to diseases in natural populations, where host and pathogens have had a long interdependent association, disease levels may be expected to be low and to vary only moderately over time. A change in cultural practices may produce unexpectedly strong

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changes in disease occurrence in a crop. The genetic diversity found in the Malawian bean landraces inevitably encourages more pathogenic variation but acts as a buffer in ensuring that no single bean anthracnose race dominates as this may destabilize the delicate balance that exists, and thus threaten the survival of certain genotypes. Any selection towards more resistant genotypes is checked by increase in corresponding virulent races and any build up of more virulent races is minimized by an increase in corresponding resistant host genotypes. This lies at the crux of the dynamic equilibrium referred to above, and explains the diversity of the bean anthracnose races that exist in Malawi.

In the quest for more economic crop production, new management systems for specific crops are continuously developed. Some of these are counter-productive in that they establish conditions favoring higher disease risk. One trend in resistance breeding is towards incorporation of vertical resistance genes. This may lead to reduction in cultivars more vulnerable to new physiological races. The introduction of another cultivar with another resistant gene may cause it to be overcome by another virulence factor in a different race. The pathosystem enters a vicious cycle, the boom-and-bust cycle, to the detriment of both farmers

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and plant breeders. The pathogenic variability of bean anthracnose in Malawi, inevitably, underscores the importance of the need to understand the dynamics of the landraces before attempting to improve them. Some agricultural practices disturb natural stabilizing mechanisms. Epidemics of plant diseases are often a result of that disturbance.

Other bean varieties, (not from Malawi) have also been identified as sources of resistance. Charlevoix, Montcalm, and Seafarer were resistant to the two Malawian isolates.

The differential varieties used for race identification have revealed some good sources of resistance to some isolates used in this study and could be useful in breeding programs. Of great interest are varieties Cornell 49-242, Evolutie and ABL36 which proved to be resistant to all isolates. The performance of Cornell 49-242 reinforces the usefulness of the 'ARE' gene in breeding for resistance to C. lindemuthianum in areas from which the isolates tested in this study came.

The use of racial mixtures in screening for resistance to C. lindemuthianum has been shown to be a very efficient procedure. There is need, however, to identify the reaction of each pure isolate, if only a few need to be mixed, so that selections can be made for

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the best combinations that represent the closest total reaction if all the isolates were combined.

In the genetic study, there is evidence for a simple inheritance mechanism controlling resistance to BA3 isolate, while a more complicated system, involving two genes, operates for the other isolate, BA4.

The Malawian variety 1212 D seems to possess a single dominant gene for resistance to BA3 but possibly two genes for resistance to the BA4 isolate. These results indicate that 1212 D could be a good source of resistance to the two isolates. The single dominant inheritance observed for BA3 in the crosses involving 1212 D suggest that this gene system could easily be incorporated in breeding for resistance to this isolate. Resistance could be incorporated and fixed within a short time. However, selections in the early generations (F_2 and F_3) may be less efficient because all heterozygotes would show resistance alongside the true breeding dominant homozygotes.

Breeding for resistance to the BA4 isolate can also be achieved by using 1212 D as the resistant parent. Although the mechanism of inheritance for BA4 is not as simple as for BA3, the independent segregation shown in this study would facilitate a less complicated breeding strategy.

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The results of this genetic study have also shown that there is independent association in factors conditioning resistance to BA3 and BA4 isolates. All the joint segregation for reaction to the two isolates in two crosses studied fit the theoretical ratios assuming independence. No unusually high frequencies of parental phenotypes were observed in any of the crosses. This means that the genes controlling resistance to BA3 and BA4 isolates segregate independently.

This is important because it shows that breeding for resistance to the two isolates can be less complicated than would have been the case had joint segregation shown linkage.

Variety 1212 D has also been observed to confer some resistance to halo blight (Pseudomonas syringe P.v. Phaseolicola) and angular leaf spot (Isariopsis griseola) (20, 49). This makes the variety a potentially useful parent for disease resistant breeding in Malawi. One disadvantage of 1212 D is that it is a navy bean and may not appeal to many of the Malawian farmers and consumers who prefer the red kidneys. The size and color problems may limit using it in its pure form and lead to using it as a parent in crosses. Being a Malawian variety it holds adaptational advantages to certain geographical and climatological areas of Malawi.

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In order to increase bean production, all factors that limit production need to receive careful evaluation. It becomes apparent, however, that not all factors can be dealt with at one time. In most cases, there is inadequate financial, institutional and personnel support to deal with all problems of production; at the same time such an approach may slow progress. This inevitably leads to an approach that is based on well defined priorities depending on what factors limit production more significantly and how best to solve them. In Malawi, bean diseases are the single most important factor limiting production and C. lindemuthianum is one of the most widespread and destructive among them. Control methods that involve the use of chemicals are less attractive because of the expense involved as well as their associated environmental problems.

The use of disease-free seed and other cultural practices, though helpful, have a prospect of limited success because farmers keep their own seeds and they may not follow sanitary practices. The ultimate solution to controlling this seed-borne disease is to grow resistant varieties. This study has identified some sources of resistance in the landraces. The few resistant landrace lines offer some opportunities in breeding for resistance. The results of screening of

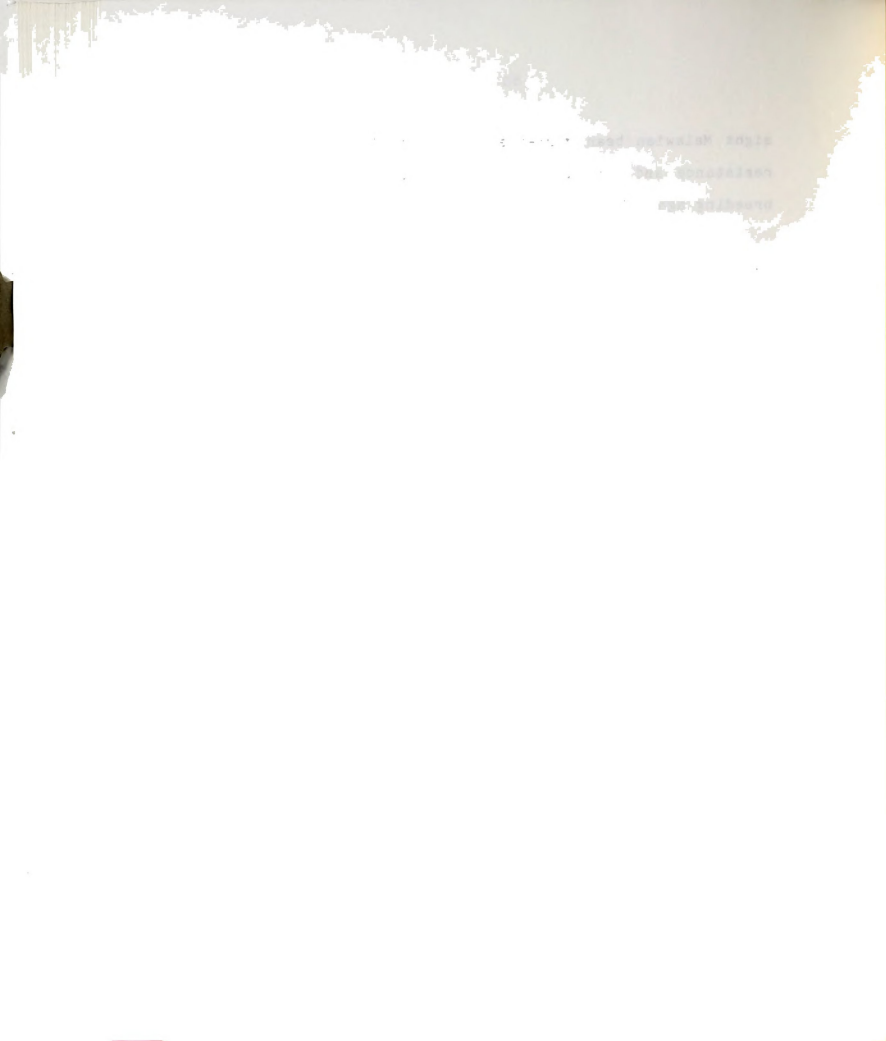
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eight Malawian bean varieties show additional sources of resistance and comprise another useful focal point for breeding against the disease.

This study has also reinforced the existence of pathogenic variability of C. lindemuthianum in Malawi. This means that any proposed breeding program should aim at producing varieties that offer broad resistance. Some of the differentials have shown such resistance. Cornell 49-242, Evolutie and ABl36 were resistant to all the tested isolates. The use of any variety will, however, depend on other acceptable characteristics. It has to be agronomically suitable and acceptable to the consumer.

Obviously these resistant sources differ in their performance for various traits. Those that excel in most of the desired traits will have to be selected for further evaluation, selection and/or hybridization. For instance, the variety Montcalm, which offers resistance to two of the Malawian isolate and is reportedly resistant to some Malawian halo blight pathotypes, may be selected over Cornell 49-242 on account of its seed coat color (red), since consumers generally prefer large red to small black seeds.

Consideration should also be given to how much improvement a given resistant variety needs. For instance, an existing variety may be chosen over a land-



race line on the basis that a landrace line may require more improvements in other traits before becoming useful as a variety even though both might be resistant to bean anthracnose.

The breeding strategy will be based on selection and hybridization. The selection step is now possible because this study has identified landrace lines and varieties that offer good sources of resistance to bean anthracnose. The foreign varieties will have to be grown under Malawian conditions in order to determine their agronomic performance in that environment. Further selections will then need to be made. In some cases, some selections may perform well enough to be released as varieties. In situations where no single variety performs well in all major traits, crosses will have to be made to combine the desired traits and develop superior recombinants through selection.

The presence of pathogenic variation in the isolates tested suggests that screening for races of anthracnose will have to be a continuous process. These isolates came from just a few of the bean growing areas and it would not be surprising to find additional variation in some of the areas where no isolates were collected and even within those from where the current tested isolates came. The search for resistance sources will have to be an ongoing process.

The ultimate success of any bean improvement program in Malawi will depend on an understanding of the genetic, agronomic and socio-cultural factors that limit bean production. This is a complex phenomenon and will require continued research in order to reach a synthesis of understanding.

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LITERATURE CITED



LITERATURE CITED

1. Albersheim, P. and Valent, B. S. (1978). Host pathogen interactions in plants. J. Cell Biology 78:627-643.
2. Anderson, A. J. and Albersheim, P. (1972). Host Pathogen Interactions. Comparison of the abilities of problems isolated from three varieties of *Phaseolus vulgaris* to inhibit the endopolygalacturonases secreted by three races of Colletotrichum lindemuthianum. Physiological Plant Pathology 2:339-346.
3. Andebrhan, T. and Wood, R. K. S. (1980). The effect of ultraviolet radiation on the reaction of *Phaseolus vulgaris* to species of Colletotrichum. Physiological Plant Pathology 17:105-110.
4. Andrus, L. F. and Wade, B. L. (1942). The factorial interpretation of anthracnose resistance in beans. USDA Agric. Tech. Bul. 810, 29 pp.
5. Ayonoadu, U. W. U. (1974). Races of bean anthracnose in Malawi. Turrialba 24:311-314.
6. Bailey, J. A. (1974). The relationship between symptom expression and phytoalexin concentration in hypocotyls of *Phaseolus vulgaris* infected with Colletotrichum lindemuthianum. Physiological Plant Pathology 4:477-488.
7. Bailey, J. A., Rowell, P. M., and Arnold, G. M. (1980). The temporal relationship between host cell death, phytoalexin accumulation and fungal inhibition during hypersensitive reactions of *Phaseolus vulgaris* to Colletotrichum lindemuthianum. Physiological Plant Pathology 17:329-339.
8. Bailey, J. A. and Rowell P. M. (1980). Viability of Colletotrichum lindemuthianum in hypersensitive cells of *Phaseolus vulgaris*. Physiological Plant Pathology 17:341-345.
9. Bailey, J. A., Mansfield, J. W., eds. (1982). Phytoalexins. New York, Halsted Wiley.

10. Barrus, M. F. (1911). Variation of varieties of beans in their susceptibility to anthracnose. *Phytopathology* 1:190-195.
11. Barrus, M. F. (1915). An anthracnose--resistant red kidney bean. *Phytopathology* 5:303-311.
12. Berard, D. F., Kuč, J. and Williams E. B. (1973). Relationship of genes for resistance to protection by diffusates from incompatible interactions of Phaseolus vulgaris with Colletotrichum lindemuthianum. *Physiological Plant Pathology* 3:51-56.
13. Blondel, A. (1962). L'anthracnose du haricot -- Etude des races physiologique francais du Colletotrichum lindemuthianum. Thesis. Fac. Sci. Paris, 160 pp.
14. Burkholder, W. H. (1918). The production of anthracnose--resistant white marrow bean. *Phytopathology* 8:353-359.
15. Burkholder, W. H. (1923). The gamma strain of Colletotrichum lindemuthianum (Sacc. et Magn.) B. et. C. *Phytopathology* 13:316-323.
16. Butler (Sir), E. J., and Jones, S. G. (1949). *Plant Pathology*, pg. 603, MacMillan & Co., Ltd., London.
17. Cardenas, F., Adams, M. W. and Anderson, A. (1964). The genetic system for reaction of field bean (Phaseolus vulgaris L.) to infection by three physiological races of Colletotrichum lindemuthianum. *Euphytica* 13:178-186.
18. Chaves, G. (1980). Anthracnose. In: H. F. Schwartz and H. F. Galves (Eds.), *Bean Production Problems: Disease, insect, soil and climatic constraints of Phaseolus vulgaris*. Centro Internacional de Agricultura Tropical, series 09EB-1, Cali, Colombia. pp. 22-35.
19. CIAT 1981. Germplasm screening and genetic improvement in: Bean Program 180 Ann. Rept. Centro Internacional de Agricultura Tropical, Cali Colombia, pp. 9-39.

10. *Barbus*
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11. *Barbus*
barbus

20. Correa, F. J. (1984). Angular leaf spot (Isariopsis griseola Sacc.) of red kidney beans in Michigan. M.S. Thesis. Michigan State University, 82 pp.
21. Darvill, G. A., and Albersheim, P. (1984). Phytoalexins and their elicitors--a defense against microbial infection in plants. Ann Rev. Plant Physiology. 35:243-275.
22. Commonwealth Mycological Institute. (1985). Distribution maps of plant diseases. Map No. 177 Edition 5.
23. Dey, P. K. (1919). Studies in the physiology of parasitism. Infection by Colletotrichum lindemuthianum. Ann. Past. 33:305-312.
24. Dixon, R. A., Dey, P. M., Lawton, M. A. and Lamb, C. S. (1983). Phytoalexin induction in French bean. Intercellular transmission of elicitation in cell suspension cultures and hypocotyl reactions of Phaseolus vulgaris. Plant Physiology 71:251-256.
25. Edgerton, C. W. (1915). Effects on temperature on Glomerella. Phytopathology 5:247-259.
26. Edge, O. T. and Mughgho, L. K. (1974). Agronomy experiments on Phaseolus beans. Lilongwe, University of Malawi, Bunda College of Agriculture. Research Bulletin No. 5 pp. 69-97. In: Lopez S. J. (1983). Bibliography on bean research in Africa Cali, Colombia. Centro Internacional de Agricultura Tropical. 177 p.
27. Edge, O. T., Mughgho, L. K., Rao, Y. P. and Msuku, W. A. B. (1981). Bean production in Malawi. In: Potential for field beans in Eastern Africa: Proceedings of a regional workshop held in Lilongwe, Malawi, 9-14 March 1980. Centro Internacional de Agricultura Tropical, Series 03EB-1, Cali, Colombia, pp. 54-97.
28. English, D. P., Jurale, J. B. and Albersheim, P. (1971). Host pathogen interaction. Parameters affecting polysaccharide--degrading enzyme secretion by Colletotrichum lindemuthianum grown in culture. Plant Physiology 47:1-6.

50. Correo,
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29. Elliston, J. E., Kuč, J. and Williams, E. B. (1971). Induced resistance to bean anthracnose at a distance from the site of the inducing interaction. *Phytopathology* 61:1110-1112.
30. Fouilloux, G. (1975). L'antracnose du haricot: Etude des relations entre les pathotypes anciens et nouveaux. Etude de nouvelles sources de résistance totale. In: Reunion Eucarpia Haricot. Versailles, France, Centre National de Recherches Agronomiques. pp. 81-92.
31. Fouilloux, G. (1976). Bean anthracnose: New genes of resistance. *Ann. Rept. Bean Improv. Coop.* 19:36-37.
32. Fouilloux, G. (1979). New races of bean anthracnose and consequences on our breeding programs. In: International symposium on diseases of Tropical food crops, Louvain-la-Neuve, Belgium, August, 1978. pp. 221-235.
33. Goth, R. W. and Zaumeyer, W. J. (1965). Reaction of bean varieties to four races of anthracnose. *Plant Dis. Repr.* 49:815-818.
34. Hargreaves, J. A., and Bailey, J. A. (1978). Phytoalexin production by hypocotyls of Phaseolus vulgaris in response to constitutive metabolites released by damaged bean cells. *Physiological Plant Pathology* 13:89-100.
35. Hargreaves, J. A. (1981). Accumulation of Phytoalexins in cotyledons of French bean (Phaseolus vulgaris L.) following treatment with Torton (2-octylphenyl polyethoxyethanol) surfactants. *The New Phytologist* 87:733-741.
36. Hubbeling, N. (1978). Selection for resistance to anthracnose. Particularly in respect to the "Ebnet" race of Colletotrichum lindemuthianum. *Ann. Rept. Bean Improv. Coop.* 19:49-50.
37. Hubbeling, N. (1977). The new jota race of Colletotrichum lindemuthianum. *Ann. Rept. Bean Improv. Coop.* 20:58.

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38. Hwang, S. W., Anderson, G. C., Papavizas and Converse, R. H. (1968). Cryo-preservation and pathogenicity of selected isolates of Colletotrichum lindemuthianum, Rhizoctonia and Phytophthora. Phytopathology 58:362-364.
39. Kelly, J. D. (1981). New sources of resistance to the delta race of anthracnose. Ann Rept. Bean Improv. Coop. 24:47-48.
40. Kimati, H. and Galli, F. (1970). Glomerella cingulata (Stonem) Spauld. et. v. Schrenk. f. sp. Phaseoli n.f. fase ascogena do agente causal de anthracnose do feijoeiro. Anais da Escola Superior de Agricultura "Luis de Queiroz," 27:411-437.
41. Kinyua, G. K. (1979). The occurrence of race alpha of Colletotrichum lindemuthianum in Kisii District Kenya. Thika, Kenya, National Horticultural Research Station, 3 p. In: Jorge, L. S. 1983. Bibliography on bean research in Africa, Cali, Colombia. Centro Internacional de Agricultura Tropical. p. 177.
42. Kruger, J., Hoffman, G. M., and Hubbeling, N. (1977). The kappa race of Colletotrichum lindemuthianum and sources of resistance to anthracnose in Phaseolus beans. Euphytica 26:23-26.
43. Lauritzen, J. I. (1919). The relation of temperature and humidity to infection by certain fungi. Phytopathology 9:9-35.
44. Leakey, C. L. A., and Simbwa-Bunnya, M. (1972). Races of Colletotrichum lindemuthianum and implications for bean breeding in Uganda. Ann. App. Biol. 70:25-35.
45. Mastenbroek, C. (1960). A breeding programme for resistance to anthracnose in dry shell haricot beans, based on a new gene. Euphytica 9:177-185.
46. McRostie, G. P. (1919). Inheritance of anthracnose resistance as indicated by a cross between a resistant and a susceptible. Phytopathology 9:141-148.
47. McRostie, G. P. (1921). Inheritance of disease resistance in the common bean. J. Amer. Soc. Agron. 13:15-32.

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48. Mather, R. S., Barnett, H. L., and Lilly, V. G. (1950). Sporulation of Colletotrichum lindemuthianum in culture. Phytopathology 40:104-114.
49. Msuku, A. B. (1984). Pathologic Variation in Malawian isolates of Pseudomonas syringae pv. phaseolicola (Burk.) Young, Dye, and Wilkie, and implications for breeding disease resistant beans. Ph.D. Thesis. Michigan State University, 90 pp.
50. Muhalet, C. S., Adams, M. W., Saettler, A. W. and Ghaderi, A. (1981). Genetic system for the reaction of field bean to beta, gamma and delta races of Colletotrichum lindemuthianum. J. Amer. Soc. Hort. Sci. 106:106-604.
51. Peregrine, W. T. H. (1971). A Preliminary note on chemical control of bean anthracnose in Malawi. Pans 17(1):47-50. In: Jorge, L. S. (1983). Bibliography on bean research in Africa. Cali, Colombi. Centro Internacional de Agricultura Tropical. p. 177.
52. Rahe, J. E., Kuč, J., Chuang Chien-Mei and Williams, E. B. (1969). Induced resistance in Phaseolus vulgaris to bean anthracnose. Phytopathology 59:1641-1645.
53. Rahe, J. E. and Kuč, J. (1970). Metabolic nature of the infection limiting effect of heat on bean anthracnose. Phytopathology 60:1005-1009.
54. Rahe, J. E. (1972). Phytoalexin nature of heat-induced production against bean anthracnose. Phytopathology 63:572-577.
55. Rathmell, W. G. (1973). Phenolic compounds and phenylalanine ammonia lyase activity in relation to phytoalexin biosynthesis in infected hypocotyls of Phaseolus vulgaris. Physiological Plant Pathology 3:259-267.
56. Saettler, A. W. (1982). Bean diseases and their control. In: Dry bean production--Principles and practices. Extension Bulletin E-1251, Michigan State University.

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57. Schnock, M. G., Hoffman, G. M., and Kruger. (1975). A new physiological strain of Colletotrichum lindemuthianum infecting Phaseolus vulgaris L. Hort. Sci. 10:140.
58. Schwartz, H. F., Pastur Corrales, M. A. and Singh, S. P. (1982). New sources of resistance to anthracnose and angular leaf spot of beans (Phaseolus vulgaris L.) Euphytica 31:741-754.
59. Shao, F. M. (1984). Progress report on bean pathology work at UAC, Mbeya--Research trials conducted during the 1982-3 season. Bean Newsletter for Eastern Africa 2:14-16.
60. Skipp, R. A. and Daverall, B. J. (1973). Studies on cross-protection in the anthracnose disease of bean. Physiological Plant Pathology 3:299-313.
61. Skipp, R. S., Selby, C. and Bailey, J. A. (1977). Toxic effects of phaseollin on plant cells. Physiological Plant Pathology 10:221-227.
62. Tu, J. C. (1981). Anthracnose (Colletotrichum lindemuthianum) on white bean (Phaseolus vulgaris L.) in Southern Ontario: Spread of the disease from an infection focus. Plant Disease 65:477-480.
63. Tu, J. C. (1983). Epidemiology of anthracnose caused by Colletotrichum lindemuthianum on white bean (Phaseolus vulgaris) in Southern Ontario: Survival of the pathogen. Plant Disease 67:402-404.
64. Van Rheenen, H. A., Hasselbach, O. E., and Muigai, S. G. S. (1981). The effect of growing beans together with maize on the incidence of bean diseases and pests. Neth. J. Pl. Path. 87:193-199.
65. Walker, J. C. (1950). Plant Pathology. McGraw-Hill Book Co., Inc., New York, pp. 699.
66. Whitehead, I. M., Dey, P. M., and Dixon, R. A. (1982). Differential patterns of phytoalexin accumulation and enzyme induction in wounded and elicitor-treated tissues of Phaseolus vulgaris. Planta 154:156-164.

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67. Zaumeyer, W. J. and Thomas, H. R. (1957). A monographic study of bean disease, and method for their control. U.S.D.A. Agric. Tech. Bull. No. 868.

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