RACE IDENTIFICATION AND DISTRIBUTION OF BEAN ANTHRACNOSE (*COLLETOTRICHUM LINDEMUTHIANUM)* IN MAJOR BEAN GROWING AREAS OF ZAMBIA

BY

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DECLARATION

I, MATHIAS ZULU, declare that the dissertation represents my own work and that it has not previously been submitted for a degree at this or any other university.

Signature----------

Date---------- 24.05.2005

APPROVAL

This dissertation of Mr. MATHIAS ZULU is approved as fulfilling part of the requirements for the award of the degree of Master of Science in Agronomy (Crop Science) of the University of Zambia.

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ABSTRACT

A study to determine the distribution and relative importance of bean anthracnose (*Colletotrichum lindemuthianum*) and to identify and characterize races of this fungus in the major bean production areas of Northern, Luapula and Northwestern provinces of Zambia was conducted in 2004. The study was conducted in three stages that involved a field survey and two experiments, one in the field and the other in the laboratory. The objective of the study was to gain information on the type of anthracnose races prevalent in Zambia and magnitude of their virulence in order to develop appropriate and sustainable control strategies by way of breeding for resistant varieties for adoption by resource-poor farmers. The survey was employed to collect disease samples and determine the severity and incidence in Kasama, Mbala, Mpika, Samfya, Mansa, Solwezi and Mwinilunga districts. Anthracnose fungus was found distributed in all major growing areas with medium to high severity in 76 percent of the 90 fields surveyed. Angular leaf spot, common bacterial blight and rust were the other diseases found distributed in the target areas in descending order of frequency. Anthracnose was most severe in Mwinilunga while incidence was highest in both Mbala and Mwinilunga districts. All local landraces in the target area were susceptible to anthracnose attack. The work identified 14 different races of *Colletotrichum lindemuthianum* based on the 12 CIAT standard differential cultivars from 22 isolates collected from different production areas. Race determination results confirmed that there was great variability of anthracnose fungus in Zambia. Physiological races in Mansa and Mwinilunga showed closer similarities among themselves while race 65 and 73 resembled those characterised in North America. The majority of the races attacked cultivars of Andean origin though some race-specific resistance was found in genotypes from both centres of *Phaseolus vulgaris* origin. Exotic accessions Tu, AB 136 and G 2333 were resistant to all races of anthracnose characterized in the study both in the field and laboratory experiments.
Eighteen genotypes tested in the field revealed that anthracnose attack was significantly higher ($P \leq 0.05$) at flowering and podding stages of bean development at Mutanda Research Station ($12^{o}25.88$ S and $26^{o}12.59$ E). The study provided some essential information needed to develop effective breeding and/or crossing programs against bean anthracnose fungus that exhibit high pathogenic variation. It is therefore imperative that gene deployment and pyramiding are employed together with other available methods as sustainable control strategies in order to minimize losses inflicted on the bean crop.

DEDICATION

To my lovely wife Francisca and my children Masauso, Kochiwe and Thandiwe.

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CHAPTER 1

1.0 INTRODUCTION

1.1 GENERAL

Bean (*Phaseolus vulgaris L.)*, popularly known as common bean, is a member of the family *Leguminosae*, tribe *Phaseoloeae* and subfamily *Papelionoideae.* Beans have two centres of origin that include the Middle American and Andean zones where the crop has been cultivated in association with maize for many centuries (Beebe et al., 2000). The Middle American centre of origin has given rise to the small to medium seeded cultivars while the large seeded types have risen from the Andean centre of origin in South America. The two groups of cultivars from the two gene pools have contrasting agronomic, morphological and biochemical characteristics. This may suggest that even pathogens associated with these gene pools such as anthracnose may exhibit similar patterns of their genetic diversity into these gene pools (CIAT, 1992).

The Portuguese traders introduced beans into Africa probably during the past 200-300 years ago through the East African coast (Mulila, 1995). Since then, Africa has become the second most important bean-producing continent in the tropics (Allen et al., 1996) after Latin America where Brazil is the largest bean producing country in the tropical zones.

Bean is a major staple in the Southern African region where it is the second most important source of dietary protein and the third most important source of calories

(Wortmann et al., 1998). Beans are cooked and eaten as dry beans, immature green pods or fresh seeds fried in cooking oil. Beans are also used in making soups that are canned and frozen for export market. The leaves are also locally eaten as vegetables while the haulms are used as forage (Purseglove, 1968).

Bean seeds have a high nutritive value constituting 20-30 percent of protein and 50-60 percent carbohydrate. Beans are also rich in iron, folic acid and other essential minerals such as calcium and magnesium and vitamins A and B in green pods. This nutritionally 'near perfect food' quality is very important for Africa where meat based dietary protein is largely unaffordable and protein deficiency and malnutrition plague millions of people-particularly children. It is estimated that more than 3.741 million hectares of beans are cultivated in Africa by both subsistence and commercial farmers. The small-scale farmers are mostly women (Wortmann et al., 1998). In Zambia, common bean is the most important food legume crop to people of all income groups both in rural and urban areas. Bean is a relatively inexpensive source of dietary protein and also an important source of cash income.

1.2 BEAN PRODUCTION CONSTRAINTS

The principal agronomic constraints of bean production in Zambia include soil infertility, drought, insect pests and diseases. Due to these biotic and abiotic constraints, bean average yields for small-scale farmers are extremely low ranging between 300-600kg/ha. Researchers in sharp contrast to these low yields obtain 2-3

t/ha. Mbewe et al., (1991) has reported that the main cause for the low yields farmers are obtaining is due to diseases including anthracnose.

Anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus) Brios & Cav. is distributed worldwide. This disease often causes severe damage to the bean crop (Purseglove, 1968; Allen, 1991 and CIAT, 1992). In the tropical and subtropical zones under cool, wet and humid conditions, yield losses approach 100 percent especially where contaminated seed is used (Allen, 1991). Anthracnose is considered the most important disease of common bean in Malawi, Rwanda, Uganda, Tanzania and Zambia (Kayitare, 1987). According to regional priorities among bean production constraints in the Southern African Development Community (SADC), bean anthracnose ranks among ten most important constraints in bean production. In Zambia, anthracnose is considered important because the disease affects many farmers and it appears at least once in two seasons causing severe bean losses (Allen, 1991). It is clear that the disease lowers the productivity of beans and is therefore a major hindrance to improved health, prosperity and economic stability of rural people (Bailey, 1997) not only in the SADC but wherever it occurs.

1.3 MAJOR DISEASES OF BEANS IN ZAMBIA AND THEIR CAUSES

The common bean plant is host to a range of fungal, viral and bacterial diseases most of which cause severe losses by reducing quality and yields drastically when

conditions for their development are favourable. Some fungal diseases affect roots and stems while others affect foliage and pods. Diseases that affect roots generally cause root rots.

Apart from anthracnose (*Colletotrichum lindemuthianum*), other important foliage and pod fungal diseases are angular leaf spot (*Phaeoisariopsis griseola*), ascochyta blight (*Phoma exigua var. diversipora)*, Scab (*Elsinoe phaseoli*) and Rust (*Uromyces appendiculatus*). Diseases are estimated to reduce bean yields in Zambia by between 25-50 percent (Greenberg et al., 1986), as farmers cannot afford chemicals for disease control.

1.3.1 Bean Common Mosaic Virus (BCMV)

BCMV is the most important viral disease in the warm medium rainfall regions of Zambia covering Eastern, Central and Lusaka provinces. Aphids act as vectors for BCMV.

1.3.2 Common Bacteria Blight (CBB)

Common Bacteria Blight (CBB) (*Xanthomonas campestris pv. phaseoli*) is another important disease caused by bacteria occurring in the same area as BCMV.

1.3.3 Bean anthracnose (*Colletotrichum lindemuthianum*)

Anthracnose caused by numerous races of *Colletotrichum lindemuthianum* constitutes one of the most important biotic constraints to bean production and improvement in Zambia causing poor seed germination, reductions in seed size, yield, food quality and market value. The disease affects all vegetative parts of beans including seeds and usually appears on the crop four to six weeks after sowing (Kayitare, 1987; Msuku et al., 2000). Anthracnose is important and prevalent in the cooler, wetter areas of Northern, North-western and Luapula provinces where most of the beans are produced (Mbewe et al., 1991).

Kannaiyan, et al., 1987, reported estimated yield reductions of 21-321kg/ha of bean due to anthracnose. The bean grain that is sold on the market as 'seed' in Zambia has been reported to contain five percent anthracnose infection (Greenberg et al., 1986). Yield losses associated with high, moderate and low ratings for relative importance of anthracnose occurrence in bean production areas are 200, 100 and 25kg/ha respectively (Wortmann et al., 1998).

1.4 BEAN ANTHRACNOSE PATHOGENIC RACES

The fungal pathogen that causes bean anthracnose shows high levels of inherited variability as many new forms evolve over time and space. The physiological races within the genus *Colletotrichum* differ from each other primarily in their

pathogenicity. However, secondary differences may also occur in their biochemical and cultural characteristics (Ogallo, 1991). Many new races of bean anthracnose continuously arise through recombination of sexual genes during reproduction, exchange of genetic materials in somatic cells, mutations or by extra chromosomal variation (Singh, 1986). As a result of this, new races are able to infect previously resistant varieties of beans in one area while in another area the same varieties may remain resistant if exposed to different races. Some races also attack many varieties while others attack only a limited number. This poses serious limitations in breeding for bean anthracnose resistance especially where these races exist but have not yet been identified. The races that occur in the major bean growing areas of Zambia have not been documented yet.

Breeding for resistance to bean anthracnose is one of the most logical and sustainable control strategies for resource poor farmers in the tropics. However, the high level of variability exhibited by the anthracnose pathogen has seriously hampered breeding progress (CIAT, 1992). The high level of virulence diversity explains the existence of a large number of pathotypes and the consequent breakdown of host resistance in time and space. The diversity could in part be explained by plant and pathogen co-evolution as evidenced by the two centres of origin mentioned earlier.

The occurrence of races in bean anthracnose fungus is of practical value and has direct implications in breeding for anthracnose resistance, thus justifying the need

for the current research. Additionally, there are no resistance genes that are effective against all known races of this pathogen (Mahuku et al., 2002). This study was aimed at establishing races of bean anthracnose fungus and determining the severity and the incidence of the disease in Zambia. The information gathered should form a basis for integrated management control system for anthracnose. The specific objective of the study was to investigate races of the anthracnose fungus present in Zambia in order to provide a better understanding of anthracnose distribution, incidence, severity and variability of the pathogen for application of breeding for resistance as a successful control strategy in the long run.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 GEOGRAPHICAL DISTRIBUTION AND IMPORTANCE OF BEAN ANTHRACNOSE

2.1.1 Names and taxonomy

The preferred scientific name for the bean anthracnose fungus is *Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi & Cavara [teleomorph]. The causal pathogen of bean anthracnose belongs to the genus *Colletotrichum,* classified under *Deuteromycetes*; form order; *Melanconiales*; family *Melanconiaceae* and section *Hyalosporae*.The genus is characterised by production of acervuli, which are disc-shaped or cushion-shaped, waxy sub-epidermal structures with dark setae at edges or among conidiophores.

The genus *Colletotrichum* is important because most of its species including *Colletotrichum lindemuthianum* possess a very high degree of pathogenic variability in different parts of the world causing major limitations for anthracnose control (Bailey, 1997). Many physiological races occur and over seventeen race groups have been documented so far (Gathuru, 1991). The high level of pathogen diversity in *Colletotrichum lindemuthianum* may be a result of chromosome losses and

duplications as well as gene exchange with similar pathogen species hosted by different crops (Bailey, 1997).

The common names include anthracnose of bean, anthracnose of legumes, pod canker of bean, bean anthracnose and pea anthracnose.

2.1.2 Geographical distribution of bean anthracnose

Bean anthracnose is one of the most widely distributed diseases of common bean (*Phaseolus vulgaris*) and can be devastating when climatic conditions favour the pathogen in the tropical and subtropical areas of the world (Pastor-Corrales and Tu, 1989; Mahuku et al., 2002). The disease has worldwide distribution and occurs wherever bean is grown especially in cool, frequently wet and humid regions (Schwartz, 1980; Pastor-Corrales and Tu, 1989). It is present in Africa, Asia, Australia, and Europe, Latin America and North America as well as the Oceania region. In Africa its presence has so far been recorded in Burundi, Congo Democratic Republic, Ethiopia, Kenya, Libya, Madagascar, Malawi, Mali, Mauritius, Morocco, Mozambique, Nigeria, Rwanda, Senegal, South Africa, Tanzania, Uganda, Zambia and Zimbabwe.

In Zambia, anthracnose outbreaks were first recorded in 1968 (Anon, 1970) and were locally destructive in the Copperbelt Province. Currently anthracnose occurs mostly in the Northern half of the country, which includes the Northern, Copperbelt,

Northwestern, and Luapula provinces and has since increased in incidence, severity and distribution (Greenberg et al., 1986).

2.1.3 Importance of bean anthracnose

Anthracnose is an important fungal pathogen of *Phaseolus vulgaris* causing greater losses in temperate and subtropical zones than in the tropics. It has caused economic losses in North, Central and South America, Europe, Africa, Australia and Asia (Chaves, 1980).

Yield losses of 95 percent have been recorded in Colombia and over 92 percent in Malawi (Allen, 1983). Anthracnose was once considered the most important disease in the bean-producing areas of eastern USA where losses amounting to $1.5 million were reported in Michigan in 1914. The arrival of the gamma and delta races of the pathogen in Canada between 1977 and 1978 caused severe damage to beans (Tu, 1988) until clean seed and resistant varieties were used.

In Latin America, *Colletotrichum lindemuthianum* has been reported by several workers (Schwarz and Galvez, 1980) to cause severe damage in Brazil, Argentina, Mexico, Guatemala, Peru, Ecuador and Colombia. In Eastern Africa, bean anthracnose is important in Kenya, Uganda and Tanzania. It is recurrent in the Great Lakes Region of Rwanda, Burundi and the Kivu Province of Congo Democratic Republic (CIAT, 1981). In neighbouring Tanzania, yield losses of 40-80 percent

amounting to US $304 million per year have been attributed to bean anthracnose (Bailey, 1997).

In Southern Africa, bean anthracnose is important in Malawi, Mozambique, South Africa, Zimbabwe and Zambia. In this region beans is mostly grown by resource poor small-scale farmers. These farmers use their own recycled seed and rarely apply sound cultural practices. Since they do not practice chemical control of the disease, the practice of using recycled seed has contributed to the spread of the disease, as the disease is seed borne. Yield losses are reported to increase with early plant infection (CIAT, 1976).

2.1.4 Bean anthracnose race identification

There exists a wide pathogenic diversity in races of bean anthracnose in nature to which various bean cultivars differ in their reaction to infection (Mahuku et al., 2002). Bean anthracnose race identification is based on a recommended set of differential cultivars that vary in their genes for either resistance or susceptibility to one or more races of the fungal pathogen. The standard differential varieties recommended by Centro Internacional de Agricutura Tropical (CIAT) are 12, namely: Michelite, Michigan dark red kidney (MDRK), Perry marrow, Cornell 49242, Widusa, Kaboon, Mexico 222, PI 207262, TO, TU, AB 136 and G 2333 (Beshir, 1991; Ogallo, 1991).

The differential cultivars used need to be genetically pure to avoid disease reactions being confounded by mixed gene effects. Out crossing results in recombination, while mutations or varying environmental conditions such as temperature, humidity and moisture would all have different influences on reaction of host plants as well as on the pathogenicity of the pathogens themselves (Ogallo, 1991). The traditional process of identifying physiological races of pathogens involves inoculation of isolates on the differentials and comparing the resultant disease reactions on each differential cultivar with those of already known races. Matching reactions with those of already known races readily identify the races on the Greek alphabet nomenclature system, otherwise the race is considered new if it does not match at all.

The Binary System of nomenclature recommended by CIAT uses binary number 2n, where n is equivalent to the place of the differential cultivars within the series in the order from Michelite to G 2333 for the standard differentials. The CIAT process of identifying physiological races of pathogens involves inoculation of isolates on the differentials and uses the sum of standard cultivars with susceptible reaction to give the binary number of a specific race. According to Buruchara, (1991) this method has several advantages over the traditional one in that it:

(i) Allows for easy comparison of races from one place to another or from season to season.

(iii) Allows one to know whether the race is broadly pathogenic or not. The larger the binary number the broader the pathogenicity of the race.

In Ethiopia, race characterisation revealed that 15 races of the anthracnose pathogen were identified from different locations (Beshir, 2003) while more than 72 pathotypes (Bailey, 1997), were recorded in Tanzania. In another race characterisation a total number of 40 pathotypes were characterised and none of the isolates attacked AB 136 nor G 2333 while only 2.5 and 9.7 percent could attack Tu and To respectively. Most isolates were reported to attack the Andean cultivars in Tanzania (Mwalyego, 1991).

In Mexico, analysis of 59 isolates from different regions yielded 10 distinct races (Melotto et al, 2000) that were associated with host variety as well as cultivation system and also geographical location. In Himachal Pradesh, India, Sharma et al. (1999) reported having characterised 19 local races from 85 isolates none of which attacked AB 136 and G 2333 differentials. These have also been reported resistant to all European and American isolates though the latter is susceptible to races 3481, 3545, 3977 and 3933 from Costa Rica, Mexico, and Argentina (Mahuku et al., 2002). In Brazil, 25 races were identified and characterised from various regions out of which none attacked AB 136 and G 2333 differentials but broadly attacked both Andean and Middle American cultivars (Anon, 1999).

Although there is no genetic resistance that is effective against all known races, pyramiding or incorporating several resistance genes into one single line should provide durable and stable resistance. G 2333 for instance, has three resistance genes (*Co-4*, *Co-5* and *Co-7*) that may explain its resistance to the majority of characterised races world over (Mahuku et al., 2002).

2.1.5 Inoculation methods

Successful infection by *Colletotrichum lindemuthianum* in bean depends on favourable environmental conditions, the presence of susceptible varieties, pathogenicity and the concentration of inoculum (Tu, 1982). Temperature and moisture are the most important factors for infection in addition to high humidity (greater than 92 percent). In the process of identifying different races of *Colletotrichum lindemuthianum* and also developing bean varieties resistant to this fungus, conditions that allow successful infection of the bean plant i.e. inoculation has to be provided. Hence the success of identifying and developing resistant varieties and anthracnose race identification depends on the use of reliable screening techniques (Tu, 1985). Gasana, (1991) reviewed methods for use in both the field and green house. He recommended three methods of inoculation to induce disease reaction for the purpose of screening for resistance to the disease in beans.

i.) Spraying plants with inoculum.

ii.) Dipping newly germinated bean seeds in spore suspensions and

iii.) Brushing the underside of bean leaves with inoculum.

Tu, (1985) reported the brushing method was more precise in differentiating susceptible backcross progenies than either spraying or dipping.

Recently, detached leaf method has been employed successfully for development of diseases on detached single primary or trifoliate leaves, thus making simultaneous inoculations of different pathogens on a single cultivar possible. This is also an ideal method when differential cultivar seeds are limited and plants are required to set clean seed. Tu, (1985) reported that infection through spraying was variable most probably due to beading-up and running off of spore suspension due to surface tension of epicuticular wax.

2.2 BEAN ANTHRACNOSE SYMPTOMS

The disease symptoms caused by different races of pathogen are basically identical. Leaf symptoms appear initially on the lower surface as brick red to purplish-red discolorations along the veins. With time, such discoloration develop on the upper leaf surface and simultaneously brown lesions of different sizes bearing brown, black, or even purplish margins develop around small veins. On larger leaf veins these lesions expand into sunken cankers within which acervuli bearing conidia are produced. Characteristically, leaves become dark-brittle, rugged and reduced in size. Lesions also commonly develop on cotyledons, petioles, branches, stems and pods. Pod lesions are typically sunken and contain masses of salmon-pink conidia, which

are mostly oblong or cigar-shaped. Dark brown eyespots developing longitudinally along stems are early signs of stem infection. Seeds within diseased pods also become infected and seedlings that develop from infected seed show severe symptoms with increased yield losses (Allen, 1991). Symptoms of seed infection are the appearance of rusty brown spots with small brown specks on pods. Seeds acquired from seriously infected pods generally reveal brown to light chocolate spots on the seed coats. These lesions could well extend into the cotyledons on heavily infected seeds (Tu, 1988).

2.3 BEAN ANTHRACNOSE DISEASE SPREAD

The primary infection of bean anthracnose comes from the fungus present in the mature, dry and viable seed on which the fungus survives (Tu, 1983). In most parts of Africa, a common feature of bean production is through the informal seed exchange by farmers both within and between countries (Bailey, 1997). Where seed sorting to remove infected seeds is absent or poor there is a possible risk of outbreak even in areas previously known to be free of anthracnose due to use of infected seed.

Dry crop debris, and not wet or buried straw, provides secondary spread. After initial infection, free water or frequent rain, is required to dissolve the water-soluble gelatinous matrix associated with *Colletotrichum lindemuthianum* spore mass in the acervuli. High to moderate rainfall at frequent intervals, particularly when accompanied by rainstorm, wind or splashing rain, are reported essential for local

dispersal of conidia and for progression of severe anthracnose epidemics (Zaumeyer and Thomas, 1957). According to Tu, (1983) long-distance disease spreading of between 3-5 m may result from splashing raindrops blown by gusting winds in tropical storms in the same field. One diseased plant is able to effectively spread the disease to other plants up to a radius of 30 m (Tu, 1982).

The rate of anthracnose infection and development has been found to depend on the interaction between the plant, the pathogen and the environmental conditions (Tu, 1982). The pathogen is disseminated by rain splash, wind, and physical contact between plants and through seed. Plant age, intercropping, growing of varietal mixtures, temperature, wind direction, plant density and other cultural practices such as crop rotation have all been reported to influence disease spread. The infection and development of anthracnose is reported to be faster in young plant cells than older ones (CIAT, 1976). Although most young plants in susceptible varieties have been shown to be associated with higher disease severity and yield losses (Sindhan and Bose, 1981), some varieties are susceptible throughout their vegetative phase while others show increased disease severity with advancement in plant age.

Intercropping or growing bean varietal mixtures of different proportions of resistance genes or what are known as multilines have been found to limit the spread of anthracnose disease in the field (Mwalyego, 1991). Beshir and Pretorius (2003) reported that anthracnose disease incidence and severity consistently decreased in mixtures with more than 67 percent resistant varieties with regard to susceptible

varieties. Cool temperatures (17-24 °C) and high relative humidity or free moisture favour infection and spread (Chaves, 1980). Infection and development of the pathogen is delayed or prevented by temperatures outside the range of 7-28 °C (Conner et al., 2001). Anthracnose disease-free seed has been produced by use of irrigation under higher temperatures and less humid conditions in the arid tropics thereby greatly reducing disease incidence in the fields in the USA and Canada (Tu, 1988).

2.4 BEAN ANTHRACNOSE DISEASE MANAGEMENT AND CONTROL

Some potential solutions identified for control of bean anthracnose include increased access to germplasm through development of resistant cultivars, improvement of research resources and methods to improve screening, integrated disease management and access to clean seed to reduce losses (Bailey, 1997). Control measures include, regulatory, cultural, disease prevention, host resistance and physical and chemical control measures. Regulatory control usually involves quarantine of infected seed that carries the pathogen (Allen, 1983).

2.4.1 Cultural control

Cultural control aims at the reduction of initial inoculum and/or spread of secondary inoculum. Cultural control is easy to use by farmers. It involves use of anthracnose-clean seed, crop rotation, sanitation, varietal mixtures, and also use of ridges and weed management (Buruchara, 1991; Beshir and Pretorius 2003). Wherever it has

been possible to maintain strict phytosanitory standards, clean seed has been reported as one of the most effective control measures (Allen, 1983) and has been successfully used in Canada and the USA. Seed selection to remove infected seed has been reported to reduce anthracnose severity by 33 percent and increased yields by 17 percent in Tanzania (Mwalyego, 1991). Growing of varietal mixtures has been shown to buffer against diseases and stabilises bean yields (Mwalyego, 1991).

2.4.2 Disease prevention

Disease prevention strategies are effective in reducing the incidence of bean anthracnose. Development of integrated control systems to prevent disease includes several appropriate strategies including cultural practices such as optimum plant density and spacing. The architecture of the bean plant has a great bearing on anthracnose disease control. Upright and climbers on stakes suffer less from bean anthracnose, as leaves are placed high above the soil surface. Bean plants with leaves close to the ground are more prone to splashing water, which spreads the disease from plant to plant.

2.4.3 Chemical control

Chemical control depends on the use of fungicides either as seed treatment or foliar sprays. Thiram has been used effectively against seed coat infections while results of foliar sprays have been known to depend on the chemical used, timing and the frequency of application though successful control has been provided by Benomyl.

However, the use and effectiveness of chemical control has major limitations that include, environmental hazards, development of resistance and heavy expenses.

2.4.4 Control by plant resistance

Bean cultivars differ in their reaction to infection by *Colletotrichum lindemuthianum* while the pathogen also exhibits high pathogenic variability. Cross protection induced by a non-pathogenic race, low inoculum concentration of the pathogenic race and heat treatment (32-37°C) before inoculation are the other expressions of resistance that are not well understood (Chaves, 1980). Single, independent or dominant genes depending on the pathogen race may provide resistance, but conferment of resistance becomes more complex with increased number of genes involved (Haciwa, 1991).

However, there is need to address race non-specific sources to take care of the fungus that mutates or changes with natural selection and other mechanisms. Accessions possessing broad based resistance genes have been reported but control based on resistance alone can be expensive due to very high pathogen diversity (Bailey, 1997), especially in Africa where pure varieties are rarely grown as sole crops.

The study of pathogenic variation to improve understanding of resistance mechanisms would lead to increased resistance and productivity of existing

materials. The differential cultivars AB 136, G 2333, G 811 and G 2641 have been reported resistant to almost all known races so far (Schwartz, 1983).

2.4.5 Host-plant resistance

The most appropriate and practical control of bean anthracnose is by the use of resistant varieties. Several sources of resistance have been identified and used extensively in the USA, Canada, and Europe as well as in some African and Latin American countries (Mahuku et al., 2002). The major drawback to this strategy has been reported to be the possible breakdown of resistance caused by pathogen adaptation (Mahuku et al., 2002). Host plant resistance to anthracnose has largely depended on race specificity. Anthracnose pathogen fungus has been highly variable because of mutation, natural selection or other mechanism (Pastor-Corrales and Tu, 1989). According to Haciwa, (1991) the underlying resistance to *Colletotrichum lindemuthianum* is hypersensitivity. This however, is associated with increasing degrees of race specificity, which in turn increases selection pressure favouring virulent pathotypes that break resistance. This is the major drawback of host-plant resistance as a control strategy for bean anthracnose as it leads to the breakdown of resistance due to genetic changes in the pathogen through mutations (Mahuku et al., 2002).

There are about 17 different anthracnose race groups that have been identified by their differential virulence on a range of bean cultivars so far. However, of these

seven major races of the fungus are the most common (Buruchara, 1991). These include alpha, beta, gamma, delta, kappa, epsilon and lambda. Some bean cultivars may have resistance to one or more of theses races but very few are resistant to all the major races. Haciwa, 1991 stated that horizontal resistance to anthracnose could slow down the rate of mutation for pathogens, as it does not create selection pressure. The use of multilines could also play a significant role in bean anthracnose control as they reduce disease incidence because a proportion of total inoculum falls on resistant lines thereby reducing the effective dose. According to Beshir and Pretorius (2003), bean cultivar mixtures, each possessing different genes for resistance may control anthracnose in a similar way to multilines. Some differential cultivars possess host genes for resistance characterised from *Co-1* to *Co-8* with PI 207262, AB 136 and G 2333 having more than one gene each (Melotto et al., 2000). Of these genes only *Co-1* is of Andean origin. These multiple genes stand a higher chance to confer broad resistance to variable races of anthracnose.

2.5 MECHANISM OF RESISTANCE

Expression of resistance has been reported to be due to delay in appressoria penetration, cell necrosis and growth of mycelia within the dead host cells manifested as hypersensitivity (Bailey, 1982). The mechanism of resistance as a process involves compatible and incompatible interactions between *Colletotrichum lindemuthianum* and bean cultivars. It has been noted that following infection, polysaccharides from the cell walls of *C. lindemuthianum* elicit biochemical and physiological changes in host plants that result in accumulation of isoflovonoid

phytoalexins, deposition of wall bound phenolic compounds and synthesis of hydroxyproline-rich glycoproteins (HRGP) (Lawton and Lamb, 1987). The amount of HRGP produced has been known to be higher in incompatible than in compatible interactions (Esquirre-Tugaye et al., 1990).

The plant defence responses are elicitor mediated and arise from a rapid but transient induction of enzyme synthesis resulting from accumulation of m-RNAs due to activation of plant defence genes (Lawton and Lamb, 1987). These responses indicate that transcriptional activation of defence genes characteristically underlies induction of the corresponding defence response and expression of resistance. The degree of pathogen-cultivar incompatibility is positively correlated to phaseolin concentration indicating that a phytoalexin index could be used in selecting for anthracnose disease resistance in beans. The higher HRGP in incompatible than compatible interactions (Esquirre-Tugaye, 1990) could also be used as a selection criterion but such a relationship may not always explain race-cultivar specificity (Tepper et al., 1989).

CHAPTER 3

3.0 MATERIALS AND METHODS

The study was done in three stages running concurrently within one season:

(i). Field survey. The survey was for the purpose of collecting diseased plant materials, assessing anthracnose severity and incidence and determining its distribution and relative economic importance.

(ii). Field evaluation of bean anthracnose on station. This was done to identify resistant materials and

(iii). Anthracnose race identification in the field and the laboratory.

3.1 THE FIELD SURVEY

A field survey to determine anthracnose disease severity, incidence and distribution was conducted during the last week of March and the first two weeks of April 2004. The surveys were purposively conducted in the main bean producing districts in Zambia. The districts covered were Mbala, Kasama, Mpika, Samfya/ Serenje, Mansa, Solwezi and Mwinilunga (Figure 1).

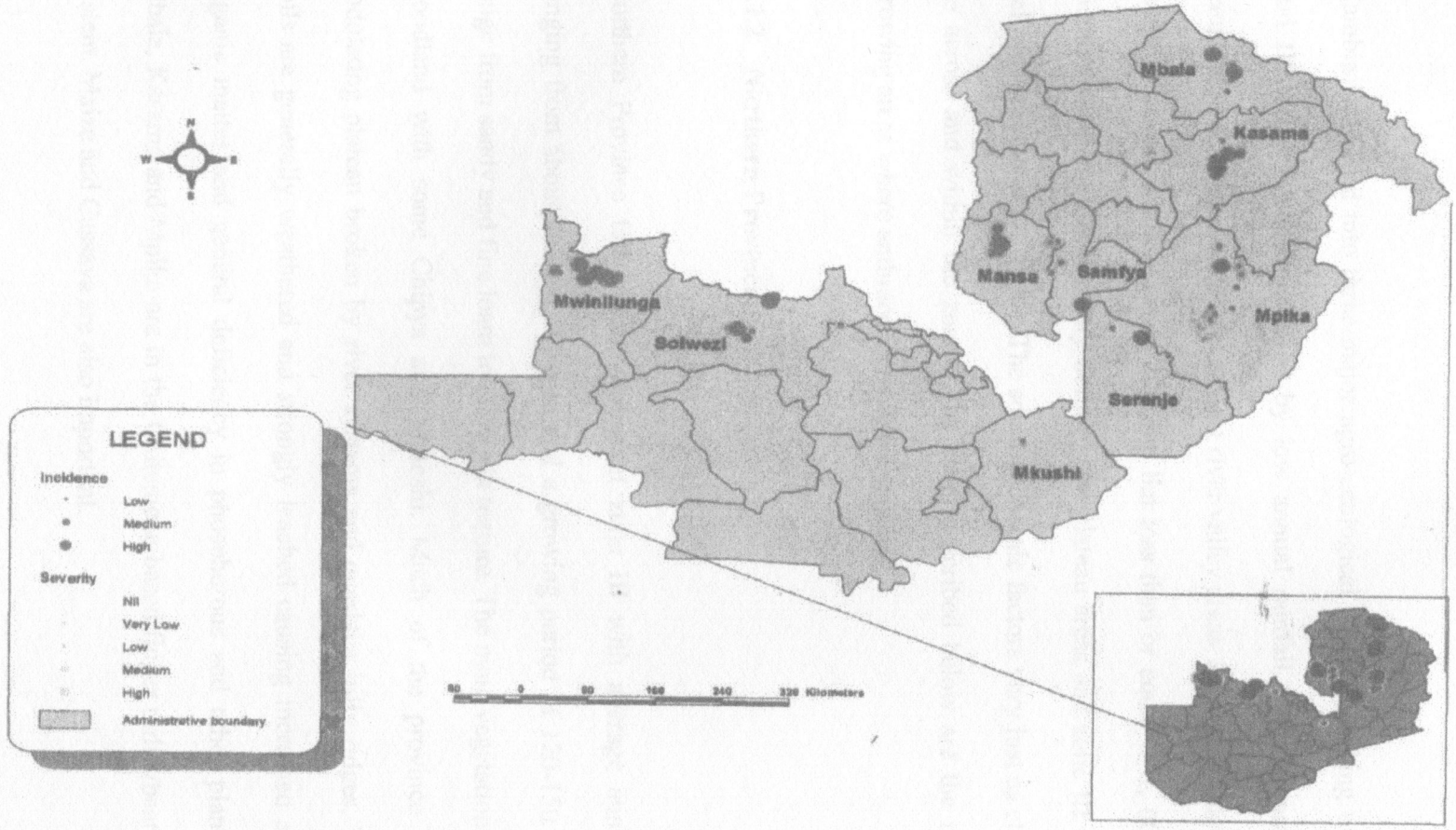

Figure 1: **Map showing sampled sites for bean anthracnose disease incidence, severity and distribution in Zambia.**

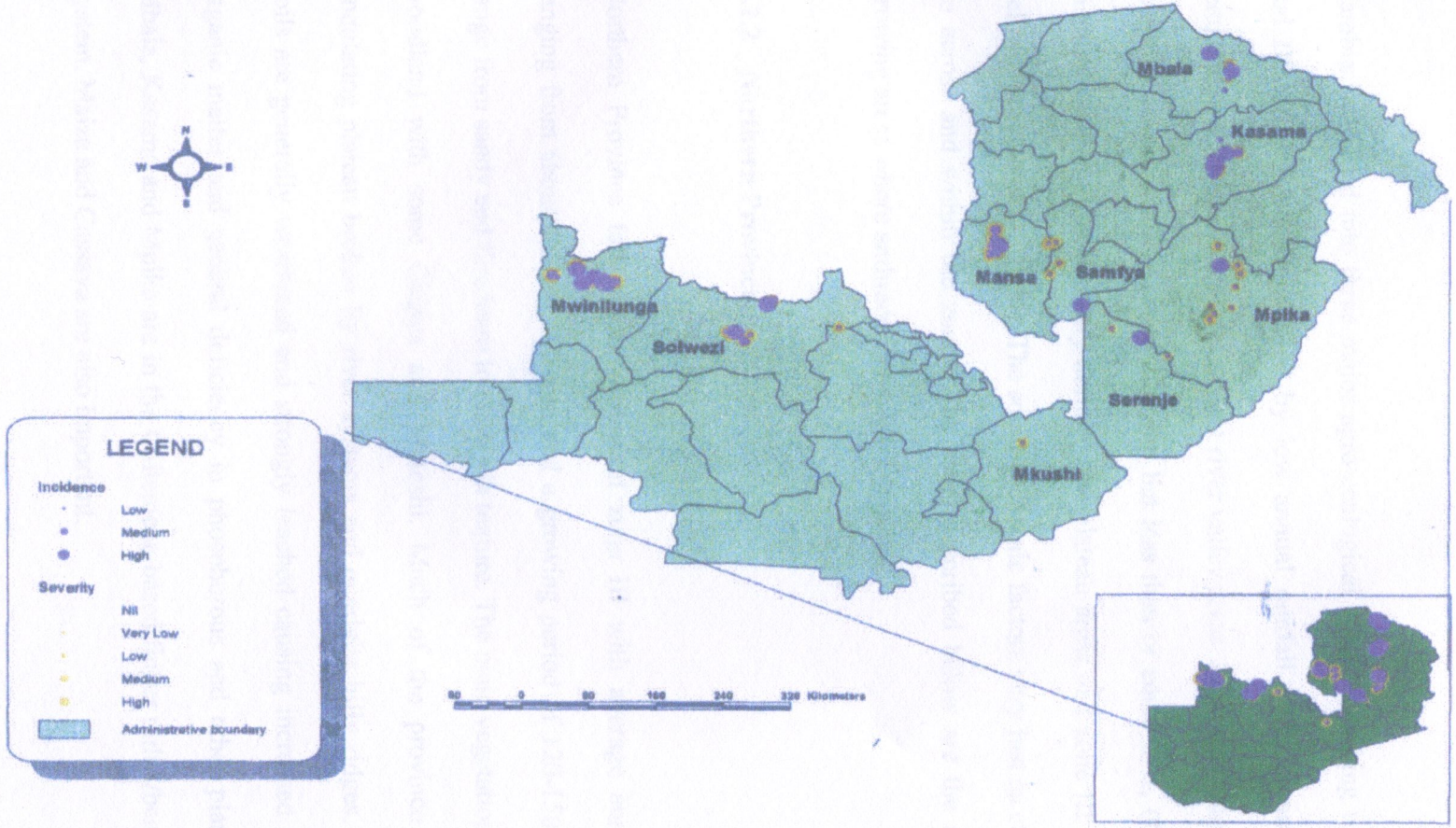

Figure 1: **Map showing sampled sites for bean anthracnose disease incidence, severity and distribution in Zambia.**

3.1.1 Brief description of the study area

Zambia is divided into three major agro-ecological zones consisting of zones I, II and III. Zone I is characterised by low annual rainfall (400-700mm) and high temperatures. This zone defines the hot river valley areas. Zone II is characterised by medium annual rainfall (above 700 mm but less than or equal to 1, 000 mm) and medium temperatures basically defining the plateau areas and zone III is described below (Bunyolo et al., 1995). The socio-economic factors vary just as ethnic groups do across and within the zones. The areas described below are the major bean-growing areas where anthracnose is also important.

3.1.2 Northern Province

Northern Province falls under ecological zone III with average annual rainfall ranging from about 1,000-1,500 mm and a growing period of 120-150 days. Soils range from sandy and fine loam to clayey in texture. The main vegetation is Miombo woodland with some Chipya and Muteshi. Much of the province is uniform undulating plateau broken by river systems and quartzite hilly ridges. The upland soils are generally weathered and strongly leached causing increased acidity, low organic matter and general deficiency in phosphorous and other plant nutrients. Mbala, Kasama and Mpika are in the Chitemene based finger millet/beans cropping system. Maize and Cassava are also important.

3.1.3 Northwestern Province

Northwestern Province also falls under ecological zone III with similar climatic conditions as Northern. The main vegetation is Miombo woodland in Solwezi and open savannah in Mwinilunga. Cassava is the staple crop while beans; pineapples, groundnuts and sweet potatoes are the major cash crops.

3.1.4 Luapula Province

Luapula Province also falls under ecological zone III with similar climatic conditions as Northern and Northwestern. The main vegetation is flood plain in Samfya and open savannah in Mansa. Cassava is the staple crop while beans, groundnuts and sweet potatoes are the major cash crops.

3.2 SAMPLING STRATEGY

A total of 90 farmers' bean fields were sampled and a questionnaire/checklist containing 30 questions each was used to obtain responses from farmers during the survey (Appendix A). A minimum of 10 and a maximum of 15 bean farmers' fields per district were sampled randomly. For each field, bean plants were inspected for symptoms of anthracnose on leaves, pods and stems depending on the stage of plant development at the time of the survey. Disease severity was visually scored in each field using a 1-9 scale (Appendices C and D). To estimate anthracnose incidence a

meter-row count method was adopted. A meter rule was placed in randomly selected locations on the ridge, mound or flat and the total number of plants over the meter rule length examined. Infected plants per unit length were expressed as a percentage of the total number of plants examined. Plant samples bearing clear-cut anthracnose symptoms were harvested and put in newspapers and later transferred into A4 paper envelopes. Each of these envelopes was labelled according to district name, field number, and date of collection, location name, longitude, latitude, altitude, anthracnose severity and incidence.

3.2.1 Bean anthracnose disease assessment

The principal criterion for evaluating anthracnose disease was based on both incidence and severity. The number of plants infected expressed as a percentage of the total number of plants per unit area gave disease incidence while the area of plant tissues affected by anthracnose causing organism expressed as a percentage of the total amount of tissues gave anthracnose severity. A correlation analysis was done to study the relationships between anthracnose disease and other diseases and environmental factors.

The Global Positioning System (GPS) equipment was used for specific field locations (longitude and latitude) as well as altitude of each field. The samples collected from the target areas were taken to the laboratory at the University of Zambia for isolation and confirmation of pathogens and race identification while the

rest of the information was analysed using Statistical Package for Social Scientists (SPSS). The locations were converted to angular distances and mapped (Appendix F).

3.3 FIELD BEAN ANTHRACNOSE RACE IDENTIFICATION

A total of 13 differentials were sourced through Dr Buruchara and Dr Chirwa of CIAT Uganda and Malawi respectively, from Professor Pretorius of South Africa. Twelve of these were standard CIAT bean anthracnose differential cultivars. These were sent to Mutanda Research Station (12^0 25.88 S and 26^0 12.59 E), in Solwezi, Zambia just in time for planting though rather late in normal planting seasons.

The differential cultivars were assessed for disease reaction at Mutanda Research Station through natural infection. The cultivars were planted in single rows of 10 seeds each planted on ridges spaced 1m apart and 10 cm between plants.

Evaluations were done at three bean development stages which included pre-flowering to flowering, podding to pod filling and physiological maturity. The disease assessment was done 21 Days After Planting (DAP), 45 DAP and 75 DAP which corresponds to R5-R6, R7-R8 and R9 bean developmental stages (Appendix B). Differential varieties planted in the field were evaluated on a scale of 1-9 (Appendix D).

3.4 FIELD SCREENING EXPERIMENT

Field race studies and anthracnose evaluations were carried out at Mutanda Research Station (12°25.387 S and 26 ° 13.002 E and altitude 1341meters above sea level (m.a.s.l) in the 2003/2004 season on acidic ferrasols with a pH of 4.0-5.0. The anthracnose evaluation nursery was planted at on 7th February in a Randomised Complete Block Design consisting of 18 entries replicated four times. Anthracnose Evaluation Nursery lines were made from a mixture of small seeded and large seeded bean type provided by CIAT for Southern Africa Bean Research Network Regional Trials. Solwezi Rose and Mbala Local provided the local susceptible check varieties while Cornell 49242 and G 1030 were the resistant checks. Plots sizes were 3 rows, 4 m long and 0.6 m apart. Within row plant spacing was 10 cm. The trial site was chosen because it had gone through a fallow period of two years without being used for bean production. The site could still have traces of natural bean anthracnose inoculum. A pre flowering spray of suspected anthracnose inoculum suspension from infected bean trash and plant parts was applied to increase the disease pressure in the field.

Parameters recorded included date of planting, plant stand at emergence and at harvest, days to 50 percent flowering, days to physiological maturity, a hundred seed weight, disease severity scores of anthracnose, angular leaf spot, common bacteria blight on a 1-9 scale and bean grain yield. Data analysis was done using MSTAT C statistical package.

3.5 LABORATORY EXPERIMENTS

3.5.1 Isolation, culture and multiplication of pure isolates

The field samples with characteristic bean anthracnose symptoms on pods were collected from Mbala, Kasama, Mpika, Samfya, Mansa, Solwezi and Mwinilunga. Infected pods were surface sterilised with 1 percent hypochlorite solution, rinsed twice in sterile water before isolation and culturing on Potato Dextrose Agar (PDA). The ingredients of PDA were dissolved in a litre of distilled water and the solution autoclaved at 121°C for about 15-21 minutes before cooling and pouring thinly onto petri dishes under the laminar flow hood.

Pure cultures were made from the sporulated fungi on petri dishes. Single conidial cultures were incubated at 25°C. After the single conidial colonies sporulated, distilled water was added and with a spatula the conidia were carefully scraped to make a suspension. The conidial suspension was cleared and filtered through a fine wire sieve mesh. The conidia in the suspension were counted using a haemocytometer in the central square, containing 16 small squares, each corner and the centre one (Pastor-Corrales et al., 1994). The number of conidia counted was then multiplied by 50 and 1000 to give a result in 10^6. The required final concentration of 1.2 x 10^6 conidia/ml was achieved by using the formula V1 x C1=V2 x C2 where V1= required initial suspension volume (ml) and C2= Final conidia concentration (1.2 x 10^6) and C1 and V2 are initial conidial concentration and final standard volume (200ml) respectively as described by Pastor-Corrales et al.

(1994). Pathak (1987) also described a similar method for standardizing *Colletotrichum lindemuthianum* conidia spore concentration.

3.5.2 Bean anthracnose race identification and resistance testing

The procedure of race identification and resistance testing in the laboratory started with sterilising river sand at 200°C for 6 hours (ISTA standards) at Mutanda Research Station. The sterilised sand was mixed with water in a ratio of 9:1 by volume before being put in small plastic pots (7.5 cm in diameter) where seeds of differential cultivars were planted and covered with polythene bags to germinate.

Five seeds were planted per pot and replicated three times on 9th October and 12th November 2004 respectively. Inoculations were done 7-9 days after planting. For each differential cultivar, the stem and cotyledons of bean seedlings with fully expanded primary leaves were sprayed with conidiae suspension of different isolates with a spore load of 1.2×10^6 conidia/ml. The inoculated plants were then incubated in a humid chamber set at 20 ± 4°C and relative humidity of 90-100 percent with 12 hr light and 12 hr darkness.

3.5.3 Bean anthracnose disease assessment and race identification

Disease assessment was done 10 days after inoculation on a 1-9 scale as described by van Schoonhoven and Pastor-Corrales (1987). Two distinct plant reactions were considered using the binary system as either resistant (rating 1-3) or susceptible (rating 4-9) (Appendix C). The binary system was applied as follows: The differential cultivars were assigned to a specific order from 1-12 each with a binary value if susceptible. In the example given below (Table 1) isolate A was virulent on differential varieties MDRK, Perry Marrow, Widusa and Mexico 22 giving race 86 while isolate B attacked Michelite, MDRK, Perry Marrow, Widusa and Mexico 22 and PI-207262 identifying race 207.

Table 1: Bean anthracnose differential cultivars, their order and binary value used for *Colletotrichum lindemuthianum* race identification when susceptible: an example

Order	Differential cultivar	Binary value	Differential cultivar reaction to different isolates	
			A	B
1	Michelite	1	R (0)	S (1)
2	MDRK	2	S (2)	S (2)
3	Perry Marrow	4	S (4)	S (4)
4	Cornell 49242	8	R (0)	S (8)
5	Widusa	16	S (16)	R (0)
6	Kaboon	32	R (0)	R (0)
7	Mexico 222	64	S (64)	S (64)
8	PI-207262	128	R (0)	S (128)
9	TO	256	R (0)	R (0)
10	TU	512	R (0)	R (0)
11	AB 136	1024	R (0)	R (0)
12	G 2333	2048	R (0)	R (0)
	Race[1]		**86**	**207**

[1] The designation of races is obtained by adding the binary values of susceptible reactions (S) of all the twelve varieties to a given isolate; race **86** is obtained by adding (2 + 4 + 16 + 64) and race **207** by adding (1 + 2 + 4 + 8 + 64 + 128).

CHAPTER 4

4.0 RESULTS

4.1 THE FIELD SURVEY

The results of the survey revealed that 47 percent of the respondents had been growing beans for the past 5-10 years. More than 53 percent of the beans in the sampled area were grown between 1301 and 1500 meters above sea level (m.a.s.l.) mainly on acidic highly weathered sandy-to-sandy loam soils. The bulk of the bean crop in the surveyed area was grown in February and 75 percent of the fields were sown sole while the rest were either intercropped with maize, cassava or sweet potatoes in small plots of 0.1-0.5 hectares (ha). The largest farm surveyed was 8 ha at Katandano Zambia National Service (ZNS) camp in Solwezi, followed by 3 ha and 2 ha in Mwinilunga and Mansa districts respectively. The bean crop at Katandano was planted late and at the time of the survey very few disease symptoms had set in despite the wet weather. It was also shown that there was very low variety diversity in the areas surveyed with only a total of six genotypes. The bean varieties that were found in the surveyed areas were Chambeshi, Mbala local, Solwezi Rose, Lusaka, Serenje white and Kabulangeti. In Mbala, out of 10 fields sampled, 90 percent of the crop was Mbala local while the remaining 10 percent was Kabulangeti. Of these only Mbala local was the most frequent (41 percent), followed by Solwezi Rose (30 percent), Kabulangeti (14.4 percent) and Chambeshi (7.8 percent). The other varieties were Lusaka (5.5 percent) and Serenje White (1.1 percent).

4.1.1 Bean anthracnose disease prevalence

Anthracnose was present in bean crops in all the surveyed districts indicating the importance of this disease in the entire major bean growing areas of Zambia. All locally grown bean cultivars were susceptible to bean anthracnose, angular leaf spot, rust, and common bacterial blight. The bean anthracnose causal pathogen is illustrated in Figure 2. Figures 3-8 and 9-10 illustrate effects of bean anthracnose, extent of damage and severity in addition to other types of bean diseases found on different varieties in the various fields surveyed respectively.

From a total of 90 bean fields randomly surveyed 76 percent were infected with bean anthracnose. Across districts, Mbala had the highest prevalence with the disease being found only after 10-20 percent of each of the individual fields had been sampled on average. Anthracnose severity and incidence was ranked highest in Mwinilunga district (Table 2) followed by Mbala, Kasama and Mansa. Of the four common diseases found in the field, angular leaf spot was the second most prevalent accounting for 43 percent of all the fields surveyed, ranking medium to high after anthracnose. Rust was the third most important disease in the surveyed area while common bacterial blight was fourth. Up to 70 percent of the respondents viewed bean stem maggot damage to be a problem while 23 percent pointed at poor soils and those who cited lack of improved seed as one of the major problems limiting bean production accounted for only 7 percent of the farmers.

In Mansa most of the seeds (60 percent) were sourced from the market while 20 percent came from own saved seed.

Plant densities were rated as: Low (133,333-166,660 plants/ha), Medium (166,667 – 199,999 plants/ha and High (above 200,000 plants/ha). The survey results showed that plant densities used were medium wherever the crop was planted on ridges, a factor that could contribute to disease prevention in these areas. However, plant densities were high on mounds and on the flat. Plant densities were highest in Mwinilunga and medium in Mpika, Samfya, Kasama and Mansa and low to medium in Mbala on average. The environmental factors such as rain and relative humidity varied across regions and cultural practices. Districts that experienced continuous and consecutive rainy days during parts of the growing season ranged between 3 and 9 with Mwinilunga and Mansa raining daily and consecutively for more than a week whilst the crop was in the field. This could probably explain the higher disease levels in the above named districts compared to say Mpika where it was drier prior to the survey time.

Disease control methods were found to vary across regions with Northern region applying delayed planting and mixed/ intercropping while in the Luapula and Northwestern regions no disease control methods were used at all (Table 2). The reason for the lack of application of any disease control strategies in these areas was attributed to farmers' inability to recognize the disease in the field. Kabulangeti was the only variety that was found distributed across all the three provinces and in all

the seven districts surveyed. The variety Solwezi Rose was found in the highest proportions in Solwezi and Mwinilunga in Northwestern though it was at least found in all the provinces but not in Mbala, Kasama and Mansa districts. The highest and possibly the coolest district in the surveyed areas was Mbala, followed by Mwinilunga and Mpika. These are the areas where the highest prevalence of bean anthracnose is expected. Anthracnose was most severe in Mwinilunga and Mansa on a 1-9 scoring scale system where 87 percent of the bean fields had a score above 7 (Table 3).

Table 2: Farmers perception of anthracnose disease and control methods practiced in each district of the surveyed areas

Disease Control Method		Names of Districts						
		Mbala	Kasama	Mpika	Mansa	Samfya	Solwezi	Mwinilunga
1.Delayed planting	Northern	3	2	2				
	Luapula				4	3		
	Northwestern						3	4
	Total	3	2	2	4	3	3	3
2.Mixed Cropping	Northern	7	8	2				
	Luapula				2			
	Northwestern						2	
	Total	7	8	2	2		2	
3.Early Planting	Northern		5	6				
	Luapula				3	4		
	Northwestern						3	1
	Total		5	2	3	4	3	1
4.None	Northern							
	Luapula				6	3		
	Northwestern						3	7
	Total				6	3	3	7

Table 3: Severity, incidence and distribution of anthracnose in the major bean growing areas of Zambia surveyed in 2004.

Province	District Name	Number of fields sampled	Mean Anthracnose Incidence (%)	Mean Anthracnose Severity (1-9)	Mean altitude (masl)	Main plant Parts Affected
Northern	Mbala	10	60.6	4	1606.4	Pod/Leaf
Northern	Kasama	15	46.5	4	1319.2	Pod/Leaf
Northern	Mpika	10	20.7	2	1399.4	Leaf
Luapula	Mansa	15	31.9	4	1260.0	Pod/Leaf
Luapula	Samfya/Serenje[1]	10	27.9	3	1326.0	Leaf
Northwestern	Mwinilunga	15	69.6	7	1417.6	Pod/Leaf/Stem
Northwestern	Solwezi	15	31.0	4	1367.0	Leaf

[1]These two administrative districts were taken as one for the purpose of this survey only and wherever Samfya appears it includes survey points in northern Serenje.

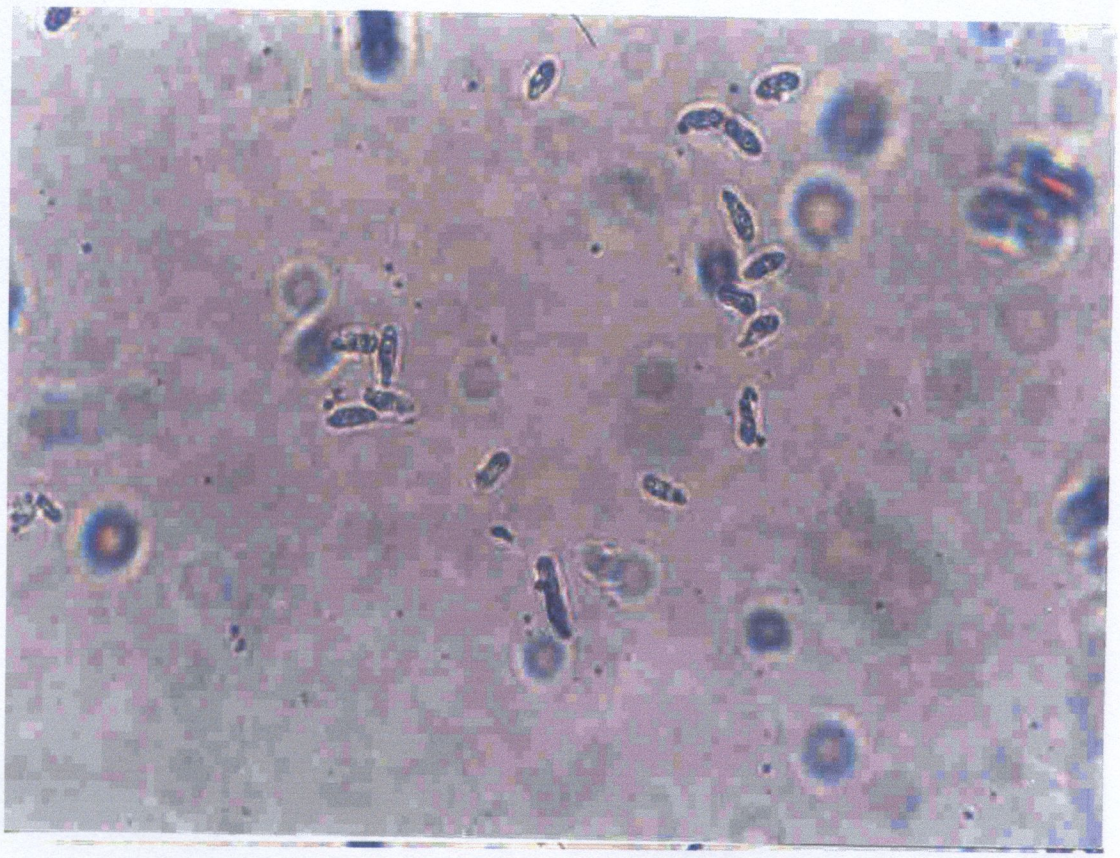

Figure 2: ***Colletotrichum lindemuthianum*** **conidia - the pathogen that causes bean anthracnose**

Figure 3: **Early anthracnose symptoms on bean pod and leaf on Mbala Local variety in Mansa**

Figure 4: **Water soaked symptoms on pods of a white bean variety in Mansa**

Figure 5: **Severe symptoms of anthracnose on bean pod, leaf, petiole and stem on Kabulangeti bean in Mansa**

Figure 6: **Leaf and pod symptoms of bean anthracnose on Solwezi bean variety in Mwinilunga**

Figure 7: Bean anthracnose on Kabulangeti bean variety in Kasama

Figure 8: Large bean field with patches of bean anthracnose infestations in Mansa

Figure 9: Bean rust (*Uromyces appendiculatus)*

Figure 10: **Angular leaf spot (*Phaeoisariopsis griseola*) on bean leaves and pods**

4.1.2 Correlation coefficient analysis

Correlation analysis results revealed that anthracnose disease severity was positively correlated ($P \leq 0.05$, $r = 0.249$) with other leaf spot diseases and with anthracnose incidence ($P \leq 0.01$, $r = 0.451$). The anthracnose incidence was positively correlated with altitude ($P \leq 0.01$, $r = 0.356$) while altitude was in turn positively correlated with severity. The numbers of days with continuous rains during the season were positively correlated with anthracnose incidence ($P \leq 0.01$, $r = 0.301$).

The other disease severity was positively correlated ($P \leq 0.01$, $r = 0.207$) with plant density (Table 4).

Table 4: Correlation analysis of some important parameters of the field survey[1]

PARAMETER	Anthracnose incidence	Severity score	Other diseases	Disease control method	Plant density
Anthracnose disease severity	**r = 0.451****	**r = 0.641****	**r = 0.249***	**r = 0.246***	**r = 0.301****
Altitude	**r = 0.356****	**r = 0.301****			
Other diseases					**r = 0.207****

[1] N = 90

** Correlation is significant at the 0.01 probability level (Pearson Correlation - 2 tailed).

* Correlation is significant at the 0.05 probability level (Pearson Correlation - 2 tailed).

4.2 FIELD EXPERIMENTS

Results of the field trial (Table 5) showed that anthracnose disease was present at Mutanda at low to medium levels and progressively increased from early vegetative stage through podding to maturity stage. All the varieties tested were free of the disease in the early stage of the crop development except Mbala Local and G 3010, which had slight infections. The disease became more evident at flowering stage with more varieties showing symptoms while those affected earlier showed increased disease incidence with Mbala local being rated 4 on a 1-9 scale. Towards the end of the podding stage each of the 18 varieties tested exhibited some symptoms of infection but the severity was higher ($P \leq 0.05$) on six that had scores ranging from 4 to 4.5 on average. The resistant check (Cornell 49242) remained relatively resistant to anthracnose across the three bean development stages that were evaluated. So did Rao 55, PAN 122, RAB 482, Fleetwood and ZAA 5/2. However, Mbala local and Solwezi Rose, which were local variety checks, were significantly ($P \leq 0.05$) more susceptible to anthracnose. Cornell 49242 was however, significantly ($P \leq 0.05$) susceptible to common bacterial blight (Figure 11).

4.2.1 Bean anthracnose race identification in the field

The total annual rainfall at Mutanda was 1370.3mm (Appendix E) with a total of not less than 200mm in the months of December to March.

Field results showed that all differential cultivars except Perry Marrow and Kaboon were resistant to races locally present after 21 DAP (Table 4). The disease progressed with time and at 45 DAP, Widusa, Cornell 49242, Tu, AB 136 and G 2333 differentials were still resistant to anthracnose while at 75 DAP only Cornell 49242, Tu, AB 136 and G 2333 showed complete resistance out of the13 tested (Figure 12).

Table 5: Severity of anthracnose and CBB on selected bean parameters scored at three different growth stages at Mutanda farm

Variety Name	[a] Grain Yield ($kgha^{-1}$)	Plant Vigour (1-5)	100 Seed Weight (g)	R5 Anthracnose (1-9)	R7-R8 Anthracnose (1-9)	R9 Anthracnose (1-9)	Days to 50% Flowering	Days to 50% Maturity	R9 CBB (1-9)
202-402	493 bc	4.5 abc	19.5 ef	1.0 b	2.0 bc	4.3 a	42 bcdef	74 ab	2.0 f
BRB 80	453 bcde	3.5 b	18.5 efg	1.0 b	2.0 bc	3.5 a	41 cdef	72 abc	4.5 bcde
BRB45	394 cdef	4.0 bcd	16.8 gh	1.0 b	2.8 ab	4.0 a	40 ef	73 ab	3.5 cdef
KID 31	382 cdef	4.5 abc	35.5 b	1.0 b	2.5 abc	4.5 a	39 f	69 cdef	5.0 bcd
CORNELL 49242	411 cde	4.0 bcd	19.3 ef	1.0 b	1.0 c	3.5 a	47 a	69 cdef	7.5 a
DOR 814	403 cde	4.3 abcd	17.8 fgh	1.0 b	2.5 abc	3.5 a	45 ab	70 cde	4.5 bcde
EXL 52	507 abc	3.8 cd	17.8 fgh	1.0 b	1.0 c	2.5 a	45 ab	70 cde	2.5 ef
FLEETWOOD	590 ab	4.3 abcd	19.0 ef	1.0 b	1.8 bc	2.0 a	44 abc	74 ab	3.0 def
G 3010	379 cdef	3.8 abc	13.8 i	1.5 ab	3.0 ab	4.0 a	44 abc	71 bcd	4.0 cdef
PAN 122	404 cde	4.3 abc	20.5 e	1.0 b	1.0 c	3.0 a	40 ef	74 ab	2.0 f
PAD 3	257 fg	4.3 abcd	26.5 d	1.0 b	2.0 bc	3.3 a	41 cdef	68 efg	6.5 ab
RAB 482	470 bcd	4.3 abcd	19.5 ef	1.0 b	1.0 c	3.0 a	42 bcdef	72 abc	5.5 abc
RAO 55	648 a	4.8 ab	18.0 fgh	1.0 b	1.5 bc	3.3 a	46 abc	74 ab	3.8 cdef
UBR (92) 25	486 bc	4.0 abc	16.0 h	1.0 b	1.0 c	2.5 a	44 abc	75 a	3.0 def
PVA 1082	328 def	4.5 abc	38.3 a	1.0 b	1.8 bc	4.5 a	44 abc	70 cde	4.5 bcde
ZAA 5/2	419 cde	5.0 a	33.0 c	1.0 b	1.8 bc	2.5 a	40 ef	68 efg	4.5 cde
MBALA LOCAL	316 efg	4.5 abc	26.3 d	2.0 a	4.0 a	4.5 a	40 ef	67 efg	4.0 cdef
SOLWEZI ROSE	174 g	4.3 abcd	33.8 bc	1.0 b	2.5 abc	3.8 a	41 cdef	66 g	4.5 bcde
MEAN	417.31	4.25	22.75	1.08	1.94	3.39	42.44	70.74	4.15
CV%	24.02	13.74	6.34	32.67	55.47	40.32	5.86	2.77	39.55
LSD (0.05)	142.3	0.83	2.05	0.50	1.53	NS	3.53	2.78	2.33

[a] Values followed by the same letters are not significantly different from each other according to Duncan's Multiple Range Test.

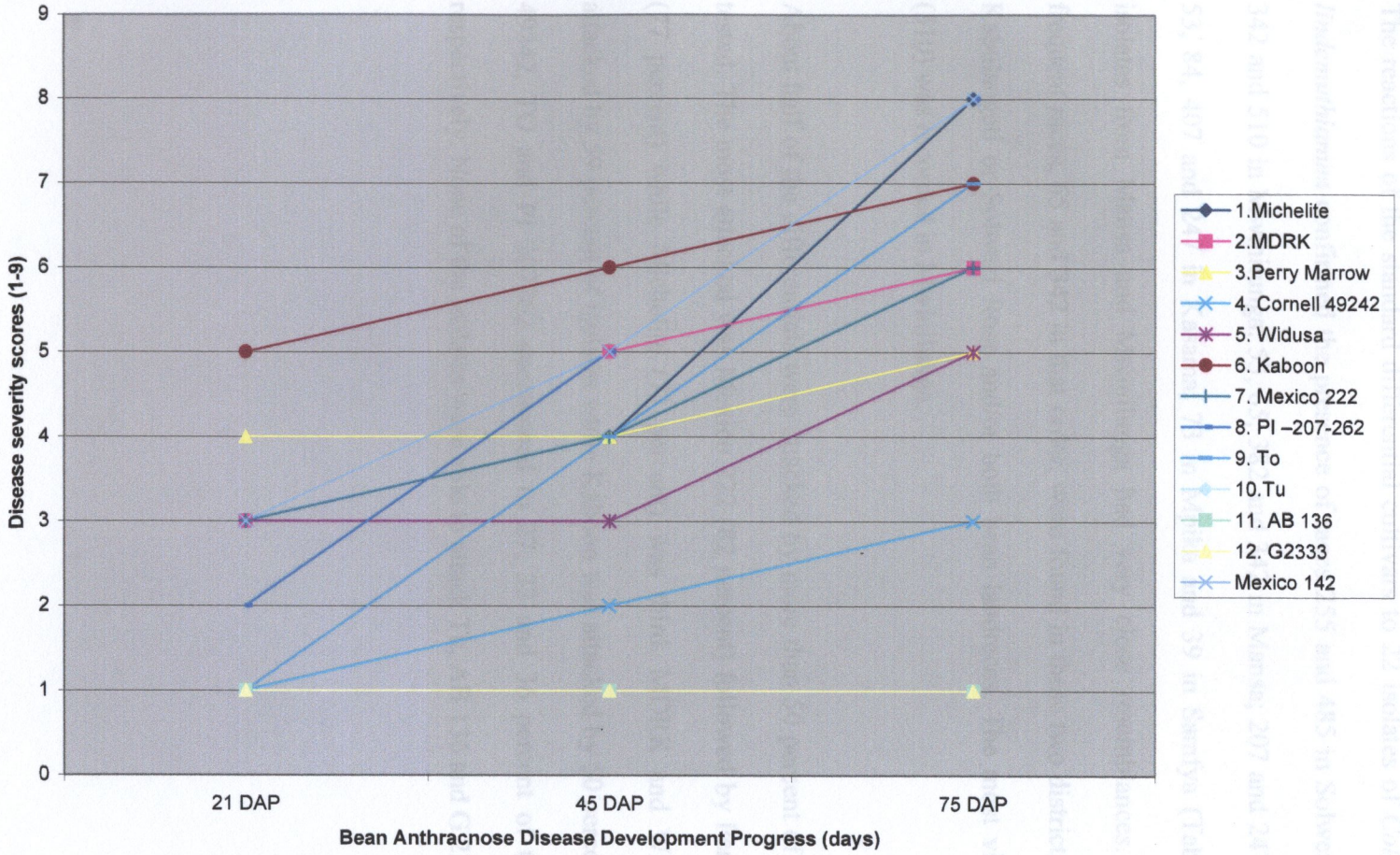

Figure11: Reaction of different cultivars to anthracnose at three different growth stages at Mutanda in 2004 season

4.3 LABORATORY RACE IDENTIFICATION OF BEAN ANTHRACNOSE FUNGUS

The reactions of the standard differential cultivars to 22 isolates of *Colletotrichum lindemuthianum* confirmed the presence of races 255 and 485 in Solwezi; 65, 255, 342 and 510 in Mwinilunga; 53, 65, 382 and 342 in Mansa; 207 and 247 in Mbala; 53, 84, 407 and 247 in Kasama; 73 in Mpika and 39 in Samfya (Table 6). Test isolates from Mansa and Mwinilunga had very close resemblances. The most frequent races, 65 and 342 in that order, were found in these two districts hosted by Kabulangeti or Solwezi Rose and/or both bean landraces. The most virulent race (510) was recorded in Mwinilunga.

About half of the differentials were attacked by more than 50 percent of all isolates tested. The most attacked was Mexico 222 (82 percent) followed by Perry Marrow (77 percent) while Michelite (73 percent) was third. MDRK and Widusa were attacked by 59 percent of isolates while Kaboon was attacked by 50 percent. Cornell 49242, TO and PI 207262 succumbed to 27, 32 and 36 percent of the isolates respectively. None of the isolates were able to attack Tu, AB 136 and G 2333.

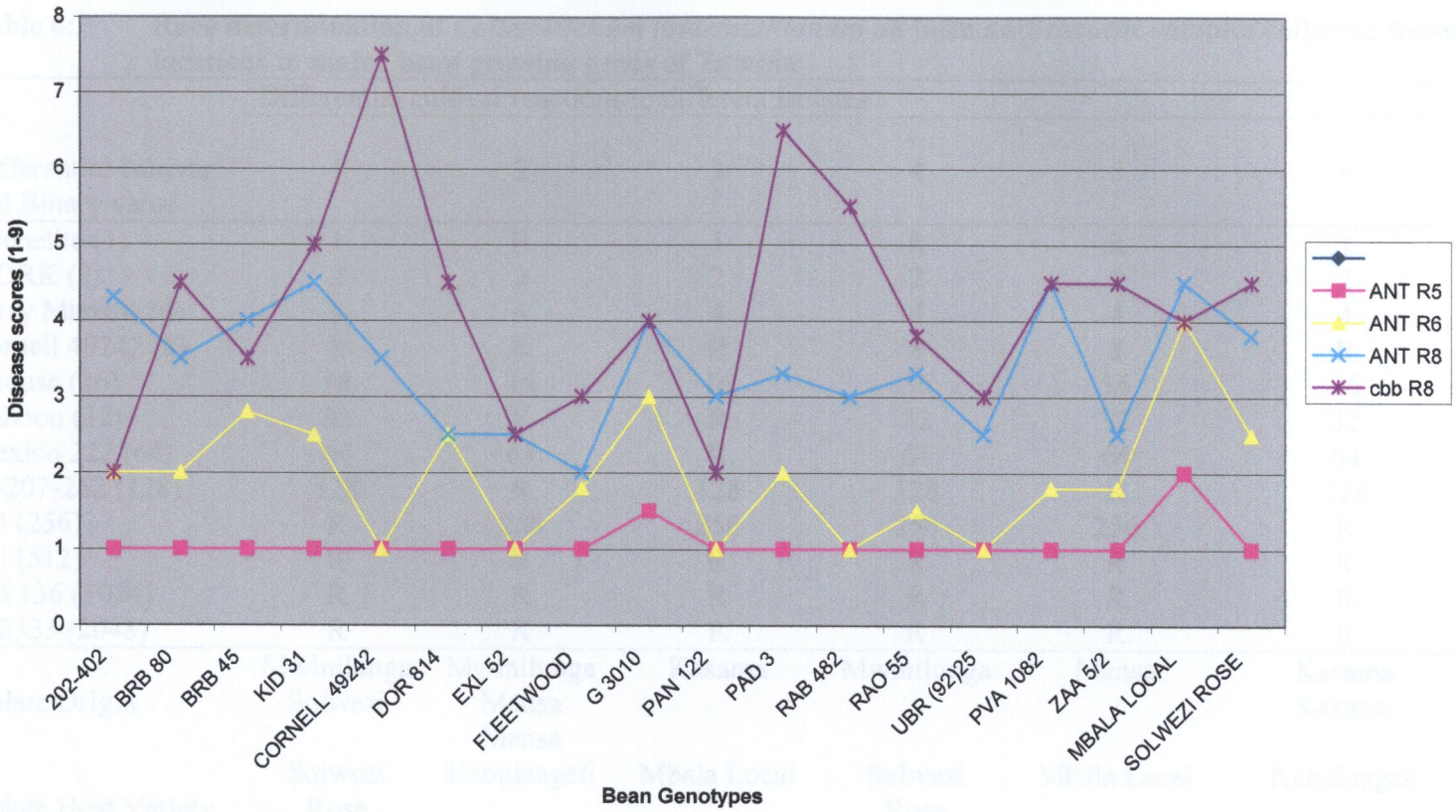

Figure 12: Severity of bean anthracnose and Common bacterial blight at different growth stages of different bean genotypes

Table 6: Race determination of *Colletotrichum lindemuthianum* on bean anthracnose samples collected from different locations in major bean growing areas of Zambia

	Differential cultivar reactions to different isolates						
Differential cultivar and Binary value	1	2	3	4	5	6	7
Michelite (1)	1	R	1	R	R	1	R
MDRK (2)	2	2	2	2	2	2	R
Perry Marrow (4)	4	4	4	4	4	4	4
Cornell 49242 (8)	8	R	R	8	8	R	R
Widusa (16)	16	16	16	16	16	16	16
Kaboon (32)	32	R	R	32	32	32	R
Mexico 222 (64)	64	64	R	64	64	64	64
PI-207-262 (128)	128	R	128	128	R	128	R
TO (256)	R	256	256	256	256	R	R
TU (512)	R	R	R	R	R	R	R
AB 136 (1024)	R	R	R	R	R	R	R
G 2333 (2048)	R	R	R	R	R	R	R
Isolate Origin	Mwinilunga Solwezi	Mwinilunga Mansa	Kasama	Mwinilunga	Mansa	Kasama Kasama	Kasama
Isolate Host Variety	Solwezi Rose	Mansa Kabulangeti	Mbala Local	Solwezi Rose	Mbala Local	Kabulangeti	Kabulangeti
Race Designation	255	342	407	510	382	247	84
Number of Isolate Per Reaction	2	3	1	1	1	2	1

Table 6: Continued....

	Differential cultivar reactions to different isolates						
Differential cultivar and Binary value	8	9	10	11	12	13	14[1]
Michelite (1)	1	1	1	1	1	1	1[2]
MDRK (2)	2	R	R	2	R	2	R[3]
Perry Marrow (4)	4	4	R	4	4	4	R
Cornell 49242 (8)	R	R	R	8	R	R	8
Widusa (16)	16	R	R	R	16	R	R
Kaboon (32)	32	32	R	R	32	32	R
Mexico 222 (64)	64	64	64	64	R	R	64
PI-207-262 (128)	128	128	R	128	R	R	R
TO (256)	R	256	R	R	R	R	R
TU (512)	R	R	R	R	R	R	R
AB 136 (1024)	R	R	R	R	R	R	R
G 2333 (2048)	R	R	R	R	R	R	R
Isolate Origin	Mbala	Solwezi	Mwinilunga Mwinilunga Mwinilunga Mansa	Mbala	Kasama Mansa	Samfya	Mpika
Isolate Host Variety	Mbala Local	Solwezi Rose	Kabulangeti Solwezi Rose	Mbala Local	Kabulangeti Mbala Local	Mbala Local	Mbala Local
Race Designation	247	485	65	207	53	39	73
Number of Isolate Per Reaction (22)[4]	1	1	4	1	2	1	1

Key to Table 6: **Continued....**

[1] Total number of distinct races identified

[2] Binary Number = Susceptible

[3] R = Resistant

[4] Total number of Isolates tested

CHAPTER 5

5.0 DISCUSSION

The results for the survey and field experiments are based on natural infection in the field where the disease established itself from local inoculum representing a range of strains of the pathogen that occurred at each given locality without discrimination. The infection occurred continuously at any crop development stage without discrete artificial application of arbitrary levels of inoculum. The pathogen strains were also naturally aggressive and not selected for their exceptional virulence or grown under artificial environments.

However, for race characterization in the laboratory, it was imperative that the pathogens from different districts were isolated and grown artificially and applied at specific concentrations to the plants under controlled environments. These attributes thus make the study appropriate and relevant to conditions obtained in the target environments.

5.1 THE FIELD SURVEY

5.1.1 Bean anthracnose incidence and severity

The survey provided data that helped to describe geographic distribution, epidemiology and relative importance of anthracnose and other diseases in the target areas. During field surveys, anthracnose symptoms were found mainly on bean crops that had at least attained V5 to R9 development stages (Appendix B). In Mbala and

Kasama it was possible to record symptoms on bean plants in the V4 development stages indicating that anthracnose attack in these areas initialised early. The number of plants affected by anthracnose per unit area was highest in Mwinilunga district followed by Mbala and Kasama. The area of plant tissues affected by the anthracnose pathogen expressed as a percentage of the total amount of tissues followed a similar pattern as the incidence but Mansa was second highest after Mwinilunga. The probable reason for this trend could be that the higher altitude and frequent wetness in Mwinilunga, Mbala and Kasama could have had the effect of increasing both incidence and severity due to generally lower ambient temperatures in these areas as a result of altitude and increasing humidity. These results are also in agreement with those reported by Wortmann et al., (1998) suggesting that cool wet and humid environments favour bean anthracnose disease development and spread.

The bean cultural production practices being practiced by farmers in both Mwinilunga and Mbala could be responsible for the higher disease prevalence. The survey showed that bean fields in these areas tended to have increased plant density due to use of mounds or planting on the flat respectively. Plant to plant disease spread was possibly favoured by closed canopy and humid conditions due to increased plant density.

The positive correlations revealed between anthracnose disease severity with other leaf spot diseases and with anthracnose incidence could be attributed to the fact that the same environment that favoured anthracnose also favoured other leaf diseases.

The positive correlations between the number of days with continuous rains during the season and anthracnose severity and incidence and severity with plant density confirm that cold wet weather and higher plant densities favoured anthracnose development in the fields. Mpika and Samfya districts recorded low to medium values due to both drier and less humid conditions during the growing period. At the time of the survey the disease was found to be most severe at pod development stage (R8) followed by flowering stage (R6). Anthracnose has been reported to appear in the field between 4-6 weeks after emergence (Kayitare, 1987; Msuku et al., 2000). However, some of the bean plants in these districts were less than 4 weeks at the time of the survey partly explaining the low disease severity.

The largest source of seed planted was from own saved seed, which could have been highly infected after years of recycling. This could have contributed significantly to some of the high disease incidence and severity especially in Mwinilunga, Mansa and Kasama districts.

5.1.2 Bean anthracnose distribution in Zambia

Anthracnose was distributed across the entire major bean growing area in region III of Zambia confirming the importance of the disease in this part of the country. A spot check conducted in Mkushi (region II) showed both low incidence and severity suggesting that anthracnose may not be important in drier areas.

5.2 FIELD EXPERIMENTS

5.2.1 Severity of bean anthracnose on differential cultivars

The results showed that local anthracnose races attacked both Andean and Middle American differentials. These results also suggest that a mixture of races occurred at Mutanda giving a maximum binary value of 503. Disease infection and development has been shown to be a function of time and possibly age as well as variety of the plant exposed as supported by the above findings. Tu, AB136 and G 2333 which are all of Middle American origin showed complete resistance to local races possibly because they have several resistance genes. The single and dominant *Are* resistance gene in black seeded Cornell 49242 that confers resistance to alpha, beta, gamma, epsilon and lambda may not be durable in the presence of many virulent anthracnose races. However, in the current experiment it was resistant to all local races. Most Middle American differentials except Mexico 222 showed increase in disease severity with increase in plant age but less susceptibility until after 45 DAP. These results suggest that the disease in these genotypes could cause fewer losses on yield compared to Andean cultivars as symptoms came in after flowering. However, all Andean differentials (Widusa, Kaboon, Michigan Dark Red Kidney (MDRK) and Perry Marrow) exhibited increase in disease severity with increase in plant age starting from 21DAP. The differential variety Kaboon was found highly susceptible to anthracnose at an early age but had a stable disease reaction through 45 and 75 DAP. This result is not in complete agreement with those reported by Schwartz et al., (1983), which showed that Kaboon was resistant to the majority of both Andean

and Middle American races. However, the result agrees with Landes and Hoffman, (1973) that the penetration rate of anthracnose infection was faster in younger than older cells of the epidermis.

Michelite exhibited, to the contrary, an increase in susceptibility to anthracnose with increase in plant age but showed resistances at 21-45 DAP. Sindhan and Bose (1981), reported similar results with some bean varieties citing cultivar exceptions to results reported by Landes and Hoffman, (1973).

5.2.2 Field bean anthracnose race identification

Using the traditional system or the Greek System of race nomenclature, this result indicates that all differential cultivars of Andean origin were either of intermediate resistance or became susceptible suggesting the probable presence of about ten race groups namely; Alpha, Beta, Gamma, Delta, Lambda, Mexican II, Zeta, Teta, Eta and Mu races at Mutanda in Solwezi. Alpha Brazil, Kappa and Brazil II were possibly absent since Cornell 49242 that is normally susceptible to these races was resistant. Similar results were obtained by Greenberg et al., (1986), who suggested the possible presence of lambda, gamma, epsilon, and alpha and delta races in Mbala, the only other site where similar work has been done before in Zambia.

5.3 EVALUATION OF FIELD EXPERIMENTS

5.3.1 Effect of bean anthracnose on bean yield

When 18 bean genotypes were grown together in the field Solwezi Rose and Mbala local gave the lowest grain yields despite being adaptable varieties. This was probably because they were both attacked by anthracnose in their early vegetative development stages. These results are in agreement with Chaves (1980) who reported crop losses of up 100 percent when anthracnose infection occurred on young bean plants.

5.3.2 Effect of bean anthracnose on bean seed size

The results of the field experiment in which eighteen genotypes were sown showed that large to medium seed size beans were more prone to anthracnose attack than the smaller seeded ones apart from G 3010. This probably indicates that sources of resistance against anthracnose in this region could be found in Middle American gene pool small seeded cultivars. The resistance exhibited by small seeded varieties in the trial may also indicate that the races of anthracnose present in Zambia were mostly of Andean gene pool though a mixture could be expected. The smaller seeded cultivars also showed delayed maturity periods with higher yields compared with the medium sized ones probably because they remained physiologically active longer and accumulated more dry matter.

5.3.3 Effect of anthracnose on late-planted beans

In the same experiment mentioned above, late planting and probably disease infection resulted in lower seed weights particularly for Solwezi beans and PVA 1082 which under normal circumstances weigh more than 40g/100 seeds and are classified as large. These results conform to those reported by CIAT, 1992 that seed size; number as well as seed quality reduces due to bean anthracnose disease. The crop was planted late and grew under cool, wet and humid conditions due to free moisture in the form of both rain and dew which conditions could have favoured anthracnose attack. Terminal drought could also have resulted in immature shrivelled seeds.

5.4 LABORATORY BEAN ANTHRACNOSE RACE IDENTIFICATION

A total of 14 physiological races of *Colletotrichum lindemuthianum* were identified and characterised on the 12 CIAT standard differential cultivars using binary system of nomenclature from 22 isolates. This confirms the great variability of bean anthracnose fungus in Zambia. There were marked similarities between races occurring in Mansa and those in Mwinilunga. For instance, Mexico 222 had similar reactions to almost all isolates from these districts. These two districts also accounted for 43 percent of all the races characterised. The high variability in races from Mansa district could be probably because more than 50 percent of the seed was sourced from the market and could have been infected. The other reason could arise

from cross border seed exchanges since both districts share borders with the Democratic Republic of Congo which is another important bean growing country with similar production environments.

The bean host variability was very low in the regions of survey, which could partly explain the limited total number of isolates tested. Some races were found to be location specific while others were found in more than one district or region without regard to agronomic practices. Limited race spread in some districts could have been as a result of farmers growing their own saved seed without sorting.

5.5 POSSIBLE SOURCES OF RESISTANCE GENES

The differential varieties G 2333, AB 136 and TU all of Middle American origin showed complete resistance to all races of anthracnose probably based on possessing several resistance genes. The single resistance gene in black seeded Cornell 49242 was also found with intermediate resistance that could easily be incorporated into commercial varieties and land races, but its resistance alone may not be durable. In the current study races, 73, 207, 255, 382 and 510 attacked Cornell 49242. These results indicate clearly that the major sources of horizontal resistance to some Zambian virulence of *Colletotrichum lindemuthianum* are of Middle American origin and mostly small seeded. Kaboon was the only Andean cultivar that exhibited race-specific type of resistance. The breeding implications of these results indicate that to overcome the pathogenicity of Zambian races characterised so far G 2333;

AB 136 and TU could be used as potential sources of resistance genes. These genotypes provided the broadest genetic diversity of host resistance with the necessary combinations of resistance genes. The use of such genes would avoid releasing bean varieties that are compatible with the pathogen.

The virulence diversity results in Table 6 can be used to combine resistance genes that would withstand pathogen populations present in any of the given areas in the study. Resistance in the above cultivars could be due to higher quantities of plant metabolites produced such as isoflovonoids, phaseolin, phytoalexins and hydroxyproline-rich glycoproteins which are inhibitory to *Colletotrichum lindemuthianum* compared with susceptible plants (Schwartz et al., 1980).

Production of resistant cultivars would combine economic feasibility and ease of adoption of resistant varieties by farmers with environment and sustainable agriculture in managing anthracnose.

CHAPTER 6

6.0 CONCLUSION

According to survey results the major bean diseases in the target area were anthracnose, angular leaf spot, rust and common bacterial blight. The results obtained in this study clearly demonstrate that at least 14 different races of *Colletotrichum lindemuthianum* that cause bean anthracnose were identified in Zambia. The study also provides evidence that these races are distributed in all major production areas causing anthracnose with extremely varied severity and incidence with Mwinilunga and Mansa providing current "hot spots" with the highest race diversity.

The presence of different pathogenic races of *Colletotrichum lindemuthianum* in Zambia shows that there is need for the development of varieties resistant to several strains of this pathogen. The current study provides evidence that at least three exotic differential cultivars (Tu, AB 136 and G 2333) could provide effective resistance genes against all races characterised so far.

Anthracnose poses a real threat to the productivity of local bean landraces popular with farmers in Zambia. All the local bean landraces were found susceptible to anthracnose disease. Almost all the beans grown for sale and consumption in the country come from these local land races. There is need therefore to develop a

breeding programme for resistance to anthracnose in order to improve and sustain local bean production.

The results of this study however represent only one season's work in limited locations with limited isolates. More samples collected at bean pod filling stages in all districts would be required to facilitate more isolates and ease pathogen isolation. Multilocation tests under varying disease pressures conducted seasonally for 3-4 years are recommended in future research for more representative data and to monitor new races. It is also recommended that work to characterise more races with the help of molecular markers should be considered in future research.

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APPENDICES

Appendix A: Questionnaire and Checklist on bean anthracnose survey in major bean growing areas of Zambia conducted between March and April 2004.

1. Name of Region or Province----------
   1. Northern
   2. Luapula
   3. Northwestern

2. Name of District.....

   1. Mbala 2. Kasama 3. Mpika 4. Mansa

   5. Samfya 6. Solwezi 7. Mwinilunga

3. Name of Farmer or Respondent ----------

4. Sex: 1. Male 2. Female

5. Age: 1. 15-25 2. 25-30 3. 31-40 4. 41-50

   5. 51-60 6. 61-80

6. How many years has the respondent been growing beans?

   1. < 5 2. 5-10 3. >10 yrs

7. What is the distance from the district centre?

   1. 10-20 km 2. 21-30 km

   3. 31-40 km 4. 41-50 km.

5. More than 50 km

8. What is the location's altitude? (Please use GPS)

1. < 1000 m 2. 1000-1300 m. 3. 1301-1500 m

4. above 1500 m

9. What is the soil type on which bean is grown?

1. Sandy soil 2. Sandy loam 3. Clay soil

4. Loam 5. Clay loam

10. What variety (ies) of bean is (are) grown in your area?

1. Chambeshi 2. Mbala Local 3. Solwezi Rose

4. Lusaka 5. Kabulangeti 7. Serenje white

8. Pembela 9. Other-Specify

11. What is the colour of the bean variety grown?

1. Red 2. Yellow 3. White 4. Purple

5. Brown 6. Cream 7. Khaki 8. Mixed

12. What is the seed size of the beans grown?

1. Large 2. Medium 3. Small

13. When was the crop planted?

1. November 2. December 3. January

4. February 5. March

14. What is the cropping pattern?

1. Sole beans 2. Beans + Maize

3. Beans + Cassava 4. Beans + Sweet potatoes

15. At what stage of crop development is the disease most severe?

1. Seedling stage. 2. Flowering stage

3. Podding stage 4. Maturity stage.

16. What is the total estimated area observed---------- (ha)?

1. 0.1-0.5 2. 0.51-0.75 3. 0.76-1.00.

4. 1.10-2.0. 5. More than 2.0

17. What percent of the total area is actually sampled?

1. 10 percent 2. 20 percent 3. 30 percent.

4. 40-50 percent 5. > 80 percent

18. How would you rank the severity of bean anthracnose disease?

1. Low 2. Medium 3. High.

19. What other bean diseases are present in the field?

1. Angular leaf spot 2. Rust

3. Common Bacterial Blight 4. Ascochyta blight 5. None

20. How would you rank the named disease in question 19 above?

1. Low 2. Medium 3. High

21. What methods do you use to control bean diseases?

1. Delayed planting 2. Mixed cropping /Intercropping

3. Early planting 4. Mixed cultivars 5. Spraying. 6. None

22. What other major problems limit bean production in this area?

1. Poor soils 2. Bean stem maggot

3. Lack of improved seed 4. Lack of market

5. Weeds 6. Other- specify.

23. Do you sort seeds prior to planting to avoid seed borne diseases?

1. Yes. 2. No.

24. What is the source of bean seed planted in your field?

1. From own saved seed 2. From shops and seed companies.

3. PAM. 4. NGOs 5. Market 6. Friends and relatives

25. What is the anthracnose severity score (1-9 Scale)?

26. How do you rate anthracnose incidence observed in the field?

1. 0-20 percent. 2. 21-40 percent 3. 41-60 percent

4. 61-80 percent 5. More than 80 percent

27. Was crop rotation considered?

1. Yes 2. No

28. What Planting Method was used?

1. Flat 2. Mounds 3. Ridges

29. What was the plant density like?

1. Low 2. Medium 3. High

30. How many continuous rainy days did you experience during the current cropping season?

1. 3 days 2. 4-5 days 3. 7 -9days

4. 10-14 days 5. More than 2 weeks

Appendix B: The developmental stages of the common bean plant.

Stage	Description
V0	Germination: Water absorption by the seed, emergence of the radicle, and transformation into the primary root.
V1	Emergence: Cotyledons appear at soil level and begin to separate. The epicotyl initiates its development.
V2	Primary leaves: Totally opened primary leaves.
V3	First trifoliate leaf: The first trifoliate leaf opens and the second trifoliate leaf appears.
V4	Third trifoliate leaf: The third trifoliate leaf opens and the buds on the lower nodes produce branches.
R5	Preflowering: The first flower bud or the first raceme appears. Flower buds in determinate varieties are formed on the last stem or branch node. In indeterminate varieties racemes are first observed on the lower nodes.
R6	Flowering: The first flower opens.
R7	Pod formation: The first pod appears being more than 2.5 cm long.
R8	Pod filling: The first pod begins to fill (seed growth). At the end of the stage seeds lose their green colour and begin to show varietal characteristics. Defoliation initiates.
R9	Physiological maturity: Pods lose their pigmentation and begin to dry. Seeds develop their typical varietal colour.

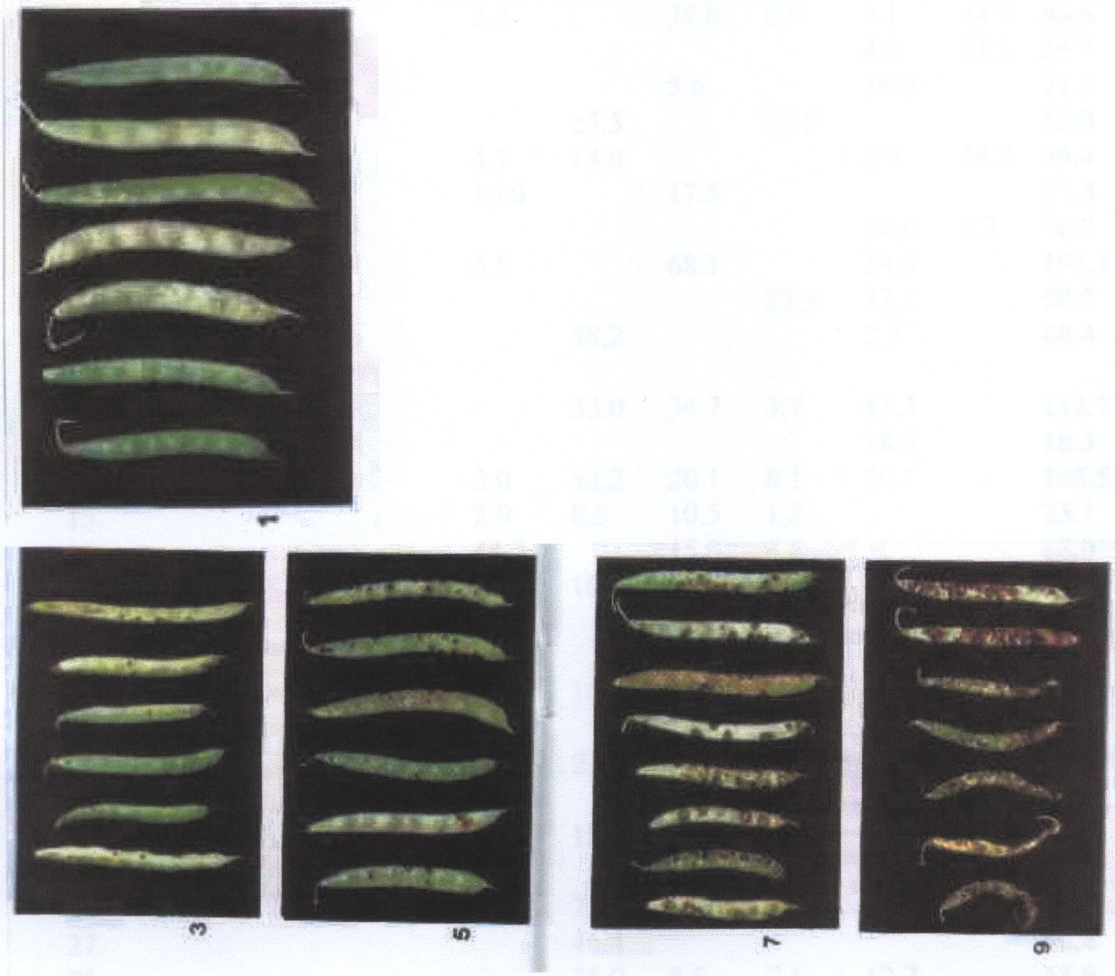
SOURCE: van Schoonhoven, A. and Pastor-Corrales, M.A. (1987).
V= Vegetative, R=Reproductive.

Appendix C: Scoring scale (1-9) used to evaluate the reaction of bean germplasm to anthracnose pathogen

Scale	Description
1	No visible symptoms.
3	Presence of very few and small lesions, mostly on the primary vein of the leaf's lower side or on the pod that cover approximately 1 percent of the surface area.
5	Presence of several small lesions on the petiole or on the primary and secondary veins of the leaf's lower side. On the pods, small (less than 2 mm) round lesions with or without reduced sporulation cover approximately 5 percent of the pod surface area.
7	Presence of numerous enlarged lesions on the lower side of the leaf. Necrotic lesions can also be observed on the upper leaf surface and petioles. On the pods the presence of medium-sized (larger than 2 mm in diameter) lesions are evident but also some small and large lesions generally with sporulation that cover approximately 10 percent of the pod surface area may be found.
9	Severe necrosis on 25 percent or more of the plant tissue is evident as a result of lesions on the leaf, petioles, stem, branches, and even on the growing point, which results in death of much of the plant tissues. The presence of numerous, large, sporulating sunken cankers can result in pod malformation, low seed number and death of the pod.

SOURCE: van Schoonhoven, A. and Pastor-Corrales, M.A. (1987).

Appendix D: Standard figure for scoring scale (1-9) used to evaluate the reaction of bean germplasm pods to anthracnose pathogen (*Colletotrichum lindemuthianum*).



SOURCE: van Schoonhoven, A. and Pastor-Corrales, M.A. (1987).

Appendix E: Rainfall Summary at Mutanda Research Station for 2003/2004 season

Day	Month										
	July	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	**Totals**
1.					3.5		39.0	6.5	3.1	34.5	**86.6**
2.									4.2	21.5	**25.7**
3.							5.6		16.0		**21.6**
4.						25.5		26.5			**52.0**
5.					4.7	14.0			2.0	18.7	**39.4**
6.					10.0		17.5				**27.5**
7.									20.0	6.7	**26.7**
8.					5.5		68.1		29.5		**103.1**
9.								23.3	27.2		**50.5**
10.						58.2			2.2		**60.4**
11.											
12.						33.0	34.7	3.7	41.3		**112.7**
13.									18.3		**18.3**
14.					3.0	44.2	20.1	8.1	30.1		**105.5**
15.					2.9	8.5	10.5	1.2			**23.1**
16.					44.2		15.0	8.8			**68.0**
17.				73.5		10.1		87.5			**171.1**
18.											
19.				1.0							**1.0**
20.				3.0		10.6					**13.6**
21.					25.0		4.0	32.0			**61.0**
22.						2.5		6.7	51.1		**60.3**
23.											
24.						15.8					**15.8**
25.							2.5	4.6	1.5		**8.6**
26.						7.4		39.0	38.7		**85.1**
27.						46.4					**46.4**
28.						35.0	8.5	7.6	12.7		**63.8**
29.				1.0	14.5						**15.5**
30.				1.5	1.0		4.5				**7.0**
31.											
2003/2004 Total (mm)				***80.0***	***114.3***	***311.2***	***230.0***	***255.5***	***297.9***	***81.4***	**1,370.3**
Total Rain days				5	10	13	12	13	15	4	**72**
2002/2003 Total (mm)			***13.5***	***71.3***	***131.8***	***167.5***	***130.2***	***226.4***	***176.0***	***75.0***	**991.7**
Total Rain days			2	5	12	17	12	17	15	6	**86**
Difference (mm)			13.5	8.7	17.5	143.7	99.3	29.1	121.9	6.4	

Appendix F: Survey Locations in major bean growing areas in Zambia

MWINILUNGA

Sample Serial #:	District name:	Location Name	Latitude: S	Longitude: E	Altitude: masl	Anthracnose severity (1-9)	Anthracnose incidence %
MWN1	Mwinilunga	Miluna	11°44.874	24°27.415	1406	1	18
MWN2	Mwinilunga	Ndumba	11°44.565	24°28.165	1385	7	84
MWN3	Mwinilunga	Unknown	11°44.296	24°35.442	1441	7	76
MWN4	Mwinilunga	Unknown	11°44.316	24°35.418	1437	9	88
MWN5	Mwinilunga	Samuteba	11°45.699	24°40.643	1497	7	50
MWN6	Mwinilunga	Samuteba	11°45.675	24°40.692	1497	7	95
MWN7	Mwinilunga	Samuteba	11°45.660	24°40.615	1501	7	89
MWN8	Mwinilunga	Samuteba Sc	11°44.605	24°39.370	1322	9	94
MWN9	Mwinilunga	Nyang'ombe	11°43.888	24°33.988	1445	7	86
MWN10	Mwinilunga	Kawiko	11°40.896	24°24.928	1399	7	28
MWN11	Mwinilunga	Kawiko	11°40.900	24°24.901	1397	7	42
MWN12	Mwinilunga	Kawiko	11°40.453	24°24.824	1396	9	92
MWN13	Mwinilunga	Kampemba	11°45.300	24°23.934	1380	3	45
MWN14	Mwinilunga	Kampemba	11°45.273	24°24.000	1378	9	100
MWN15	Mwinilunga	Kampemba	11°45.116	24°24.047	1383	7	57
SOLWEZI							
SOL1	Solwezi	Research St	12°25.387	26°13.002	1341	5	45
SOL 2	Solwezi	Kyafukuma	12°00.096	26°26.446	1477	5	80
SOL 3	Solwezi	Farm Inst.	12°01.001	26°26.238	1521	5	47
SOL 4	Solwezi	Mulimbi	12°01.956	26°26.956	1489	1	18
SOL 5	Solwezi	Kang'wena	12°22.925	27°19.400	1296	3	15
SOL 6	Solwezi	ZNS Farm	12°01.505	26°28.848	1469	3	13
SOL 7	Solwezi	Katandano	12°02.135	26°27.948	1248	5	60
SOL 8	Solwezi	Mutanda R.	12°25.880	26°12.590	1341	3	9
SOL 9	Solwezi	Unknown	12°21.323	26°15.489	1356	3	17
SOL10	Solwezi	Musolokoto	12°23.815	26°14.101	1303	3	18
SOL11	Solwezi	Kajongo	12°23.251	26°12.228	1349	3	12
SOL12	Solwezi	Mulimbi	12°23.155	26°11.247	1351	3	20
SOL13	Solwezi	Mutoma	12°23.162	26°11.168	1353	9	84
SOL14	Solwezi	Yakulanda	12°23.721	26°13.908	1311	3	10
SOL15	Solwezi	Agric.Camp	12°23.555	26°14.439	1301	3	17

Research St = Mutanda Research Station
Mutanda R = Mutanda River

Appendix F: Cont.

MANSA

Sample Serial #:	District name:	Location Name	Latitude: S	Longitude: E	Altitude: Masl	Anthracnose severity (1-9)	Anthracnose incidence %
MAN1	Mansa	TAS1	$11^0$13.886	28^0 56.703	1253	1	18
MAN2	Mansa	TAS2	$11^0$14.579	28^0 57.231	1253	3	20
MAN3	Mansa	Muwang'uni	$11^0$15.193	28^0 52.765	1215	1	17
MAN4	Mansa	Chikuwe	$11^0$14.596	28^0 52.968	1269	1	18
MAN5	Mansa	Chalowa	$11^0$15.193	28^0 56.703	1247	1	15
MAN6	Mansa	Chilambe	$11^0$18.790	28^0 51.395	1249	7	49
MAN7	Mansa	Sando1	$11^0$19.291	28^0 50.600	1264	1	19
MAN8	Mansa	Sando2	$11^0$19.299	28^0 50.447	1270	7	76
MAN9	Mansa	Kalasa Rd	$11^0$19.363	28^0 49.943	1286	7	56
MAN10	Mansa	Unknown	$11^0$05.775	28^0 53.961	1304	1	12
MAN11	Mansa	Chikotwe	$11^0$09.039	28^0 53.143	1263	5	65
MAN12	Mansa	Lubende	$11^0$16.459	29^0 16.058	1252	3	20
MAN13	Mansa	Unknown	$11^0$09.290	28^0 58.401	1263	3	15
MAN14	Mansa	TAS3	$11^0$14.299	28^0 57.409	1254	7	58
MAN15	Mansa	Kalimankonde	$11^0$13.388	28^0 56.627	1258	3	20

SAMFYA

SaS 1	Samfya/Serenje	Sondashi	$11^0$43.570	29^0 30.121	1223	3	17
SaS 2	Samfya/Serenje	Unknown	$11^0$59.834	29^0 33.832	1211	3	18
SaS 3	Samfya/Serenje	Yamba	$11^0$58.770	29^0 31.944	1253	3	16
SaS 4	Samfya/Serenje	Mukoso	$11^0$35.313	29^0 26.646	1223	5	18
SaS 5	Samfya/Serenje	Unknown	$11^0$40.322	29^0 27.648	1230	3	10
SaS 6	Samfya/Serenje	Unknown	$12^0$47.760	30^0 24.968	1456	5	75
SaS 7	Samfya/Serenje	Unknown	$12^0$47.005	30^0 25.212	1431	5	80
SaS 8	Samfya/Serenje	Unknown	$12^0$51.986	30^0 24.230	1456	1	9
SaS 9	Samfya/Serenje	Unknown	$13^0$30.480	29^0 47.277	1564	1	16
SaS 10	Samfya/Serenje	Unknown	$13^0$47.368	29^0 02.873	1214	3	20

MPIKA

MPK 1	Mpika	FTC	$11^0$49.235	31^0 27.057	1404	5	60
MPK 2	Mpika	Kaole	$11^0$57.214	31^0 25.361	1445	3	20
MPK 3	Mpika	Munamala	$11^0$59.108	31^0 21.360	1430	1	16
MPK 4	Mpika	ZNS-Mpika	$11^0$59.924	31^0 19.921	1357	1	18
MPK 5	Mpika	ZNS-Mpika	$11^0$57.948	31^0 20.095	1359	1	20
MPK 6	Mpika	Lubanga	$12^0$05.384	31^0 14.476	1338	3	15
MPK 7	Mpika	Mufubushi	$12^0$07.142	31^0 14.537	1416	3	19
MPK 8	Mpika	ZCA-Mpika	$11^0$42.948	31^0 27.286	1415	1	13
MPK 9	Mpika	ZCA-Mpika	$11^0$42.209	31^0 27.942	1412	3	14
MPK10	Mpika	Unknown	$11^0$42.300	31^0 18.538	1418	3	12

Appendix F: Cont.

KASAMA

Sample Serial #:	District name:	Location Name	Latitude: S	Longitude: E	Altitude: Masl	Anthracnose severity (1-9)	Anthracnose incidence %
KAS 1	Kasama	Chamfubu	$09^0$52.679	$31^0$21.678	1341	7	48
KAS 2	Kasama	Lushinga	$09^0$53.152	$31^0$19.648	1366	5	81
KAS 3	Kasama	Mulambe	$10^0$03.599	$31^0$15.902	1425	5	56
KAS 4	Kasama	Misamfu	$10^0$10.717	$31^0$12.529	1412	1	18
KAS 5	Kasama	Musa	$10^0$19.054	$31^0$15.902	1281	9	90
KAS 6	Kasama	KFTI 1	$10^0$19.061	$31^0$12.003	1278	3	40
KAS 7	Kasama	KTFI 2	$10^0$19.066	$31^0$12.017	1281	5	19
KAS 8	Kasama	KTFI 3	$10^0$19.121	$31^0$12.019	1285	3	59
KAS 9	Kasama	Musa	$10^0$20.016	$31^0$13.326	1294	3	16
KAS10	Kasama	Onole	$10^0$20.160	$31^0$13.565	1320	5	80
KAS11	Kasama	Unknown	$10^0$20.150	$31^0$18.570	1318	5	64
KAS12	Kasama	Tafimbwa	$10^0$22.447	$31^0$14.720	1280	5	52
KAS13	Kasama	Unknown	$10^0$22.403	$31^0$14.903	1282	1	15
KAS14	Kasama	Farm Inst.	$10^0$22.391	$31^0$13.326	1283	1	10
KAS15	Kasama	Nseluka	$09^0$58.752	$31^0$15.002	1342	3	50
MBALA							
MBA 1	Mbala	Senga	$09^0$10.001	$31^0$16.400	1524	5	81
MBA 2	Mbala	Katito1	$09^0$03.003	$31^0$23.092	1715	1	34
MBA 3	Mbala	Katito2	$09^0$02.387	$31^0$22.869	1742	1	12
MBA 4	Mbala	Buningi	$08^0$56.154	$31^0$21.610	1701	3	87
MBA 5	Mbala	Outbound	$08^0$52.150	$31^0$16.358	1487	3	15
MBA 6	Mbala	Unknown	$08^0$58.902	$31^0$21.002	1741	5	89
MBA 7	Mbala	Kaziwe Sch.	$09^0$12.261	$31^0$21.128	1542	7	91
MBA 8	Mbala	Unknown	$09^0$12.280	$31^0$22.130	1550	7	83
MBA 9	Mbala	Musombizi1	$09^0$18.346	$31^0$16.750	1521	5	86
MBA10	Mbala	Musombizi2	$09^0$17.384	$31^0$16.956	1541	3	28

Survey Area lay between Latitude 08052.150 - 13047.368 and Longitude 24^0 23.934-31^0 27

Appendix G: Field experiment analysis of variance (ANOVA) Tables

Appendix G1: Analysis of Variance of percent stand count at harvest of different bean genotypes

Source of Variation	Degrees of freedom	Sum of Squares	Mean square	F-Value	Prob.
Replication	3	1350.11	450.037	8.54**	0.0001
Genotype	17	4917.50	289.256	5.498**	0.0000
Error	51	2688.39	52.714		
Non-additivity	1	11.30	11.302	0.21	
Residual	50	2677.09	53.542		
Total	71	8956.00			
Mean			74.667		
CV%	9.72				

*Significantly different at 0.05 probability level.

**Significantly different at 0.01 probability level.

Appendix G2: **Analysis of Variance of days to 50 percent flowering of different bean genotypes**

Source of Variation	Degrees of freedom	Sum of Squares	Mean square	F-Value	Prob.
Replication	3	18.00	6.000	0.97	0.4149
Genotype	17	369.78	21.752	3.51**	0.0003
Error	51	316.00	6.196		
Non-additivity	1	35.63	35.627	6.35	0.0150
Residual	50	280.37	5.607		
Total	71	703.78			
Mean			42.444		
CV%	5.86				

*Significantly different at 0.05 probability level.

**Significantly different at 0.01 probability level.

Appendix G3: Analysis of Variance of days to Maturity of different bean genotypes

Source of Variation	Degrees of freedom	Sum of Squares	Mean square	F-Value	Prob.
Replication	3	22.38	7.458	1.95	0.1338
Genotype	17	504.24	29.661	7.74**	0.0000
Error	51	195.37	3.831		
Non-additivity	1	5.12	5.122	6.35	0.2514
Residual	50	190.25	3.805		
Total	71	721.99			
Mean			70.736		
CV%	2.77				

**Significantly different at 0.05 probability level.

*Significantly different at 0.01 probability level.

Appendix G4: Analysis of Variance of 100 seed weight of different bean genotypes

Source of Variation	Degrees of freedom	Sum of Squares	Mean square	F-Value	Prob.
Replication	3	5.83	1.944	0.93	0.4311
Genotype	17	3843.5	226.088	108.61**	0.0000
Error	51	106.17	2.082		
Non-additivity	1	1.12	1.1821	0.53	0.2514
Residual	50	105.05	2.101		
Total	71	3955.50			
Mean			22.750		
CV%	6.34				

*Significantly different at 0.05 probability level.

**Significantly different at 0.01 probability level.

Appendix G5: Analysis of Variance of anthracnose disease severity at Pre-flowering stage of different bean genotypes

Source of Variation	Degrees of freedom	Sum of Squares	Mean square	F-Value	Prob.
Replication	3	0.61	0.204	1.63	0.1948
Genotype	17	4.50	0.265	2.11*	0.0205
Error	51	6.39	0.125		
Non-additivity	1	4.65	4.647	133.39**	0.0000
Residual	50	1.74	0.035		
Total	71	11.50			
Mean			1.083		
CV%	32.67				

*Significantly different at 0.05 probability level.

**Significantly different at 0.01 probability level.

Appendix G6: Analysis of Variance of anthracnose disease severity at flowering stage of different bean genotypes

Source of Variation	Degrees of freedom	Sum of Squares	Mean square	F-Value	Prob.
Replication	3	1.67	0.556	0.48	0.6993
Genotype	17	46.78	2.752	2.37**	0.0092
Error	51	59.33	1.163		
Non-additivity	1	5.31	5.311	4.92	0.0312
Residual	50	54.02	1.080		
Total	71	107.78			
Mean			1.944		
CV%	55.47				

*Significantly different at 0.05 probability level.

**Significantly different at 0.01 probability level.

Appendix G7: Analysis of Variance of common bacterial blight disease severity at pod filling stage of different bean genotypes

Source of Variation	Degrees of freedom	Sum of Squares	Mean square	F-Value	Prob.
Replication	3	35.15	11.718	4.34**	0.0084
Genotype	17	140.57	8.269	3.06**	0.0001
Error	51	137.60	2.698		
Non-additivity	1	0.44	0.444	0.16	
Residual	50	137.15	2.743		
Total	71	313.32			
Mean			4.153		
CV%	39.55				

*Significantly different at 0.05 probability level.

**Significantly different at 0.01 probability level.

Appendix G8: Analysis of Variance of grain yields of different bean genotypes

Source of Variation	Degrees of freedom	Sum of Squares	Mean square	F-Value	Prob.
Replication	3	57920.50	19306.833	1.92	0.1379
Genotype	17	849785.28	49987.369	4.97**	0.0000
Error	51	512621.50	10051.402		
Non-additivity	1	39157.10	39157.104	4.14	0.0473
Residual	50	473464.40	9469.288		
Total	71	1420327.28			
Mean			417.306		
CV%	24.02				

*Significantly different at 0.05 probability level.

**Significantly different at 0.01 probability level.