BIOLOGY AND CONTROL OF BEAN ANTHRACNOSE IN ETHIOPIA

by

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Chapter 1

Introductory statements

Common bean (*Phaseolus vulgaris* L.) is an important food legume crop and provides an essential part of the diet of Ethiopians. It is grown as a subsistence crop under traditional farming systems, usually as an intercrop with maize, sorghum or coffee. In Ethiopia, common beans are grown between 1200-2000 m above sea level under diverse climatic conditions in at least four climatic regions. These include the central rift valley and the Hararghe lowlands representing the semi-arid areas, Hararghe highlands, southern Ethiopia representing the mid-altitude, cooler areas, and western Ethiopia representing the sub-humid regions. This wide range of geographical and ecological conditions is associated with a diversity of bean diseases.

Diseases are often a significant constraint in bean production, with anthracnose, caused by *Colletotrichum lindemuthianum* (Sacc. & Magn.) Briosi and Cav., being an important disease in Ethiopia, particularly in a cool and humid environment. Substantial yield losses have been experienced in susceptible varieties or when infected seed is used for planting.

The study was initiated on the premise that an understanding of the biology of the pathogen and epidemiology of bean anthracnose will lead to improved management strategies. The major objective was to investigate certain aspects of bean anthracnose in Ethiopia that could be manipulated for disease control, particularly in resource poor farming systems. Specific objectives were to:

- Assess the geographical distribution of anthracnose and other major diseases of common bean in Ethiopia;
- Collect anthracnose isolates and determine the genetic composition of the pathogen population in Ethiopia;
- Evaluate the effect of bean cultivar mixtures on the development of anthracnose in time and space;

- Determine yield losses caused by anthracnose and options for chemical control;
- Study the genetic resistance of a diverse collection of common bean cultivars to representative isolates of *C. lindemuthianum*.

The thesis is structured in a literature review followed by chapters each addressing the above objectives. Chapters were formatted as individual articles, thus some repetition was unavoidable.

The study started in 2000 and was organized in three phases. During the first phase, course work was undertaken in the Department of Plant Pathology at the University of the Free State and the experimental framework was designed. Purification of isolates of *C. lindemuthianum* was conducted in the laboratory and differential cultivars were multiplied in the greenhouse. During the second phase (December 2001 and May 2002), field work was conducted in Ethiopia of which the main activities included a survey, and field and greenhouse experiments at Ambo and Bako. The third phase comprised data organization and analysis, and writing of the thesis.

Chapter 2

An overview of the biology and control of bean anthracnose caused by *Colletotrichum lindemuthianum*, with special emphasis on Ethiopia

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is an important food legume crop in many parts of the world. The annual global production has been estimated at 18 million tons grown on 27 million hectares, with an average yield of 696 kg/ha (FAOSTAT database May 2003; http://www.ciat.cgiar.org/). In Ethiopia, beans grown as a subsistence crop under traditional farming systems and often intercropped with maize, sorghum and coffee, provide an essential part of the daily diet (Habtu *et al.*, 1995).

Diseases are major constraints in the production of bean. Of particular concern is anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. & Magn.) Briosi and Cav. (teleomorph = *Glomerella cingulata* (Stonem.) (Bailey *et al.*, 1992). Anthracnose is the most important disease of bean in Ethiopia and can cause complete yield losses on susceptible cultivars when contaminated seed are planted. The impact of epidemics is further enhanced when favourable environmental conditions prevail (Pastor-Corrales and Tu, 1989).

Symptoms attributed to pathogens within the genus *Colletotrichum* are commonly referred to as *anthracnose* and typically include depressed, black lesions which are subcuticular or angular bearing erumpent, pink spore masses (Sutton, 1992). The understanding of pathogen development in relation to the host is a prerequisite for conducting and interpreting epidemiological studies (Bailey *et al.*, 1992). It also helps to study the physiological, genetic and molecular aspects of *C. lindemuthianum* to develop appropriate strategies for anthracnose control (Maeli *et al.*, 2000). Pastor-Corrales and Tu (1989) summarized the etiology of this fungus.

This review presents a summary of current knowledge on the biology, epidemiology, physiology and control of bean anthracnose.

OCCURRENCE AND DISTRIBUTION OF THE PATHOGEN

Anthracnose was first described from plant specimens obtained in Germany in 1875 (Walker, 1957). Since then the disease has become one of the most important and widely distributed throughout the world. It has been reported in European countries (Hubbeling, 1977; Tu and Aylesworth, 1980), Canada (Tu, 1983), China (Tu and Aylesworth, 1980), Latin America (CIAT, 1988), and the USA (Zaumeyer and Thomas, 1957). It is also widely distributed in tropical regions under warm and moist weather conditions and has been reported in eastern Africa, particularly Kenya, Uganda, Tanzania and Ethiopia (CIAT, 1986). Although plant residues contribute to pathogen survival and dissemination (Chaves, 1980), infected seed plays an important role in the international distribution of the antracnose pathogen (Chaves, 1980). This is especially true for many African countries where farmers continuously use infected seed, contributing to the distribution of the pathogen.

ECONOMIC IMPORTANCE

Yield losses

Losses due to anthracnose seed infection in susceptible cultivars have been estimated at 95% (Large, 1966), and up to 100% when infection occurs on young bean plants (Chaves, 1980). In Tanzania, on a highly susceptible variety, the disease partially defoliated the crop at mid-flowering and incurred yield losses as high as 92% (Allen, 1983). In Ethiopia, anthracnose has been shown to reduce yield by as much as 62% (Beshir, 1997). Besides anthracnose, diseases such as golden mosaic virus, web blight, rust, angular leaf spot and ascochyta blight are also important in bean production (Pastor-Corrales and Tu, 1989; Pastor-Corrales *et al.*, 1995) and losses caused by a single disease are usually difficult to determine.

Origin, distribution, production and usage of common bean in Ethiopia

Common bean was introduced to Ethiopia by the Portuguese in the 16th century (Assefa, 1985). In Ethiopia, food legumes are cultivated in the north, central and southwest regions and cover approximately 12-14% of the total cropping area (Assefa, 1985). Legume crops are second to cereals as a source of human and animal food. The most important legume crops are faba bean (*Vicia faba* L.), field pea (*Pisum sativum* L.), chickpea *(Cicer arietinum* L.), lentil (*Lens culinaris* Medik.) and common bean (*P. vulgaris* L). Common bean is widely grown in the east of Ethiopia (Hararghe highlands), south and southwest (Sidama), west (Keffa and Wollega) and in the rift valley (Shimelis *et al.*, 1990).

In Ethiopia, common bean is extensively consumed in a boiled form (mixed with sorghum or maize) or as a vegetable in traditional dishes. It can also be mixed with other pulses to prepare a local soup (Kassahun, 1990). Besides its local consumption, white pea bean is used for export purposes. In 1973, 10% of the country's total export earnings originated from common bean (Ayele, 1990). This contributed 86% of all exports in the pulses and oil seed sector (IAR, 1991).

Under small scale farming conditions, bean production is low and the average yield has been estimated at 600-700 kg/ha in contrast to the 2500-3000 kg/ha which can be achieved with better management (Amare, 1987). More recently, the FAOSTAT database of May 2003 (http://www.ciat.cgiar.org/) reported that on average, 750 kg beans are produced per hectare in Ethiopia, with a total annual production of 90 000 tons. This average is slightly higher than that for the entire world (696 kg/ha) and Africa (629 kg/ha). Low productivity has been associated with the lack of pure seed (Ayele, 1990), poor soil fertility (Ohlander, 1980), moisture stress (Kidane, 1987), pests (Ferede and Tsedeke, 1986), weeds (Etagegnehu, 1987) and diseases (Ohlander, 1980; Habtu, 1987). Anthracnose is the most important disease of bean in Ethiopia (Habtu, 1987).

ANTHRACNOSE SYMPTOMS

Dillard (1988) described anthracnose symptoms as follows. severely infected seeds show brown to black blemishes and sunken lesions (Fig.1A). Seedlings grown from infected seeds may have dark-brown to black sunken lesions in the cotyledons (Fig. 1B) which usually result in premature stunting of plants. Furthermore, the stem may be girdled, thus killing the seedling. Under moist conditions, small, pink masses of spores are produced in the lesions. Spores produced on cotyledon and stem lesions may spread to the leaves. On the lower leaf surface, linear, dark brick red to black lesions occur on the veins (Fig. 1C) and as the disease progresses, discolouration appears on the upper leaf surface. Leaf symptoms are not obvious and are easily overlooked when examining bean fields. Infected pods produce small, reddish brown to black blemishes, which are distinctly circular (Fig. 1D). Mature stem lesions are surrounded by a circular, reddish brown to black border with a greyish black interior (Fig. 1E). The pathogen can eventually infect the entire crop stand (Fig. 1F).

TAXONOMY OF THE PATHOGEN

Bean anthracnose is caused by the fungal pathogen *C. lindemuthianum*. Its perfect stage, *Glomerella cingulata* (Kimati and Galli, 1970), is rarely found in axenic culture or in nature. Thus, the imperfect name C. *lindemuthianum* is commonly used. The pathogen falls under the genus *Colletotrichum*, classified under Deuteromycetes, order Melanconiales, family Melanconiaceae and section Hyalosporae (Clements and Shear, 1957; Alexopoulos, 1962). Typical morphological characteristics in culture are colony appearance (Fig. 2A), conidia (Fig. 2B), mucilage showing disc-shaped or cushion-shaped, waxy structures (Fig. 2C), and dark spines or setae at the edges or among the conidiophores (Fig. 2D) which are brown, septate, and slightly swollen at the base to taper gently to the rounded paler apex. Acervuli have pale, salmon-colour spore masses.

Colletotrichum lindemuthianum differs from other species in the genus by its growth and dark pigmentation in culture (Von Arx, 1957; Baxter *et al.*, 1985). The conidiophores are simple and elongate. Conidia are unicellular, hyaline, and

cylindrical with both ends obtuse or with a narrow and truncate base. Conidia are uninucleate, and usually have a clear vacuole-like body near the centre. A conidium germinates in six to nine hours and produces one to four germ tubes. The germ tubes form appressoria at their tips during pathogenesis (Walker, 1957; Zaumeyer and Thomas, 1957).

The genus *Colletotrichum* contains several species of economic importance. Von Arx (1957) revised the classification of the fungi in the genera *Colletotrichum* and *Gloeosporium,* recognising 13 species. Since then, more species have been discovered and reported (Kirangu, 1983) including *C. lindemuthianum, C. destructivum, C. fuscum, C. fusarioides, C. phyllachoioides, C. paludosum, C. atramentarium, C. crassipes, C. graminicola, C. dematium, C. spinaceae, C. coffeanum and C. trifolli.* Virtually all species in this genus are pathogenic to many crop species of economic importance. They cause diseases such as fruit rots, wilting, leaf spots, necrosis and anthracnose. Some of the crops affected are beans, soybeans and cowpeas (Von Arx, 1957; Kirangu, 1983).

Traditionally, the taxonomy of *Colletotrichum* has been based on conidial shape and size, appressorial shape, colony morphology, and growth rate (Kemp *et al.*, 1991; Yang and Sweetingham, 1998). Conidia of *C. gloeosporioides* are cylindrical and colonies are gray to dark gray. By contrast, conidia of *C. acutatum* are fusiforum, colonies often have pink to carmine pigmentation and have a slower growth rate than *C. gloeosporioides* (Sutton, 1992). As stated above conidia of *C. lindemuthianum* are unicellular, hyaline, and cylindrical with both ends obtuse or with a narrow and truncate base. Conidiogenous cells of *Colletotrichum spp.* are usually aggregated in conidiomata. The former appear to be homologous with setae, the production of which is controlled by environmental factors.

HOST RANGE

Colletotrichum lindemuthianum has a wide host range and appears to be well adapted over an array of ecological habitats. It occurs not only on common bean but also on other crops, both in temperate and tropical areas (Walker, 1957). The

distribution and success of this pathogen are associated with its host range, favourable environmental conditions such as temperature, rainfall and relative humidity, and aspects of the host plant that influence its survival, e.g. infected seed and crop residues (Zadoks and Schein, 1979; Gunipert, 1989; Switch and Whittington, 1983).

Colletotrichum lindemuthianum has been isolated from lima beans (*Phaseolus lunatus* L.), scarlet runner beans (*P. coccineus*), tepary beans *(P. acutifolius var. latifolius* L. (Walp.)), mung beans (*P. aureus*), cowpea (*Vigna unguiculata*), Kudzu beans (*Dolichos bifloris* L.), and broad beans (*Vicia faba* L.) (Walker, 1957; Jefferies *et al.*, 1990). Koch (1996) reported that representative *Colletotrichum* isolates were collected from *Medicago sativa* L. in South Africa. The cultural morphology on potato-carrot agar and pathogenicity on *M. sativa, Glycine max* L., *Nicotiana tabacum* L. and *P. vulgaris* were compared. Out of these, five species of *Colletotrichum* were distinguished, largely on the basis of their conidial shape.

INFECTION PROCESS

Adhesion of spores

The first essential feature of successful pathogenesis is the attachment of spores to the plant surface (Hamer *et al.*, 1988). According to Young and Krauss (1984), spores of *C. lindemuthianum* suspended in water adhered to bean hypocotyls within one hour. When the wax was removed from surfaces of bean hypocotyls, the number of adhering spores was reduced by 80%. Adhesion ensures that a pathogen remains in contact with its host for as long as it is necessary for penetration, whether mechanical or enzymatic (Bailey *et al.*, 1992). Adhesive competence depends on conidia and the leaf surface. Mercure *et al.* (1994) reported a significant difference in the ability of 14-day-old conidia to adhere to the leaves of 5- and 8-week-old plants.

Spore germination and penetration

Colletotrichum species have two main infection strategies, i.e. subcuticular and intercellular hemibiotrophism. The infection process of *C. lindemuthianum* on *P. vulgaris* (Mercer *et al.*, 1975; Elliston *et al.*, 1976; O'Connell *et al.*, 1985; Perfect,

1999) follows the latter approach. Conidia adhere to plant surfaces and undergo complex differentiation, including germination, the development of germ tubes, appressoria and infection pegs and finally, penetration of the host through natural opening e.g. stomata, wounds or directly through the cuticle (Bailey *et al.*, 1992; Agrios, 1997). According to different authors the conidia germinate within 2-6 h (O'Connell *et al.*, 1985), 18 h (O'Connell *et al.*, 1985) and 12 h (Mercer *et al.*, 1975) to produce germ tubes, and then proceed to form appressoria, which penetrate the cuticle directly.

Following penetration, the pathogen develops beneath the cuticle by forming an intramural network of hyphae, before spreading rapidly throughout the tissue with both inter- and intracellular hyphae, killing the host cells in advance. Without any detectable detrimental changes to the host, *C. lindemuthianum* is transformed within a few hours to a highly aggressive and destructive pathogen. The morphology of the pathogen also changes. Instead of procuring large intracellular primary hyphae, thin secondary hyphae form which grow both intracellulary and intramurally, causing extensive degradation of cell walls and death of cells (O'Connell *et al.*, 1985). The mycelium masses then form acervuli with a water-soluble gelatinous matrix (Sindhan and Bose, 1981), which later rupture the host cuticle and form lesions that turn dark brown (Mercer *et al.*, 1975).

EPIDEMIOLOGY OF ANTHRACNOSE

Survival

According to Pastor-Corrales and Tu (1989) *C. lindemuthianum* can overwinter either in seed or infected crop residues. However, its longevity in infected pods and seed varies considerably, depending on environmental conditions. Moisture is an important factor that influences the survival of the fungus. The fungus survived two to five years on pods and seeds that were air-dried and kept in storage at 4°C or on dry infected plant materials left in the field. It can survive as dormant mycelium within the seed coat and is capable of withstanding temperatures of –15°C to –20°C for a limited period.

Tu (1983) and Dillard and Cobb (1993) determined that *C. lindemuthianum* could overwinter in field soils and debris in Michigan, and that the overwintering population was sufficient for initiating an epidemic in beans the following season. However, Araya *et al.* (1987) considered the level of overwintering inoculum in Michigan insufficient to cause disease the following year and did not recommend rotation.

Sources of inoculum

Other than infected seed and plant residues, insects, clothing and animals may also disperse *C. lindemuthianum* to healthy plants (Chaves, 1980). Although the pathogen may survive in plant residue or in bean straw, seed plays an important role in the international distribution of the pathogen. The fungus may remain viable in seed for three to five years. The majority of farmers in east Africa retain their seed from a previously grown crop, and most probably contribute to the carry over and spread of the disease (Leaky and Simbwa-Bunnya, 1972).

Lesions on the cotyledons often serve as sources of secondary inocula. The conidia are water-borne and are washed down on to the hypocotyls and subsequently the stems. The primary leaves also serve as foci of secondary infection.

The major sources of inoculum for *Colletotrichum* are conidia produced in acervuli and ascospores produced in and released from perithecia. In young acervuli and perithecia, conidia and ascospores are encased in a moist hydrophilic mucilaginous material, or spore matrix (Bailey *et al.*, 1992). Dissemination of spores from young acervuli occurs in water droplets, whilst wind can distribute dry spore masses from the older acervuli and ascospores from perithecia (Nicholson and Moraes, 1980). The matrix maintains spore viability under conditions of low humidity (Nicholson and Moraes, 1980) and protects spores from extreme temperature and ultra-violet light, and from toxic plant metabolites (Nicholson *et al.*, 1986). McRae and Stevens (1990) have reported that the presence of a mucilaginous matrix in conidial inoculum will hasten the onset of symptoms.

Dissemination

Various studies have shown that the spread of anthracnose from the initial infection focus in the field depends on the speed and direction of the wind. According to Ntahimpera *et al.* (1996) prevailing wind associated with rain splash is an important factor determining the dissemination of anthracnose. Long-distance dissemination (3-5m) may result from raindrops being blown by gusting winds (Tu, 1983). Araya Fernandez *et al.* (1987) reported that the number of foci of initial inoculum in the field was linearly related to the incidence of anthracnose on leaves, but not to pod infection. Similarly, under field conditions during the rainy season, incidence was higher on leaves, whereas during the dry season, incidence was higher on pods. Tu (1983) remarked that the disease spreads rapidly by spores carried in splashing raindrops, or through human activities or implements that come in contact with diseased plants. Hence, the average distance of spread is 3-4.6 m per rainstorm of more than 10 mm precipitation. Thus, in a growing season, one diseased plant can effectively spread the disease to other plants within a 30 m radius.

Environmental factors

Optimum conditions for *C. lindemuthianum* development include high relative humidity (92%), temperatures between 18 and 22°C (Tu, 1983; Maeli *et al.,* 2000) and moderate rainfall at frequent intervals (Tu, 1983). Infection is favoured by moderate temperatures between 13-26°C, with an optimum at 17°C. High humidity (>92%) or free moisture must be present for infection to occur successfully. Moderate rainfall at frequent intervals also is essential for the local dissemination of conidia and development of anthracnose epidemics (Pastor-Corrales and Tu, 1989; Pastor-Corrales *et al.*, 1995).

CONTROL OF ANTHRACNOSE

Chemical control

Various chemicals have been tested for the control of bean anthracnose. Pastor-Corrales and Tu (1989) have shown that seed coat infections are controlled effectively with ferbam and ziram. However, internal seed contamination is not reduced. Formulations with benomyl and thiophanate-methyl applied to seeds at 5.2 g/kg achieved more than 95% control (Pastor-Corrales and Tu, 1989).

Preventative spraying with systemic fungicides has been attempted with limited success. Maneb and zineb at 3.5 g/l (Bailey *et al.*, 1992), benomyl at 0.55 g/l (Beshir, 1997), captafol at 3.5 kg/ha (CIAT, 1988), carbendazim at 0.5 kg/ha (CIAT, 1988), and fentin hydroxide at 1.2 g/l (Pastor-Corrales and Tu, 1989) are reportedly effective in the control of bean anthracnose.

In Ethiopia, varying levels of anthracnose severity were maintained by spraying benomyl at a rate of 0.4 kg/ha at different time intervals (Beshir, 1997). Combination and rotation of fungicides are more effective than continually using a single compound (Dekker, 1995). Pastor-Corrales and Tu (1989) recommended spraying foliage at flowering initiation, late flowering, and pod fill to achieve satisfactory disease control. Sindhan and Bose (1981) reported that out of 13 fungicides tested, benomyl, carbendazim, carboxin, and ziram were effective as seed dressing and foliar sprays. These treatments increased seed germination and seed yield while reducing disease incidence.

Cultural control

Various cultural practices can be helpful in reducing the incidence of anthracnose. Anthracnose-free bean seed has been produced and used in different regions of the world to control the disease. Pathogen-free seed was produced from susceptible cultivars with surface or furrow irrigation in semi-arid regions. However, infected plant debris must be removed from the field soon after harvest. It is also important to restrict the activity and movement of humans and agricultural implements in a field when foliage is wet from rain or dew (Timmer and Brown, 2000). Based on the fact that the fungus could survive for two years in dry debris and seed (Jefferies *et al.*, 1990) a three-year crop rotation cycle has been suggested to control anthracnose (Tu, 1983).) Field sanitation and shifting planting dates as control strategies to reduce losses caused by anthracnose have been described by Zaumeyer and Thomas (1957), Chaves (1980), Ferraz (1980) and Schwartz *et al.* (1983).

Bean anthracnose can be managed by using mixtures of susceptible and resistant bean cultivars (Tu, 1983). This practice reduces the amount of inoculum that is readily available to infect susceptible plants (Ntahimpera *et al.*,

1996). The latter authors reported that disease incidence was consistently lower in plots with 25% and 50% resistant cultivars as opposed to 10% resistant cultivars. Other researchers have also confirmed the role of cultivar mixtures to restrict the spread of the disease (Switch and Whittington, 1983; Tu, 1983; Mohamood *et al.* 1991; Pyndji and Trutman, 1992; Chakraborty *et al.*, 1995; Madden, 1997).

Biological control

Colletotrichum spp. can also be managed biologically (Young and Krauss, 1984). The classical approach to control anthracnose is based on reducing the initial inoculum. In this regard, antagonistic microorganisms have been applied to foliage to suppress or inhibit disease development (Korsten and Jeffries, 2000). *Bacillus subtilis* (Cohn.) Praznowski has been shown to be a promising antagonist for *C. lindemuthianum* (Bailey *et al.*, 1992). It produces spores, which can withstand adverse conditions while also producing antifungal and antibacterial compounds (O'Connell *et al.*, 1985). A similar approach for the control of anthracnose using yeast fungi has also been reported (Pastor-Corrales and Tu, 1989). In addition, a spore suspension of *Trichoderma viride* has been used as a foliar spray to control *Colletotrichum* spp. (Bailey *et al.*, 1992).

Breeding for resistance

Host resistance is the most cost-effective approach to control bean anthracnose and an important objective is to improve resistance in high-yielding and widely adapted cultivars. Incorporation of a gene for antracnose resistance, through backcrossing, has assisted in the development of high yielding varieties such as Centralia and Dresden in Canada (Tu, 1992). A number of resistance genes and sources have been identified and used in breeding for anthracnose resistance (Kelly *et al.*, 1994). Resistance sources have been used extensively in the United States, Canada, Europe, and in some countries of Africa and Latin America (Pastor-Corrales and Tu, 1989). Other reports indicated anthracnose-resistant bean germplasm in Spain (Fernandez *et al.*, 2000) and Colombia (Zeven, *et al.*, 1999), or the selection of introductions for direct cultivation (Pastor-Corrales *et al.*, 1994).

Variation in the pathogen. Many plant pathogens show inherited variability, as evidenced by new forms, which evolve and infect previously resistant hosts. It is an accepted fact that the more variable a pathogen is, the more difficult it is to breed for resistance and maintain that resistance over time and space. Different variants within a species differ from each other primarily on the basis of their pathogenicity (Habtu *et al.*, 1996; Agrios, 1997). Pathogenic variants or physiologic races can be detected and characterized by their reactions on a set of host varieties referred to as host differentials. The procedure generally follows the collection and purification of single-spore isolates, inoculation of the race differentiating set with standard concentrations of these isolates, and identification of a race according to the resistance/ susceptibility reaction pattern of the differentials.

Pathogenic variability in *C. lindemuthianum* was first demonstrated by Barrus in 1911 and subsequently by several other workers (Pastor-Corrales and Tu, 1989; Maeli *et al.*, 2000). In earlier studies in Uganda, evidence of pathogenic variability was found in *C. lindemuthianum* (Leaky and Simbwa-Bunnya, 1972). Initially, three differential common bean cultivars were used to identify races of *C. lindemuthianum* (Buruchara, 1991). The first nomenclature system used the Greek alphabet to designate variants. Nine *C. lindemuthianum* races (*alpha, delta, epsilon, zeta, eta, teta, kappa, lambda,* and *mu*) were subsequently identified in Brazil (Menezes and Dianese, 1988). The races that have been identified in Kenya include *alpha, delta, gamma, epsilon* and *lambda* (Kelly *et al.*, 1994).

It soon became obvious that the differential series was not large enough to allow the classification of an increasing number of races. Despite the fact that there was general agreement on the identity of some of the races of *C. lindemuthianum* reported, the apparent use of different assessment criteria and differential cultivars complicated the interpretation of results. In 1988, researchers at CIAT, Colombia, defined a group of 12 common bean differentials to be used internationally and to facilitate the exchange of information and resistant germplasm. At the same time, a binary system of race classification was proposed (CIAT, 1988).

Since then, many surveys have been conducted throughout the world to identify the prevalence and distribution of specific bean races. Of the races reported, 46 were from the United States (Kelly *et al.*, 1994), nine from Mexico (Gonzalez *et al.*, 1998), 33 from Nicaragua (Rava *et al.*, 1993) and 41 from Colombia (Restrepo, 1994) including other countries. Preliminary work indicated the presence of 14 races of *C. lindemuthianum* in Ethiopia (Beshir, 1999).

Characterization of pathogens continues to be important in developing effective management strategies. The number of options and tools to study pathogen variability is increasing with new developments in molecular biology. Numerous pathogens are difficult to identify by morphological or pathogenic characteristics. Technologies that would enable pathologists to identify pathogens rapidly and accurately would be useful in studies of pathogen epidemiology, diversity, ecology, as well as in the detection of initial inoculum in disease forecasting. Mesquita *et al.* (1998) developed a strategy for race-specific DNA diagnostics for *C. lindemuthianum* and CIAT (1986) used molecular technology for virulence assessment. Mesquita *et al.* (1998) reported that DNA-based molecular markers helped as an auxiliary tool to aid the classification of races 73, 65 and 64 of *C. lindemuthianum* in Brazil. In Mexico, Gonzalez *et al.* (1998) identified 59 isolates of *C. lindemuthianum* using molecular markers.

Variation in the host. Cultivars vary significantly in their reaction to different anthracnose races (Tu, 1992). Several resistance sources have been used in the United States, Canada, Europe, and in some countries of Africa and Latin America (Andersen *et al.*, 1963). According to Bassett (1996), the *A* and *Are* genes were originally reported to confer resistance to the *alpha,* and *lambda* and *epsilon* races of anthracnose, respectively. Currently, a *Co* designation for anthracnose resistance genes is used (Kelly and Young, 1996) and those listed by Bassett (1996) are *Co-1* (syn. *A*, in the Andean variety Michigan Dark Red Kidney, linked to RAPD marker $OF10_{530}$), *Co-2* (syn. *Are*, in the Middle-American variety Cornell 49-242, linked to RAPD markers $OQ4_{1440}$, $OH20_{450}$, $B355_{1000}$), *Co-3* (syn. *Mexique 1* in the Middle-American variety Mexico 222), *Co-3*^{2} (in the Middle-American variety Mexico 227), *Co-4* (syn. *Mexique 2* in the Middle-American variety TO), *Co-5* (syn. *Mexique 3* in the Middle-American variety TU,

G2333 and selection 1360), *Co-6* (in the Middle-American variety AB 136, linked to RAPD markers $OAK20_{890}$ and $OAH1_{780}$), and *Co-7* (in the Middle-American variety G2333 and selection 1308 of G2333).

Two independent genes for resistance were reported in the bean cultivar G2333 (Young *et al.*, 1998). One was allelic to *Co-4* in TO and named $Co\text{-}4^2$ whereas the other was given the temporary gene symbol *Co-7*. The RAPD markers $OAS13_{950}$ and $OAL9_{740}$ were linked to $Co\text{-}4^2$ and two 24-mer SCAR primers were subsequently developed for this gene. More recently, an AFLP marker study revealed a 108 bp fragment situated 9.9 cM from the $Co\text{-}1^2$ allele and which co-segregated with resistance in the Andean cultivar, Kaboon (Vallejo and Kelly, 2002).

Greenhouse and field screening. Inoculation methods to screen host resistance involve seed inoculation, seedling sprays, a dip method and assessment under natural, field conditions. Hence, germplasm are regularly screened using one or a combination of the above methods (Tu, 1992; Pastor-Corrales *et al.*, 1995). However, the most appropriate and practical screening method for bean anthracnose is under field conditions (Pastor-Corrales and Tu, 1989; Tugaye-Esquerre *et al.*, 1992).

Inoculation technique. In order to screen plant material efficiently, inoculum is often increased for field or greenhouse tests. The inoculum should preferably consist of an equal mixture of the most virulent races for a specific geographical area. In the greenhouse, bean seedlings at the one to two trifoliate stage are sprayed with a conidial suspension (recommended concentration of 1.2×10^6 conidia/ml) using an atomizing device. After inoculation, plants are covered with a plastic bag or placed in a dew chamber to maintain high relative humidity (85-100%). Depending on the available facilities, the incubation period usually varies and could be as long as seven days. Following exposure to high humidity, plants are returned to normal greenhouse conditions. For certain susceptible varieties, symptoms may be observed from the 7^{th} day of inoculation onwards. Field inoculations are performed in the late afternoon in moderate temperatures when dew is expected. This operation is usually carried out with a knapsack sprayer.

Assessment scales. Anthracnose severity is based on the percentage of leaf and/or pod area infected using a standardized CIAT scale of 1 to 9. Field reactions are periodically evaluated two weeks after the initial inoculation, including flower stage (R6) and the pod filling stage (R8). Foliage symptoms can be observed primarily to identify highly susceptible cultivars. Pods are more intensively evaluated at 70-100% formation, and again at harvest. Reactions of promising resistant and intermediate materials identified during the field evaluations should be verified in the glasshouse. Disease reactions of the seedling leaves and stems are classified according to the following scale: resistant, with no apparent infection; intermediate, with few small necrotic lesions; and susceptible, with large necrotic lesions or plant death.

The CIAT 1-9 scale, where 1= 1-10%, 2= 11-20 %, 3= 21-30%, 4= 31-40%, 5= 41-50%, 6= 51-60%, 7= 61-70%, 8= 71-80%, 9= >81%, is described as follows (CIAT, 1987; Tu, 1994; Pastor-Corrales *et al.*, 1995):

1: No visible disease symptoms.

3: Presence of very few and small lesions, mostly on the primary vein of the lower leaf surface or on the pod, that covers approximately 1% of the surface area.

5: Presence of several small lesions on the petiole or on the primary and secondary veins of the lower leaf surface. On the pods, small (less than 2 mm in diameter) round lesions, with or without reduced sporulation, covered approximately 5% 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 on the 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 and that cover approximately 10% of pod surface area may be found.

9: Severe necrosis on 25% or more of the plant tissues are evident as a result of lesions on the leaf, petioles, stems, branches, and even on the growing point, which often results in death of most of the plant tissues. The presence of numerous, large, sprouting, sunken cankers can result in pod malformation, low seed number, and death of the pod.

CONCLUSIONS

Bean anthracnose is recognized as a primary problem affecting bean production in several bean-growing countries of Africa. Due to differences in climatic conditions and cropping systems, production constraints vary from place to place. These constraints can be resolved by an understanding of bean production practices. In the Ethiopian bean production system, diseases including bean anthracnose, is an important component. The importance of anthracnose is generally accepted but few studies have been conducted to determine yield losses due to the disease in most African countries, including Ethiopia. Information on the relative importance, distribution, damage potential, and management of bean anthracnose is urgently needed. Strategies for disease management need to be devised in an integrated crop production system. Such strategies should concentrate on developing improved cultivars that combine high yield and other agronomic characters with disease resistance. To this effect knowledge of the epidemiology and control of bean anthracnose is essential.

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Fig 1. Anthracnose symptoms on infected bean plants: A: seed infection, B: infected cotyledon, C: infected leaf, D: infected pods, E: infected stem, and F: infected plot of the susceptible cultivar Mexican 142.

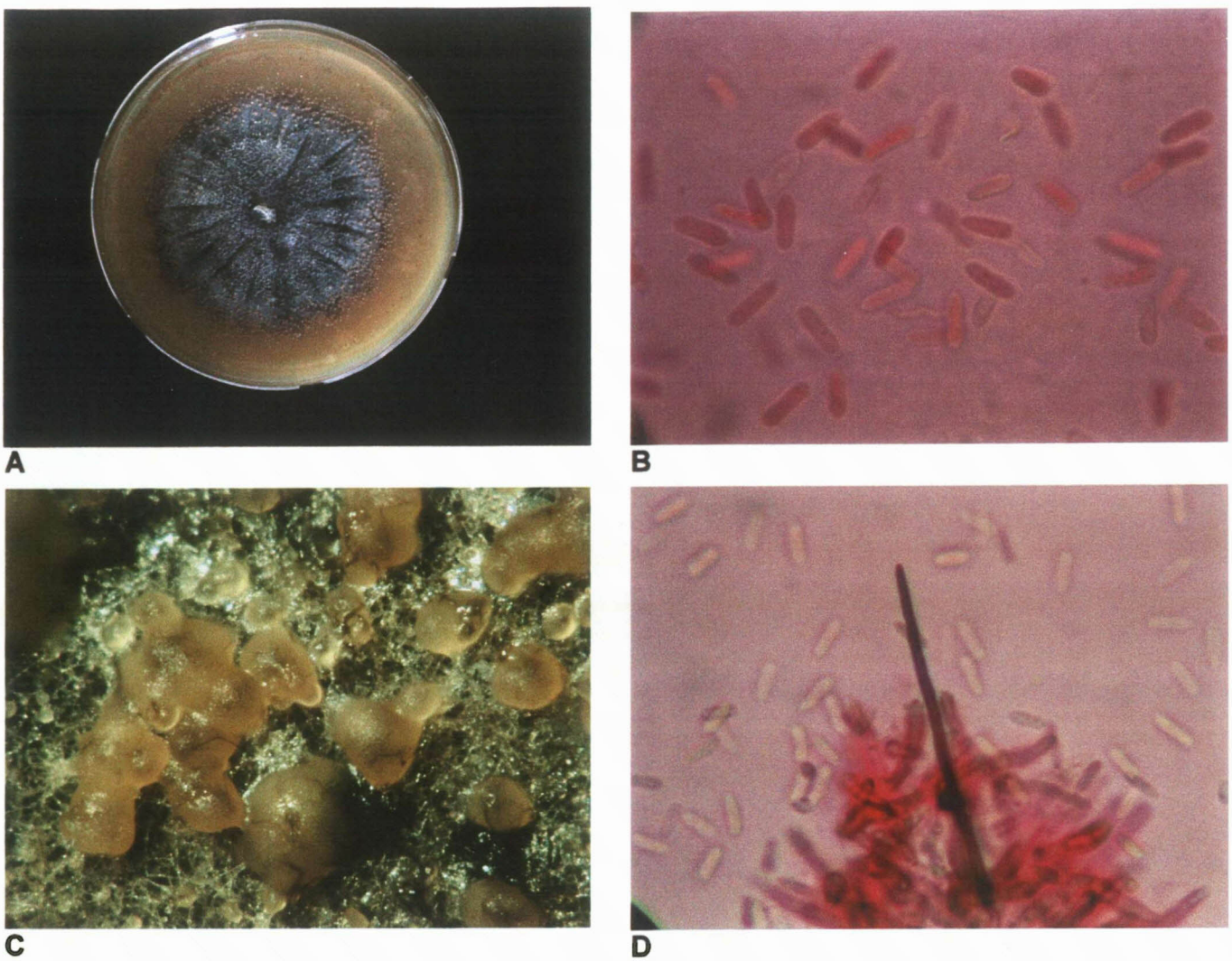

Fig 2. Cultural and morphological characteristics of *Colletotrichum lindemuthianum*: A: colony growth on potato dextrose agar; B: conidia stained with lactofuchsin (photographed at x400); C: mucilage of *C. lindemuthianum* on potato dextrose agar; D: conidia and seta stained with lactofuchsin (photographed at x400).

Chapter 3

Occurrence and distribution of anthracnose on common bean in Ethiopia

ABSTRACT

A survey was conducted to assess the distribution and prevalence of bean anthracnose in the major bean growing regions of Ethiopia in 2001. Farmers' fields were selected along roadsides and data were collected after every 20 km. The surveyed regions include humid, sub-humid, moist, sub-moist, arid and semi-arid agro-ecological zones (AEZ). Weather data indicated that the mean rainfall in the humid zone ranged from 766 to 1404 mm, minimum temperature was from 10°C to 14°C and maximum temperature ranged from 25°C to 27°C. Although prevalence and disease distribution varied among surveyed areas, anthracnose occurred more regularly than other diseases. The survey data revealed that in the humid zone anthracnose severity ranged from 42 to 67% and incidence from 22 to 76%. Severity in the sub-humid areas varied from 34 to 64% and incidence from 15 to 63%. In semi-arid and arid zones, severity and incidence were relatively low, ranging from 34 to 60% and 25 to 60%, respectively. An incidence of 37 to 81% was recorded in the semi-arid zone. Other diseases such as common bacterial blight, angular leaf spot, rust and ascochyta leaf blight were more commonly distributed in semi-arid and arid zones.

Keywords: *Colletotrichum lindemuthianum*, common bean, *Phaseolus vulgaris*, agro-ecological zones.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is an important legume food crop for millions of people in Latin America and Africa (CIAT, 1984). Common bean is an important food component to supplement protein and as a cropping system in many African countries such as Kenya (Gathuru and Mwangi, 1992), Zambia (Haciwa, 1992), Rwanda (Gasana, 1992) and Ethiopia (Habtu *et al.*, 1996) where it is intercropped with cereals, coffee or enset (Table 1). However, the average yield obtained by Ethiopian farmers is low because of various plant diseases such as common bacterial blight, rust, anthracnose, angular leaf spot, floury leaf spot, ascochyta blight, web blight, halo blight and bean common mosaic virus (Habtu and Awgichew, 1984). Of these, bean anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. and Magn.) is found in most beans growing areas with cool to moderate temperatures and high humidity. In the warm and humid regions of the country, anthracnose, angular leaf spot and floury leaf spot are more prevalent (PPRC, 1990). In the presence of dew, rain or high relative humidity, conidia of *C. lindemuthianum* produce germ tubes which penetrate the bean tissue and cause disease lesions (Pastor-Corrales and Tu, 1989).

Anthracnose has been intensively studied in North America and Europe (Hubbeling, 1957; Allen, 1983; Pastor-Corrales and Tu, 1989; Tu, 1994). Although it is still regarded as one of the more important bean diseases, its economic importance in recent years has declined in developed countries through the effective use of clean seed and resistant varieties (Awgichew, 1982; Allen, 1983; Pastor-Corrales and Tu, 1989). However, in developing countries, anthracnose remains serious and is regarded as one of the principal diseases of beans throughout tropical regions including Latin America and Eastern Africa (Singh and Gathuru, 1979; Allen, 1983; Pastor-Corrales and Tu, 1989; Braun, 1997). This prevalence of anthracnose is due to the lack of an integrated disease management program, the development of new pathogen races, or to the introduction of known races to new areas.

The pathogen is splash-dispersed over short distances and its incidence increases during rainy periods. For example, in Canada, the spread of infection from foci was measured at up to 4.6 m per rainstorm (Tu, 1994). Anthracnose can survive in seed or infected crop residues for two to five years under low moisture conditions (Davis *et al.*, 1981; Tu, 1994). The degree of seed transmission is, furthermore, highly correlated with pod infection (Tu, 1993).

Stewart and Dagnachew (1967) reported bean anthracnose in two regions of Ethiopia, namely Shoa and Keffa, whereas country-wide surveys in the early 1970's revealed the presence of anthracnose in all bean growing areas (Habtu and Gorfu, 1985; Habtu, 1994). Today, anthracnose is widespread and one of the most important diseases in the country and surveys (PPRC, 1990) have revealed differences in the prevalence and severity of anthracnose across regions and with different farming practices.

In Ethiopia, production of common bean is concentrated mainly in the south and southeastern regions of the rift valley (08°23N', 039°19'E). It is normally grown from sea level to an altitude of about 2800 m. Under Ethiopian conditions, beans are well adapted in altitudes between 700 m and 2000 m in irrigated and rainfed systems (Ohlander, 1980; Habtu and Awgichew, 1984; Habtu *et al.*, 1985). As expected, this wide range of ecological adaptation is associated with several diseases.

Different races of *C. lindemuthianum* are known to infect beans worldwide, with some causing serious economic losses (Shimelis *et al.*, 1990; Beshir, 1997). Survey data (Marlatt *et al.*, 1983; Polley and Thomas, 1991; Polley *et al.*, 1993; Clear *et al.*, 1996; Habtu *et al.*, 1996; Hughes and Gottwald, 1998; Adane *et al.*, 2000; Greer and Webster, 2001) were used not only to estimate the severity and distribution of diseases, but also to compare disease levels across agro-ecological zones based on climatic conditions. Survey data generally help to describe the geographical distribution of diseases, their relative importance, and their epidemiology.

Therefore, the major objective of the present study was to assess the distribution of anthracnose and other major diseases of common bean in Ethiopia and to place their importance in perspective.

MATERIALS AND METHODS

Sample area

Surveys were conducted in six agro-ecological zones (AEZ) of Ethiopia designated as moist (M1 and M2) with mean annual rainfall of 1051.1 – 1059.0 mm, sub-humid (SH1 and SH2) with rainfall of 1194.2 – 1404.0 mm, humid (H1) with mean rainfall of 1207.3 mm and semi-arid (SA3) with mean rainfall of 766.3 mm (CSA, 1992) (Table 2).

Fields surveyed

The survey area included 25 fields from the humid zone, 55 moist, 30 semi-arid, 30 sub-humid, 30 sub-moist, and five per-humid fields (Table 3). In total 175 fields, representing private land and research stations, were selected. Each field, visited once, was selected at intervals of 15 to 20 km along main roads as described in other survey studies (Roy *et al.*, 1994). The main weather data (rainfall, temperature [minimum and maximum] and relative humidity) for 1998, 1999, 2000 and 2001, measured at six representative locations (Ambo, Bako, Areka, Jimma, Adam-Tulu and Awassa), were also compiled. For each bean field, plants were assessed for disease at five sites.

Crop and disease assessment

Each field was assessed for crop developmental stage, disease severity and incidence of angular leaf spot (*Isariopsis griseola* (Sacc.) Ferraris), bean anthracnose (*Colletotrichum lindemuthianum*), bean rust (*Uromyces appendiculatus* (Pers.), common bacterial blight (*Xanthomonas campestris* pv. *phaseoli* (Erw. Smith) Dowson. and floury leaf spot (*Mycosphaerella phaseoli* [Drummond] Deighton. Disease severity, defined as the affected leaf area, included lesion size and associated chlorosis as a percentage of total area affected. Incidence was defined as percentage of infected plants divided by the

total number of plants observed. Diseases were assessed at full podding stage of plants (Fernandez *et al.*, 1986). Incidence was measured by determining the number of infected plants per square meter.

Severity of anthracnose was scored using a 1 to 9 CIAT scale where 1 indicates no symptoms and 9 severe infections (Schoonhoven and Pastor-Corrales, 1987):

1. No visible disease 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 covers approximately 1% 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 in diameter) round lesions, with or without reduced sporulation, cover approximately 5% of the pod surface area,

7. Presence of enlarged lesions on the lower side of the leaf. Necrotic lesions can also be observed on the upper leaf surface and on the 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 and that cover approximately 10% of pod surface area may be found,

9. Severe necrosis on 25% or more are evident as a result of lesions on the leaf, petioles, stems, branches, and even on the growing point, which often results in death of most of the plant tissues. The presence of numerous, large, sprouting, sunken cankers can result in pod malformation, low seed number, and death of the pod.

Results and Discussion

Disease prevalence and distribution varied among the surveyed areas. The distribution and prevalence of all observed diseases are indicated in Table 4. The proportion of anthracnose infected samples varied as follows: 14% in humid (H2), 31% moist (M1 and M2), 17% semi-arid (SA2), 17% sub-humid (SH1 and SH2) and 17% sub-moist (SM1 and SM2). High severity was noted at the humid

zone (H2). AEZ SA2 was associated with high incidence of disease. Anthracnose was observed in all surveyed areas, but a high incidence occurred at Bako, Didessa, Ambo, Arsi-Negele, Meki and Areka, all regions within the H2, M1 and M2 AEZ's. Average rainfall in these regions ranged from 766 to 1404 mm, humidity from 55 to 74%, minimum temperature ranged from 10° to 14° C, and maximum temperature from 25° to 27°C. According to Allen (1983) and Habtu (1994) these ranges in weather variables favour anthracnose occurrence and development. It was clear that anthracnose is not restricted to the described AEZ's as the disease was observed in both humid and moderately humid regions such as at Bako, Jimma, Ambo, and Awassa. In addition, this is consistent with previous findings that anthracnose is more severe in regions with high rainfall and moderate temperature.

Common bacterial blight was distributed in cooler areas where rainfall is erratic. In the warm and humid regions of the surveyed area angular leaf spot and floury leaf spot were prevalent. The data indicated that AEZ SA2 and SM2 (Ambo, Bako, Didessa, Awassa, Arsi-Negele, and Melkassa) were closely associated with high and intermediate levels of bacterial blight and a high level of anthracnose. SH1, SH2 and M1 (Jimma, Metu, Hararghe, and Melkassa) supported low levels of anthracnose and bacterial blight but showed intermediate to high levels of rust. SH2 and H2 (Bako, Metu, and Jimma) were associated with a high presence of angular and floury leaf spot, low level of rust and intermediate level of anthracnose.

From this survey and considering weather patterns, it was clear that conditions conducive for anthracnose prevail in several bean growing regions of Ethiopia. Research on anthracnose with the eventual aim to control this disease is thus warranted.

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Table 1. A broad classification of bean producing zones in Ethiopia

AEZ[a]	Region	Sub-region	Altitude	Season	Cropping system	Other major crops	Use	Bean cultivar
SA2	Central Rift-Valley	Ziway (08°20'N, 038°58'E), Meki (08°05'N, 038°51'E), Awassa (06°48'N, 037°43'E)	1500-1700	bimodal, single	monocrop	Maize, tef	cash	Mexican 142
M1, M2	Eastern	Pawe (11°19'N, 36°24'E), east Hararghe (09°14'N, 42°14'E)	1700-2200	unimodal single	intercrop	Maize, sorghum, chat	food cash	varietal mixtures
SH1, SH2	Sidamo, Gamo-Gofa	Hararghe, Bako (09°06'N, 37°09'E), Asosa (07°60'N, 036°75'E)	1500-1900	bimodal	monocrop intercrop	Maize, coffee, sweet potato, enset, tef	food	Red Wolaita
H1, H2	Kefa, Wollega, Illubabor, Gojjam, East Shoa	Konso (11°19'N, 36°24'E), Jimma (07°28'N, 035°37'E)	1500-1700	unimodal	monocrop intercrop	Maize, sorghum, coffee	food	Mexican 142 varietal mixtures
SM1, SM2	Eastern Ethiopia		2100	unimodal	monocrop intercrop	maize,	food	varietal
A2	Eastern Ethiopia	Kobo (12°04'N, 39°37'E), Meiso (10°12'N, 40°59'E), Babile (11°56'N, 41°44'E), Tigray (12°24'N, 39°33'E) Jijiga (07°07'N, 037o48'E)	1500	unimodal	intercrop	sorghum maize, sorghum	food	mixtures varietal mixtures

Table 2. Weather data from some representative agroecological areas in Ethiopia for the period 1998 – 2001

Weather variables	Location	AEZ[d]	Altitude (masl[e])	1998	1999	2000	2001	Mean
Rainfall[a]	Jimma	H1, H2	1730	550.1	1219.0	1583.4	1476.8	1207.3
	Bako	SH1	1660	1197.5	1012.0	592.4	1974.7	1194.2
	Areka	SH2	1750	1238.3	1523.5	1310.4	1543.7	1404.0
	Awassa	M1	1700	1428.3	926.4	794.2	1087.2	1059.0
	Adami Tulu	SA3	1650	980.4	653.5	715.7	715.7	766.3
	Ambo	M2	2225	1064.1	936.8	1063.5	1140.0	1051.1
Min Temp[b]	Jimma	H1, H2	1730	12.7	11.6	14.6	12.7	12.9
	Bako	SH1	1660	14.2	14.3	13.0	14.5	14.0
	Areka	SH2	1750	13.3	13.3	13.3	12.5	13.1
	Awassa	M1	1700	13.9	12.9	12.7	13.2	13.2
	Adami Tulu	SA3	1650	14.6	10.6	13.2	13.2	12.9
	Ambo	M2	2225	11.2	9.1	9.4	10.5	10.1
Max Temp[b]	Jimma	H1, H2	1730	26.3	26.8	26.8	26.2	26.5
	Bako	SH1	1660	27.8	26.7	26.8	29.0	26.8
	Areka	SH2	1750	23.5	25.7	26.2	25.8	25.3
	Awassa	M1	1700	27.2	27.4	27.6	27.1	27.3
	Adami Tulu	SA3	1650	27.9	25.3	27.3	27.5	27.0
	Ambo	M2	2225	26.3	25.9	26.2	26.4	26.2
RH[c]	Jimma	H1, H2	1730	60.7	63.9	65.0	65.3	63.7
	Bako	SH1	1660	66.2	64.5	62.2	63.8	64.2
	Areka	SH2	1750	-	-	-	-	-
	Awassa	M1	1700	66.6	63.4	62.3	63.2	63.9
	Adami Tulu	SA3	1650	61.9	47.8	55.7	55.9	55.3
	Ambo	M2	2225	76.7	92.9	63.0	63.4	74.0

[a] Data are for 7 months (May-November) of each year.
[b] Temperature (minimum and maximum) data average of 7 months of each year.
[c] Relative humidity.
[d] Agro-ecological zones: SA = semi-arid; M = moist; SH = sub-humid; H = humid; SM = sub-moist; A = arid
[e] Meters above sea level.
Source: National Meteorology Service Agency, Ethiopia.

Table 3. Area of suitable land in each agro-ecological zone and visited areas

AEZ[a]	Area covered by bean plants/ha/000	Fields visited	Number of sampled plants	Proportion of total sample
H2	2324	25	125	14
M1, M2	1680	55	275	31
SA2	812	30	150	17
SH1,SH2	288	30	150	17
SM1,SM2	204	30	150	17
A2	76	5	25	3
Total	5384	175	875	-

[a] = Agro-ecological zones; SA = semi-arid; M = moist; SH = sub-humid; H = humid; SM = sub-moist; A = arid.

Table 4. Distribution and importance of major bean diseases of *Phaseolus vulgaris* L. in bean growing areas in Ethiopia in 2001

Diseases	Ambo	Awassa	Arsi negele	Areka	Bako	Didessa	Hararhe	Jimma	Metu	Melkasa	Meki
Alternaria leaf spot	-	+	-	+	+	-	-	-	-	-	-
Angular leaf spot	+	+	+	+	+++	++	+	+++	+++		+
Anthracnose	+++	++	+++	+++	+++	+++	++	++	++	+	+++
Ascochyta	-	-	-	+	+	+	+	+	++	-	+
Rust	+++	+++	++	++	+++	++	++	+++	++	+	++
Cercospora leaf spot	-	-	+	+	+	-	-	+	-	-	-
Common bacterial blight	++	+++	+++	++	++	+++	+	+	+	+++	++
Floury leaf spot	+	+	-	+	+++	++	+	+++	+++	-	+
Halo blight	+++	++	+	++	++	+	-	-	-	++	-
Phoma blight	++	++	++	+	++	++	+	+	+	++	+

- = Not observed.
+ = Slight, observed only on few plants.
++ = Moderate, observed on most plants.
+++ = Severe, observed on most plants, plants defoliated.

Table 5. The mean range of anthracnose severity and incidence in representative AEZ[a] surveyed areas in Ethiopia in 2001

AEZ[a]	Range in %	
	Severity	**Incidence**
M1, M2	25 - 64. 8	22.9 - 76.0
H2	42 - 67.	34.0 - 76.1
A2	25 - 60.0	30.8 - 61.1
SA2	34 - 60.0	37.5 - 81.1
SH1, SH	34 - 64.2	5.3 - 63.4
SM1, SM2	24.- 64.0	5.8 - 55.5

[a] = Agro-ecological zones; SA = semi-arid; M = moist; SH = sub-humid; H = humid; SM = sub-moist; A = arid.

Chapter 4

Pathogenic variability in *Colletotrichum lindemuthianum* in Ethiopia

ABSTRACT

The pathogenic variability of *Colletotrichum lindemuthianum* isolates in Ethiopia was studied. A total of 260 isolates of *C. lindemuthianum* were collected; 223 from Ethiopia and 37, included as reference isolates, from Southern Africa. Collections were made from a range of altitudes (1500-2300 meter), growing conditions and cultivars of common bean (*Phaseolus vulgaris*), including known sources of resistance, as well as seeds collected from markets. Fourteen-day-old seedlings of differential cultivars were spray-inoculated with mono-conidial isolates of the fungus. Using the binomial system developed by CIAT, races 65, 73, 128, 296, 511, 589, 961 and 1027 were identified in Ethiopia and races 3, 6, 81, 390, 323, and 593 in Southern Africa. Races 128, 296, 511, 961 and 1027 were different from those earlier reported from Europe and Latin America, but 65, 73, and 589 resembled those in India, Michigan North Dakota and Brazil. Previous workers have reported the occurrence of the Southern African races 3 and 593. Among the differential cultivars only G 2333 was resistant to all Ethiopian isolates of *Colletotrichum lindemuthianum,* whereas Cornell 49-242, Kaboon, AB 136 and G 2333 were resistant to isolates from Southern Africa.

Keywords: Pathogenic variability, *C. lindemuthianum*, *Phaseolus vulgaris.*

INTRODUCTION

Fungal, viral and bacterial diseases represent an ongoing challenge to growers of common bean (*Phaseolus vulgaris* L.). Angular leaf spot, caused by *Phaeoisariopsis griseola* (Sacc.) Ferris, rust, caused by *Uromyces appendiculatus* (Pers.:Pers) Unger, white mold, caused by *Sclerotinia sclerotiorum* (Lib.) De Bary, and anthracnose, caused by *Colletotrichum lindemuthianum* (Sacc. and Magn.) Briosi. and Cav. are among the main fungal diseases affecting common bean (CIAT, 1988).

Anthracnose is the most destructive disease of bean in the tropical and subtropical areas of the world (Garirdo, 1986; Pastor-Corrales and Tu, 1989; Sharma *et al.*, 1993; Kelly *et al.*, 1994). Optimum conditions for disease development include high relative humidity, frequent precipitation, and temperatures ranging from 18° to 22° C (Pastor-Corrales and Tu, 1989). Yield losses due to anthracnose have been reported to be as high as 95% in susceptible bean cultivars (Maeli, *et al.*, 2000). In Ethiopia, anthracnose is prevalent in the cool and moist bean growing regions of the southern rift valley and western and eastern highlands of the country (Ohlander, 1980; Beshir, 1991; Habtu and Zadoks, 1995). Estimated yield losses in these areas range from 42 to 62% (Beshir, 1997). Infected seeds of susceptible cultivars serve as a primary source of inoculum (Sharma *et al.*, 1994). Most farmers save their own seed after harvest and for this reason seed-borne diseases can be devastating, particularly when there is a succession of seasons favouring the pathogen (Goth and Zaumeyer, 1965; Zadoks and Schein, 1979).

Effective disease control is limited by efficient seed transmission of the fungus (Tu, 1992), lack of cost-effective chemical control methods (Pastor-Corrales and Tu, 1989), the ability of *C. lindemuthianum* to survive for long periods in plant debris (Dillard and Cobb, 1993), and the occasional development of sclerotia (Sutton, 1992).

Anthracnose can be controlled by quarantine, establishment of tolerance levels for infected seeds, and seed production in disease free areas (Tu, 1983; Pastor-Corrales and Tu, 1989). Furthermore, seed treatment, cultural measures, fungicide spraying, and the use of resistant cultivars can be employed to eradicate *C. lindemuthianum*. Host resistance has been the most appropriate method for control of anthracnose, particularly in countries where alternative methods are difficult to implement. Resistance is the most practical and desirable strategy to pursue as the principal component of an integrated disease management strategy (Mathur *et al.*, 1950; Matenbioek, 1960). The high variability present in *C. lindemuthianum* (Pastor-Corrales and Tu, 1989; Pastor-Corrales, 1992; Restrepo, 1994; Pastor-Corrales *et al.*, 1994), has, however, resulted in a frequent breakdown of resistance in commercial cultivars.

Pathogenic variability in *C. lindemuthianum* was first reported in 1911 (Barrus, 1911). Since then, *C. lindemuthianum* has been reported to possess a high degree of variability in different parts of the world and several races have been characterized from Europe, USA, Brazil and other countries (Leaky and Simbwa-Bunnya, 1972; Menezes and Dianese, 1988; Drijfhout and Davis, 1989; Gatuhru and Mwangi, 1991; Tu, 1992; Rava *et al.*, 1993; Kelly et al., 1994; Balardian and Kelly, 1996; Pastor-Corrales, 1992; 1996; Balardin *et al.*, 1997). The virulence spectrum of *C. lindemuthianum* is mostly unknown in the Indian sub-continent where cultivars of kidney bean have become susceptible to this disease (Sharma *et al.*, 1993).

Initially, only three common bean cultivars were used to differentiate races, designated by the Greek alphabet, of *C. lindemuthianum* (Buruchara, 1992). It soon became obvious that the differential series was not large enough to allow the classification of the increasing number of pathogenic variants. Since different workers used different race classification systems, comparison of results was difficult if not impossible. To alleviate these problems bean workers at CIAT proposed an international set of 12 differential cultivars accompanied with a binary system of nomenclature for identification of races of *C. lindemuthianum* (CIAT, 1988).

In recent years, the occurrence of bean anthracnose in Ethiopia has increased significantly (Habtu, 1987), emphasizing an urgent need for resistant cultivars. With the exception of a preliminary study (Beshir, 1999), the virulence spectrum of *C. lindemuthianum* has not been well characterized in Ethiopia. Therefore, the objective of this study was to identify anthracnose races and to obtain information on specific virulence within the local pathogen population. These races could then be maintained for further screening and selection of resistant germplasm. Since anthracnose is also important in South Africa (Koch, 1996), some isolates from this region were included for comparison.

MATERIALS AND METHODS

Fungal isolates

In the present study, 223 single-conidium isolates *of C. lindemuthianum* were established from diverse agro-ecological regions of Ethiopia in 2000 and 2001. These regions covered the rift valleys of eastern Ethiopia Hararghe highlands, southern Ethiopia (Sidama and Gamo-Gofa) and western Ethiopia (Kaffa, Wollega, Bako, Ambo, Illubabor and Gojjam) (Fig.1). Isolates were collected from commercial farms, small-scale production areas, and experimental fields. In Southern Africa, infected bean leaves were collected from Bethlehem, Cedara, Delmas, Potchefstroom, Kranskop, Lesotho, and also from Zimbabwe in 2000 and 2001.

Stems, pods and leaves that showed typical anthracnose lesions were collected in paper bags and dried. All diseased samples exhibited anthracnose symptoms of either typical small, rust-colored lesions, or canker-like lesions with black rings containing pink spore masses (Schwartz *et al.*, 1980). Cultures of the isolates were sent to ARC-PPRI (Agricultural Research Council-Plant Protection Research Institute) of South Africa for confirmation of identity.

Inoculum preparation

Small pieces of infected tissue were surface sterilized with 70% ethanol for 1 min followed by 1% NaOCl solution for 2 min. Plant segments were then rinsed in sterilized distilled water, dried on filter paper for 30 min. and placed on 3.9%

potato dextrose agar (PDA). The plated samples were kept in darkness at 24°C for 10 days. All diseased collections gave rise to fungal colonies (Fig. 2) morphologically resembling *C. lindemuthianum* (Zaumeyer and Thomas, 1957; Schwartz *et al.*, 1982). From each culture a diluted suspension of conidia was then plated on PDA or water agar and allowed to germinate. After 24 h single, germinated conidia were transferred to several plates of PDA medium and incubated in darkness at 24° C for 12-13 days (Schwartz et *al.*, 1982; Pastor-Corrales and Tu, 1989).

For inoculum preparation spores from single conidial isolates were dislodged by scraping the culture surface with a sterile spatula. Suspensions were then filtered through cheesecloth and the concentration adjusted to 1.2 x 10^6 conidia/ml by means of a haemacytometer.

Plant materials

The set of 12 differential cultivars recommended by CIAT (1988) was used for the study. Cultivar names, *P. vulgaris* gene pool, known *Co* resistance genes, binary value and certain varietal characteristics, are given in Table 1. Seeds were procured from CIAT, Cali, Colombia and multiplied. Care was taken to maintain purity of lines.

Inoculation and evaluation

Seeds of differentials were planted in 15-cm-diameter plastic pots, which contained a mixture of 75% soil and 25% compost. Plants were grown at 18-22°C in an air-conditioned greenhouse. Each differential line was replicated seven times. In certain cases, differentials were grown in vermiculite in seedling trays (Fig. 3). Using an atomizer, fourteen-day-old seedlings were inoculated by spraying them with a 1.2 x 10^6 spore/ml conidial suspension until runoff. Inoculated seedlings were incubated for 4 days at 20° ± 2° C in a moist chamber with relative humidity greater 90%. Plants were then transferred to the greenhouse.

Disease severity was rated seven 7 days after inoculation on a scale of 1-9 (Schoonhoven and Pastor-Corrales, 1987; Pastor-Corrales *et al.*, 1995), where,

1. No visible disease symptoms.
3. Presence of very few and small lesions, mostly on the primary vein of the lower leaf surface.
5. Presence of several small lesions on the petiole or on the primary and secondary veins of the lower leaf.
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 on the petioles.
9. Severe necrosis on 25% or more of the plant tissues are evident as a result of lesions on the leaf, petioles, stems, branches, and even on the growing point, which often results in death of most of the plant tissues.

A resistant (R) phenotype was assigned to plants with no or limited symptoms (scores 1 to 3), whereas plants rated 4 or higher were considered to be susceptible (S) (Fig. 3).

The reaction of all isolates on the set of the differential varieties was confirmed in separate infection studies. Races were assigned according to the numerical system of nomenclature, which is based on the sum of the binary values (Table 1) assigned to CIAT differential cultivars on which an isolate is pathogenic.

RESULTS

Characterization of Ethiopian isolates

Of the 223 isolates studied eight physiologic races were identified (Table 2). According to the binary number system, these races were 65, 73, 128, 296, 511, 589, 961 and 1027. Forty eight isolates collected from Ambo, Ziway, Arsi-Negele, Bako, Alemaya and Jimma belonged to race 511; 44 isolates from Ziway, Arsi-Negele, Awassa, Ambo, Bako, Areka, Meki, Adami-Tulu, Jimma and Alemaya belonged to race 128; 40 isolates from the same area were assigned to race 73; 27 isolates from Bako, Nekemt and Jimma belonged to race 1027; 17 isolates from Awassa, Nazreth, and Shashemene were race 961; 24 isolates

from Leku, Yirgalem and Awassa belonged to race 589; 14 isolates from Nazreth area represented race 296; and nine isolates from Bulbulla, Meki and Ziway represented race 65. Race 128 and 511 were most frequently encountered and prevalent in most areas. Other races e.g. 65, 73, 296, 589, 961, and 1027 were found in more than one location (Table 3).

None of the races attacked G2333 whereas only races 511 and 1027 were virulent on Widusa and AB 136, respectively. Races 65, 73, 128, 296 and 961 were virulent on bean varieties of Middle-American origin only, whereas 511, 589 and 1027 combined virulence to differentials of Andean and Middle-American descent.

Characterization of South African Isolates

Of the 37 isolates studied, six races, viz. 3, 6, 81, 323, 390, and 593 were identified (Table 4). Eleven isolates collected from Bethlehem and Pietermaritzburg belonged to race 593, four isolates from Kranskop and Potchefstroom were race 390, seven isolates from Delmas, Cedara and Zimbabwe were race 323, Eight isolates from Kranskop, Cedara and Lesotho represented race 81, two isolates from Kranskop and Potchefstroom were assigned race 6, and five isolates from Delmas, Mlondozi, Syferbult and Lesotho belonged to race 3. Races 3 and 81 were frequently isolated from collected areas (Table 5). None of the races attacked Cornell 49-242, Kaboon, AB 136 and G2333. Virulence to the Andean derivatives Michigan Dark Red kidney (races 3, 6, 323, 390) Perry Marrow (races 6 and 390) and Widusa (races 81 and 593) occurred whereas several Middle-American cultivars were also susceptible.

Type cultures of the races have been deposited in the culture collection of the Department of Plant Sciences, University of Free the State, Bloemfontein, South Africa.

DISCUSSION

This study has revealed the existence of several pathogenic races within the populations of C. *lindemuthianum* in Ethiopia and South Africa. However, of the eight Ethiopian and six South African races identified, no corresponding variants occurred, indicating the development of two distinct populations.

Considering isolates collected from farmers' fields in Ethiopia, races 128 and 511 constituted more than 50%, indicating the importance of these races. Among the isolates collected from Ethiopia, races 65, 73 and 589 resembled those reported by Mesquite *et al.* (1998), Kelly *et al.* (1994), Balardin and Kelly (1996) and Sharma *et al.* (1999). Likewise, races 128, 511 and 961 resembled those previously identified by Beshir (1999). With regard to South African isolates collected, races 3, 81 and 593 confirm the previous findings of Koch (1996). In addition, races 6, 323 and 390 were described for the first time.

Breeding for resistance to plant diseases has a limited chance of success if resistant selections are not challenged throughout all stages with the most virulent races of the target pathogen. Failure to do so will result in the selection of lines resistant to only certain races and quite often susceptible to the prevailing or most pathogenic races in a particular geographic area. It is strongly recommended that an active anthracnose resistance breeding program is conducted in Ethiopia and that regular surveys are conducted to support the ongoing breeding efforts. To do this efficiently, sources of resistance have to be identified and their resistance genes transferred to bean cultivars adapted to Ethiopian conditions. In this respect, Pastor-Corrales *et al.* (1994) identified the cultivar G2333 as resistant to 380 isolates of *C. lindemuthianum* from 11 Latin American countries. This cultivar has been widely grown in Tanzania, Latin America, Kenya, Uganda and Burundi (Allen, 1992). Likewise, G 2333 in Ethiopia, and AB 136, G 2333, Kaboon, Cornell 49-242 in Southern Africa, can be used in breeding for anthracnose resistance.

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Table 1. Bean cultivars used for differentiating *Colletotrichum lindemuthianum* isolates

Cultivar	Origin	Gene designation	Binary value	Growth habit[a]	Colour[b]	Seed size[c]
Michelite	Middle-American	--	1	II	1	P
MDRK	Andean	*Co-1*	2	I	6	G
Perry Marrow	Andean	*Co-1*^{3}	4	II	1	G
Cornell 49-242	Middle-American	*Co-2*	8	II	8	P
Widusa	Andean	--	16	I	1	M
Kaboon	Andean	*Co-1*^{2}	32	II	1	G
Mexico 222	Middle-American	*Co-3*	64	I	1	M
PI 207-262	Middle-American	-	128	III	2	P
To	Middle-American	*Co-4*	256	I	2	M
Tu	Middle-American	*Co-5*	512	III	8	P
AB 136	Middle-American	*Co-6, Co-8*	1024	IV	6	P
G2333	Middle-American	*Co-4*^{2}, *Co-5, Co-7*	2048	IV	6	P

[a] Growth habit : I = determinate; II = indeterminate bush, erect stem; III = indeterminate bush with weak main stem and prostrate branches; IV = indeterminate climbing habit.
[b] Seed colour : 1 = white; 2 = cream-beige; 3 = yellow; 4 = brown-maroon; 5 = pink; 6 = red; 7 = purple; 8 = black; 9 = other (grey, etc).
[c] Seed size : According to weight of 100 seed in grams, P = small <25 g; M = medium 25-40 g; G = large >40 g.

Table 2. Identification of Ethiopian isolates of *Colletotrichum lindemuthianum* according to reactions produced on the standard set of differential cultivars of common bean (*Phaseolus vulgaris*)

Cultivars	Origin	Binary value	Reaction of differential cultivars[a]							
Michelite	Middle-American	1	+	+	-	-	+	+	+	+
MDRK	Andean	2	-	-	-	-	+	-	-	+
Perry Marrow	Andean	4	-	-	-	-	+	+	-	-
Cornell 49-242	Middle-American	8	-	+	-	+	+	+	-	-
Widusa	Andean	16	-	-	-	-	+	-	-	-
Kaboon	Andean	32	-	-	-	+	+	-	-	-
Mexico 222	Middle-American	64	+	+	-	-	+	+	+	-
PI 207-262	Middle-American	128	-	-	+	-	+	-	+	-
To	Middle-American	256	-	-	-	+	+	-	+	-
Tu	Middle-American	512	-	-	-	-	-	+	+	-
AB 136	Middle-American	1024	-	-	-	-	-	-	-	+
G2333	Middle-American	2048	-	-	-	-	-	-	-	-
Race designation			65	73	128	296	511	589	961	1027

[a] + = Susceptible ; - = Resistant.

Table 3. Distribution of races of *Colletotrichum lindemuthianum* occurring on *Phaseolus vulgaris* in Ethiopia in 2000 – 2001

Race	No.of isolates	Geographic location
73	40	Alemaya (09°24'N, 42°E), Ambo (08°58'N, 037°26'E), Meki (08°05'N, 038°51'E) Ziway (08°20'N, 038°58'E), Awassa (06°52'N, 038°27'E), Areka (06°48'N, 037°43'E), Arsi-Negele (07°00'N, 038°35'E), Bulbulla (08°20'N, 038°58'E), Hadiya (06°58'N, 037°44'E)
128	44	Awassa, Arsi-Negele, Ambo, Yirgalem (06°42'N, 038°21'E), Bako (09°06'N,37°09'E), Ziway, Areka, Addami-Tulu (08°20'N, 038°58'E), Meki, Jimma (07°28'N, 035°37'E), Alemaya
296	14	Melkassa (08°23'N' 039°19'E), Nazreth
589	24	Leku (06°52'N, 038°27'E), Yirgalem, Awassa
961	17	Awassa, Nazreth, Shashamane (038°35'N, 07°01'E)
511	48	Ziway, Meki, Arsi-Nagele, Ambo, Nazreth, Alemaya, Awassa, Bako, Areka, Alemaya
1027	27	Bako, Nekemt (09°06'N,37°09'E), Jimma
65	9	Bulbulla, Meki, Ziway

Table 4. Identification of South African isolates of *Colletotrichum lindemuthianum* according to reactions produced on the standard set of differential cultivars of common bean (*Phaseolus vulgaris)*

Cultivars	Origin	Binary value	Reaction of differential cultivars[a]					
Michelite	Middle-American	1	+	-	+	+	-	+
MDRK	Andean	2	+	+	-	+	+	-
Perry Marrow	Andean	4	-	+	-	-	+	-
Cornell 49-242	Middle-American	8	-	-	-	-	-	-
Widusa	Andean	16	-	-	+	-	-	+
Kaboon	Andean	32	-	-	-	-	-	-
Mexico 222	Middle-American	64	-	-	+	+	-	+
PI 207-262	Middle-American	128	-	-	-	-	+	-
To	Middle-American	256	-	-	-	+	+	-
Tu	Middle-American	512	-	-	-	-	-	+
AB 136	Middle-American	1024	-	-	-	-	-	-
G2333	Middle-American	2048	-	-	-	-	-	-
Race designation			3	6	81	323	390	593

[a]+ : Susceptible, - : Resistant.

Table 5. Distribution of races of *Colletotrichum lindemuthianum* occurring on *Phaseolus vulgaris* in Southern Africa in 2000 – 2001

Race	No. of isolates	Geographic location
593	11	Bethlehem, Pietermaritzburg
323	7	Zimbabwe, Delmas, Cedara
81	8	Kranskop, Cedara, Lesotho
3	5	Delmas, Mlondozi, Syferbult, Lesotho
390	4	Kranskop, Potchefstroom
6	2	Kranskop, Potchefstroom

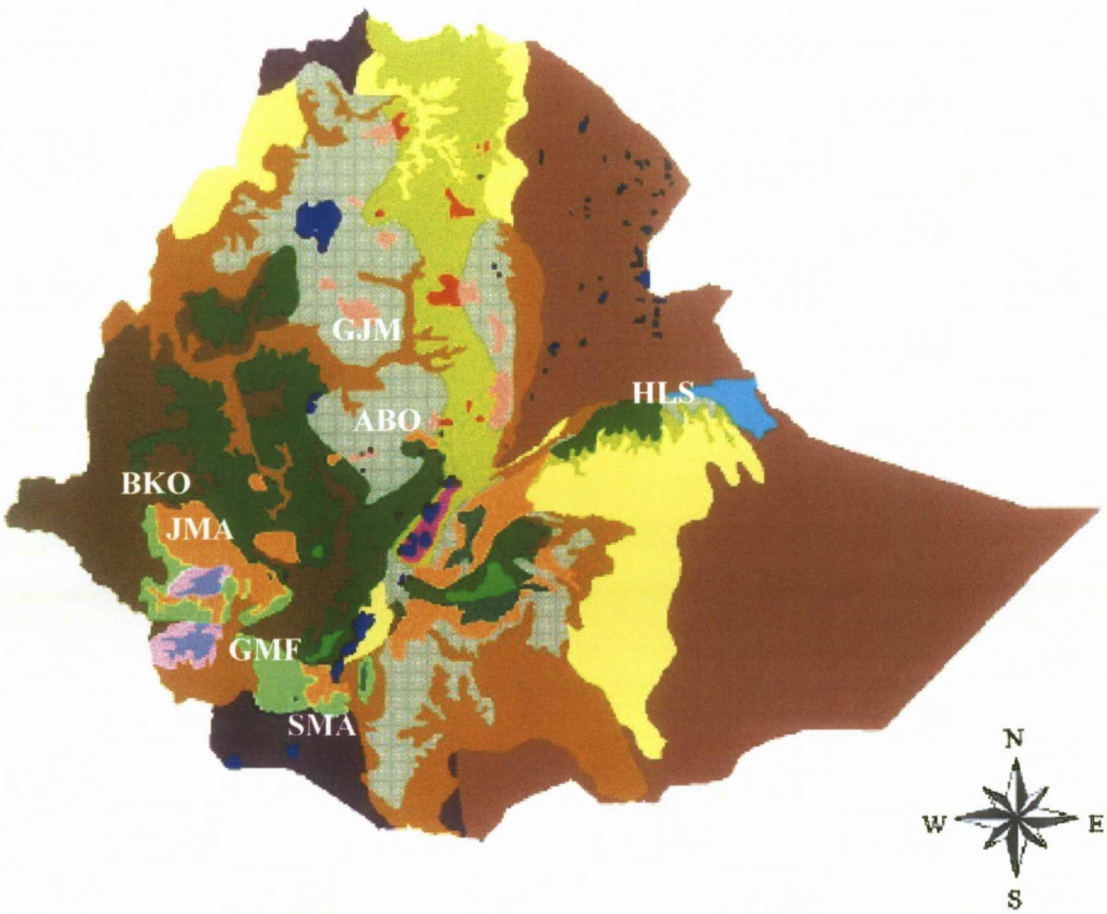

Fig 1. Map of Ethiopia showing collection sites of isolates of bean anthracnose. HLS=Hararghe highlands, GJM=Gojjam, ABO=Ambo, BKO=Bako, JMA=Jimma, GMF=Gamu Gofa, SMA=Sidama.

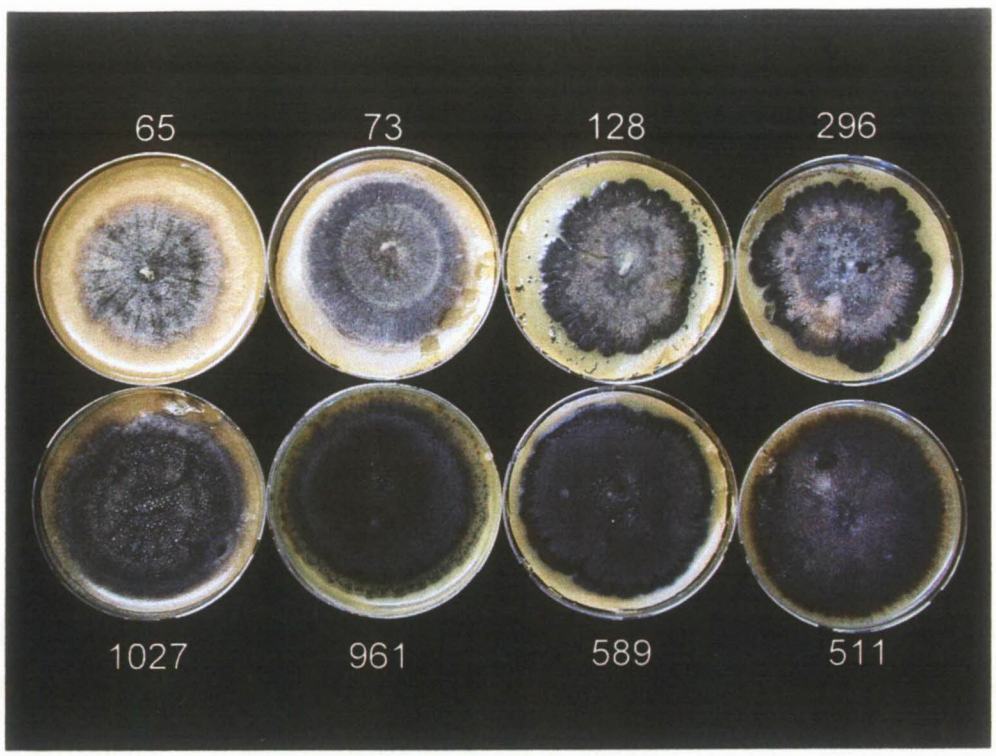

Fig 2. Growth on potato dextrose agar medium of different isolates representing races of *Colletotrichum lindemuthianum*.

Fig 3. *Collectotrichum lindemuthianum* reactions produced on the standard set of differential cultivars of common bean (*Phaseolus vulgaris*). Top: seed trays with vermiculite; centre and bottom: susceptible (arrow) and resistant reactions of differential lines.

Chapter 5

Anthracnose development in mixtures of resistant and susceptible common bean cultivars in Ethiopia

ABSTRACT

To assess the effect of cultivar mixtures on the development of bean anthracnose caused by *Colletotrichum lindemuthianum*, field experiments were conducted at Ambo and Bako in Ethiopia during 2001. Resistant and susceptible cultivars were combined, in different proportions, to achieve five mixture treatments. Plants were inoculated at the trifoliate stage by creating an infection focus in the centre of each plot. Disease progress curves for severity were constructed and analysed according to the exponential, logistic, monomolecular and Gompertz models. Results show that disease incidence and severity were consistently lower in mixtures containing 67 and 80% of the resistant cultivar, whereas, a mixture with 50:50 (susceptible : resistant) was less effective in controlling anthracnose. The monomolecular model best described disease progress in all treatments. The area under the disease progress curve was consistently lower for treatments containing a proportion of the resistant cultivar. In analysing disease gradients, the linear model gave the best fit for disease severity on distance. Infection rates and disease gradients did not discriminate clearly among treatments. Compared with a pure stand of the susceptible cultivar, yield increases were recorded in all cultivar mixtures. Multiple linear regression analysis indicated that disease levels were significantly influenced by time and space. Results of this study suggest that cultivar mixtures will contribute to reducing bean anthracnose in Ethiopia.

Keywords: *Phaseolus vulgaris*, *Colletotrichum lindemuthianum*, cultivar mixture, disease gradients, disease progress curve.

INTRODUCTION

Severe plant disease epidemics can be expected when genetically uniform or susceptible crops are grown across large areas where environmental conditions are conducive to infection (Wolfe, 1985). In contrast, a combination of genetically diverse cultivars in the same field is thought to reduce the possibility of disease epidemics (Wolfe, 1985; Cowger and Mundt, 2002). Cultivar mixtures reduce inoculum potential by limiting the spread of inoculum in space and time (Zadoks and Schein, 1979; Vanderplank, 1984). Additionally, it reduces the risk of pathogen mutation and thus increases durability of resistance.

Various mixtures of beans (*Phaseolus vulgaris* L.) of different seed size and colour have been considered to control disease and pest damage in African countries (Fried *et al.*, 1979; Pyndji and Trutmann, 1992; Geagea and Sache, 1999). In Ethiopia, beans are traditionally grown either as a mixture or intercropped with coffee, enset, chat or vegetables (Habtu *et al.*, 1995).

A mixture of genetically diverse common bean cultivars can reduce the spread of anthracnose caused by *Colletotrichum lindemuthianum* [Sacc. & Magnus.] Lams.-Scrib (Tu, 1981; Ntahimpera *et al.*, 1996). Cultivar mixtures should contain at least 25% of a resistant cultivar to be effective in anthracnose control (Ntahimpera *et al.*, 1996).

In previous chapters, the diversity and distribution of *C. lindemuthianum* in Ethiopia were described. This emphasised the existence of pathogen variability and the fact that, depending on the genetic nature of resistance, the latter may not be durable. Hence, other practical measures to reduce anthracnose development in space and time should be investigated. The aim of this chapter was to study the effect of cultivar mixtures on the development of bean anthracnose caused by *C. lindemuthianum* in Ethiopia.

MATERIALS AND METHODS

Source of inoculum

A mixture of Ethiopian isolates of *C. lindemuthianum* was used as focal infection in each plot. To prepare infected material for field inoculation, five plants of Mexican 142 were grown per 15 x 15 cm pot in a greenhouse and inoculated with a spore suspension at 1.2 x 10^6 conidia/ml (Pastor-Corrales *et al.*, 1995). Plants were subsequently incubated in a dew chamber, maintained in a greenhouse for 14 days and transplanted to the centre of each experimental plot (Fig. 1).

Field plots and experimental design

The experiments were conducted during the main cropping season (June–October, 2001) at the research stations of the Ethiopian Agricultural Research Organization at Ambo and Bako. The soil type was a black vertisol at Ambo and a clay-loam at Bako. The fields were previously planted to wheat (Ambo) and maize (Bako). The experiment was designed as a randomised complete block with six replications. Plots size was 4 m x 4 m and were surrounded by a buffer strip of wheat (*Triticum aestivum* L.) at Ambo and noug (*Guizotia abyssinica* L.) at Bako.

Five treatments consisting of different proportions (1:0, 1:1, 1:2, 1:4 and 0:1) of susceptible and resistant cultivar mixtures were included. Mexican 142 was used as susceptible component and Roba-1 as resistant. Cultivar mixtures were prepared by hand and one seed at a time was drawn randomly and planted 10 cm apart. Each plot consisted of 10 rows with 40 seeds per row. Care was taken to use Mexican 142 seeds that were free of anthracnose symptoms.

Disease assessment

Plots were subdivided as indicated (Fig. 1) and bean plants were tagged for assessment in each grid at distances of 0.2, 0.6, 1.0, 1.4 and 1.8 m from the centre. Disease severity was assessed nine times at 10 day-intervals commencing seven days after the transfer of infected plants to the centre of each plot.

Disease severity was evaluated on a 1 to 9 scale (CIAT, 1987) and expressed as the percentage of the total leaf area infected (1= 1-10%, 2= 11-20 %, 3= 21-30%, 4= 31-40%, 5= 41-50%, 6= 51-60%, 7= 61-70%, 8= 71-80%, 9= >81%). Incidence was evaluated by rating the proportion of infected plants.

1. No visible disease 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 covers approximately 1% 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 in diameter) round lesions, with or without reduced sporulation, cover approximately 5% of the pod surface area,

7. Presence of enlarged lesions on the lower side of the leaf. Necrotic lesions can also be observed on the upper leaf surface and on the 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 and that cover approximately 10% of pod surface area may be found,

9. Severe necrosis on 25% or more are evident as a result of lesions on the leaf, petioles, stems, branches, and even on the growing point, which often results in death of most of the plant tissue. The presence of numerous, large, sprouting, sunken cankers can result in pod malformation, low seed number, and death of the pod.

Yield and 100-seed weight

Eight plants occurring at the 0.2, 0.6, 0.1, 1.4 and 1.8 m sampling points of each treatment and replication were tagged to record yield (gram per eight plants). A random sample was taken from each plot to obtain 100-seed weight.

Data analysis

Disease progress curves. The area under disease progress curves (AUDPC) for incidence and severity were calculated according to the formula below (Campbell and Madden, 1990). Analysis of variance was performed on

incidence, severity, AUDPC, yield and 100-seed weight using the Number Crunching Statistical System (NCSS) (Jerry, 2000).

$$AUDPC = \sum_{i}^{n-1}\left(\frac{y_i + y_{i+1}}{2}\right)(t_{i+1} - t_i)$$

where, n = number of evaluation times, y_i = disease severity/incidence at each evaluation time, and t_i-t_{i-1} = the time interval.

Anthracnose incidence and severity data were analysed according to the logistic, Gompertz and monomolecular models of disease progress (Campbell and Madden, 1990). To determine the most appropriate model for describing the data, a high coefficient of determination (R^2) and low mean square error (MSE) were employed (Jeger, 1983; Campbell and Madden, 1990). Based on these criteria, the monomolecular model most accurately described anthracnose severity and incidence. Since disease severity rather than incidence has been thought to more precisely predict crop losses (Koch and Parlevliet, 1990; Kocks *et al.*, 1999), severity data were subjected to further analyses. Using the monomolecular model [Y= ln(1/(1-y)] disease severity data was linearized and the apparent infection rate (r) determined.

Disease gradients. Three models (power law, exponential and linear) (Campbell and Madden, 1990) were compared to rate the effect of bean mixtures on the spread of anthracnose from a focal point. Data from the fifth assessment were used to calculate disease gradients.

Regression analysis. To describe the association between disease severity and mixture treatment, evaluation time and distance from the inoculum source, multiple regression analysis was conducted using NCSS.

RESULTS

Disease progress over time

AUDPC calculated from anthracnose severity was significantly influenced by treatment, evaluation time and distance from the source of inoculum at Ambo (Table 1) and Bako (Table 2). Untransformed curves for each treatment are shown for Ambo (Fig. 2A) and Bako (Fig. 2B). At both sites, the pure susceptible and resistant stands supported the most and least anthracnose development, respectively. At the earliest date of disease assessment (seven days after inoculation) anthracnose severity levels were similar for all mixture treatments at Ambo and Bako (Table 3). Severity was consistently lower in the 1S:4R mixture at both sites. A maximum of 58% severity was reached on the susceptible control at Ambo whereas 59% was recorded at Bako on 108 days after planting.

The relationship between monomolecular transformed disease severity data against time is indicated in Fig. 3 (Ambo) and Fig. 4 (Bako). At Ambo regression coefficients of 0.006, 0.006, 0.008 and 0.009 were estimated for treatments 1S:4R, 1S:2R, 1S:1R and 1S:0R, respectively. The best fit (R^2 = 0.94 was obtained for the 50% susceptible and resistant mixture as well as the susceptible control. At Bako, disease severity in the 1S:4R mixture was significantly lower as compared to other ratios and the susceptible control. The slopes of the transformed regression lines suggested that treatments 1S:4R, 1S:2R and 1S:1R had similar rates of disease progress (regression coefficient = 0.005). Little disease progress was detected in the resistant control (regression coefficient = 0.001) and R^2 of 0.77 (Fig 4).

Disease gradients

The spread of anthracnose severity from a central infection focus among treatments varied at Ambo (Fig. 5A) and Bako (Fig. 5B). As expected, disease intensity was higher in all treatments near to the focal centre and decreased gradually towards the edges of the plots. The rate of disease decline varied among treatments. Disease severity from the infection centre for mixtures 1S:1R, 1S:2R and 1S:4R, ranged from 55 to 39, 55 to 30 and 45 to 20%, respectively at Ambo (Fig 5A). For the susceptible pure stand the severity ranged from 65 to 50

whereas in the resistant stand disease spread remained relatively low irrespective of the distance from the inoculum source. At Bako, treatments showed similar trends. At this site severity was significantly less for all mixtures as distance from the focus increased, except in the susceptible pure stand (Fig. 5B). Disease spread was lower and remained constant in the resistant control.

In evaluating the three models for description of disease gradients, both the exponential and linear models provided good fits (Figs. 6 and 7). As indicated by the linear model in Fig. 6, the rate of disease spread for 1S:4R was -0.022, -0.054 for 1S:2R, –0.074 for 1S:1R and –0.118 for the susceptible pure stand at Ambo. At Bako, disease gradients were very similar (1S:4R = -0.031, 1S:2R = -0.042, 1S:1R = -0.046 and the susceptible stand = –0.063).

Effect of cultivar mixtures on yield

Variation in yield and 100-seed weight was observed among treatments both at Ambo (Table 4) and Bako (Table 5). Seed yield significantly increased ($P \leq 0.05$) as the proportion of the resistant cultivar increased in the mixture. Significantly higher yield was obtained in all mixtures as compared to the susceptible pure stand (526.63 g/plot). Yield increase over the control was 363%, 184% and 182% for the mixture ratios of 1S:4R, 1S:2R and 1S:1R at Ambo. At Bako, the three treatments yielded significantly better with yield increases of 430%, 281% and 285% over the susceptible control. Yield of mixture 1S:4R was statistically similar to the resistant pure stand. There was no significant yield difference between mixture ratios 1S:1R and 1S:2R (Table 5). The 100-seed weights indicated that all mixtures outyielded the susceptible control at both sites (Tables 4 and 5).

The relationship between yield response and anthracnose severity as influenced by cultivar mixtures is depicted in Fig. 8. The mean severity for the susceptible pure stand was 38% while there was a decreasing trend in severity for the mixtures when the proportion of the resistant cultivar increased. Whereas yield was low in the susceptible pure stand (0.5kg/plot), an increasing trend occurred when the proportion of the resistant component increased (Fig. 8).

Model development

Results of the multiple linear regression analysis of leaf severity as influenced by treatments, assessment time and distance from the source of inoculum are indicated in Table 6 for Ambo and Table 7 for Bako.

Discussion

Based on the results of the present study, it is possible to conclude that cultivar mixtures can reduce development of bean anthracnose over time and space in the field. Anthracnose severity was consistently lower in mixtures containing 50% or more of the resistant Roba-1 cultivar. The AUDPCs in mixtures containing Roba-1 were consistently lower than that of the susceptible cultivar. According to the Lannou *et al.* (1994) calculation method, efficacy rate was 51%, 40% and 19% for 1S: 4R, 1S:2R and 1S:1R, respectively at Ambo. At Bako, these efficacy rates were 51%, 41% and 25% for 1S: 4R, 1S:2R and 1S:1R, respectively. It is clear that mixtures can reduce disease severity of anthracnose and these results support previous research in this regard (Ntahimpera, *et al.*, 1996).

Browning and Frey (1969) and Wolfe (1985) reviewed biological mechanisms that reduce disease severity or incidence in cultivar mixtures. It is believed that resistant plants in cultivar mixtures not only reduce the effective initial inoculum, but also impede dispersal of the pathogen (Browning and Frey, 1969; Wolfe, 1985). Susceptible individuals receive some protection from disease build-up because resistant plants interfere with the transmission of inoculum. In our study, lower disease severity and AUDPC were found for mixtures and the pure resistant stand than in Mexican 142. Similar results were obtained in the bean rust (*Uromyces appendiculatus* (Pers.) Ung. pathosystem. Habtu *et al.* (1995) who described a restriction in disease development in mixed stands in time and space when compared to susceptible pure stands. Furthermore, in a splash-dispersed pathosystem such as *Septoria nodorum*, epidemic development has been retarded in a heterogeneous plant population (Jeger *et al.*, 1981a, b). Similar results were found for bean angular leaf spot where the disease was reduced through supplementation of local mixtures with 25% of a resistant cultivar (Pyndji, 1987; Pyndji and Trutmann, 1992). Halo blight of bean caused by

Pseudomonas syringae pv. *phaseolicola* was similarly reduced in mixtures containing 25% or more of a resistant cultivar (ISABU, 1987). The latter study compared cultivar mixtures of different proportions of resistant and susceptible components and different models were fitted to obtain appropriate mathematical descriptions of disease spread. In the present study, the monomolecular model appeared most suitable, followed by the Gompertz model. In this regard, Ntahimpera *et al.* (1996) also found the monomolecular model to describe a considerable degree of variation, but suggested the Gompertz model as best fitting. Their model selection was further based on the fact that monomolecular disease progress is generally associated with monocyclic diseases and that anthracnose inoculum levels change during the season.

Similar to Ntahimpera *et al.* (1996) infection rates in the present study, as deducted from regression coefficients, did not provide meaningful discrimination between treatments. Although the infection rate of the susceptible control was highest of all treatments at Ambo, differences were smaller than expected. Likewise, disease gradients did not clearly differentiate among treatments. Theoretically, the susceptible control should have the flattest gradient and thus the smallest slope value. In the present study, the susceptible pure stand displayed the highest slopes at both sites. It is possible that plot size was not large enough to allow for a stepwise progression of disease over distance and that individual rain storms or background inoculum influenced assessments of mixture efficacy.

The multiple regression analysis suggested that time of assessment was critical for studying anthracnose severity at both locations. Assessment of severity 68 days after planting gave rise to relatively clear differences among cultivar mixtures suggesting a suitable evaluation time for future cultivar assessments. With respect to yield, it was demonstrated that mixtures provided a significant yield increase ranging from 182 to 362% relative to the susceptible pure stands. It is obvious that the yield gain was due to less disease in the mixtures relative to the susceptible cultivar. This is an additional benefit to farmers other than anthracnose disease management.

The use of cultivar mixtures is a common practice under Ethiopian small-scale farmers (Habtu *et al.*, 1996). Farmers grow mixed bean varieties of their own preferences, with most of these being susceptible to anthracnose. Besides, anthracnose-resistant cultivars may not always be readily available to farmers. The present study confirmed the efficacy of the traditional practice of cultivar mixtures and that farmers would benefit in terms of anthracnose losses provided that 60-80% of a resistant cultivar is included in their planting mixtures. Hence, where bean anthracnose is a recurring problem, cultivar mixtures may provide an effective, inexpensive and sustainable disease management strategy.

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Table 1. Analysis of variance of AUDPC (area under disease progress curve) for severity in bean cultivar mixtures for anthracnose control at Ambo, Ethiopia in 2001

Source of variation	df [a]	Mean square	F -ratio	P[b]
Treatment (Tr)	4	233297.8	18.01	0.000000*
Time (T)	7	224862.3	106.89	0.000000*
Tr X T	28	8057.9	14.55	0.000000*
Distance (D)	4	139899.1	66.50	0.000000*
Tr X D	16	2827.8	5.11	0.000000*
Error	199	12950.9		

[a] : Degrees of freedom.
[b] : Significant at P≤0.05.

Table 2. Analysis of variance of AUDPC (area under disease progress curve) for severity in bean cultivar mixtures for anthracnose control at Bako, Ethiopia in 2001

Source of variation	df [a]	Mean square	F	P [b]
Treatment (Tr)	4	2842180	253.21	0.000000*
Time (T)	7	18767.74	6.46	0.000141*
Tr X T	28	7013.82	1.63	0.038424*
Distance (D)	4	249383.3	85.79	0.000000*
Tr X D	16	18793.66	4.37	0.000001*
Error	199			

[a] : Degrees of freedom.
[b] : Significant at P≤0.05.

Table 3. Mean anthracnose severity (%) at nine assessment times at Ambo and Bako

Time (days)	Ambo*					Bako				
	Treatment					Treatment				
	1	2	3	4	5	1	2	3	4	5
7	10a	8ab	6bc	3cd	1d	8a	1b	1b	1b	0b
17	22a	23a	19ab	17ab	15b	26a	14b	7c	4cd	1d
27	33a	29ab	26bc	23dc	19d	39a	32b	31b	27c	2d
37	39a	31b	30b	25c	20c	39a	32b	32b	29c	1d
47	43a	37b	33bc	28c	20d	41a	36b	33c	32c	1d
57	45a	37b	34bc	30c	20d	41a	37b	36b	33c	2d
67	49a	46ab	42bc	36c	23d	40a	36b	36b	34b	1c
77	52a	47ab	40bc	33c	21d	41a	39ab	38b	37b	1c
87	58a	53ab	42bc	36c	24d	59a	43b	40b	37c	1d

* Means in a column followed by same letters are not significantly different at 5% level of probability.
Treatments: 1 = 1S:0R, 2 = 1S:1R, 3 = 1S:2R, 4 = 1S:4R and 0S:1R.

Table 4. Mean yield and 100 seed weight of resistant and susceptible bean mixtures infected by anthracnose at Ambo, Ethiopia in 2001

Mixture S:R[a]	Pod/plant	Seed/plant	SW[c] (g)	Yield/ forty plants (g)
0:1	9	5.5b*	15.6a	2094.5b
1:4	9	6.3a*	14.8a	2434.8a
1:2	7	5.0b	12.3b	1497.8c*
1:1	7	4.8d*	12.3b	1485.1c
1:0[b]	6	3.8e*	8.9c	526.6d

[a] : S : susceptible, R : resistant.
[b] : Check plot.
[c] : 100 seed weight.
* : Means in a column followed by same letters are not significantly different at 5% level of probability.

Table 5. Mean yield and 100 seed weight of resistant and susceptible bean mixtures infected by anthracnose at Bako, Ethiopia in 2001

Treatment S:R[a]	Pod/plant	Seed/plant	SW[c] (g)	Yield/forty plants (g)
0:1	10	6b*	17.6a	1987.5a
1:4	8	6a*	15.8a	2115.7a
1:2	7	5c*	13.3b	1522.7c
1:1	7	5d*	11.1b	1535.2c
1:0[b]	4		7.1d	399.3d

[a] : S : susceptible, R : resistant.
[b] : Check plot.
[c] : 100 seed weight.
* : Means in a column followed by same letters are not significantly different at 5% level of probability.

Table 6. Regression model for the response variables leaf severity (LS) and yield (Y) against the predictor variables cultivar mixtures, assessment time and distance from the source at Ambo, Ethiopia in 2001

	Model
LS	= 31.85 - 0.193*(cultivar mixtures) + 0.387*(assessment time) – 0.092*(disease from source)
Y	= 6.56+ 0.067*(cultivar mixtures) +0.022*(disease from source)

Table 7. Regression model for the response variables leaf severity (LS) and yield (Y) against the predictor variables cultivar mixtures, assessment time and distance from the source at Bako, Ethiopia in 2001

LS	= 29.19 - 0.280*(cultivar mixtures) + 0.326*(assessment time) - 0.371*(disease from source)
Y	= 14.94+ 3.476*(cultivar mixtures)+ 8.235*(disease from source)

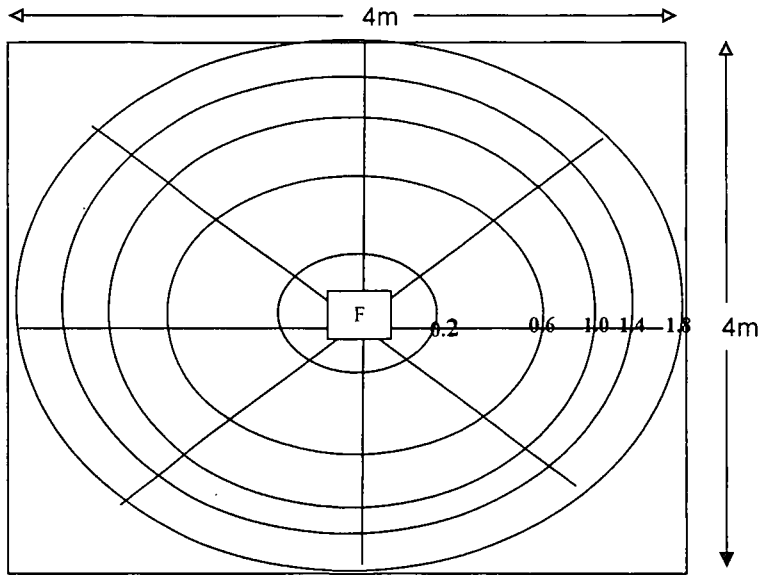


Fig 1. Diagram of an individual plot. F represents focus of infection.

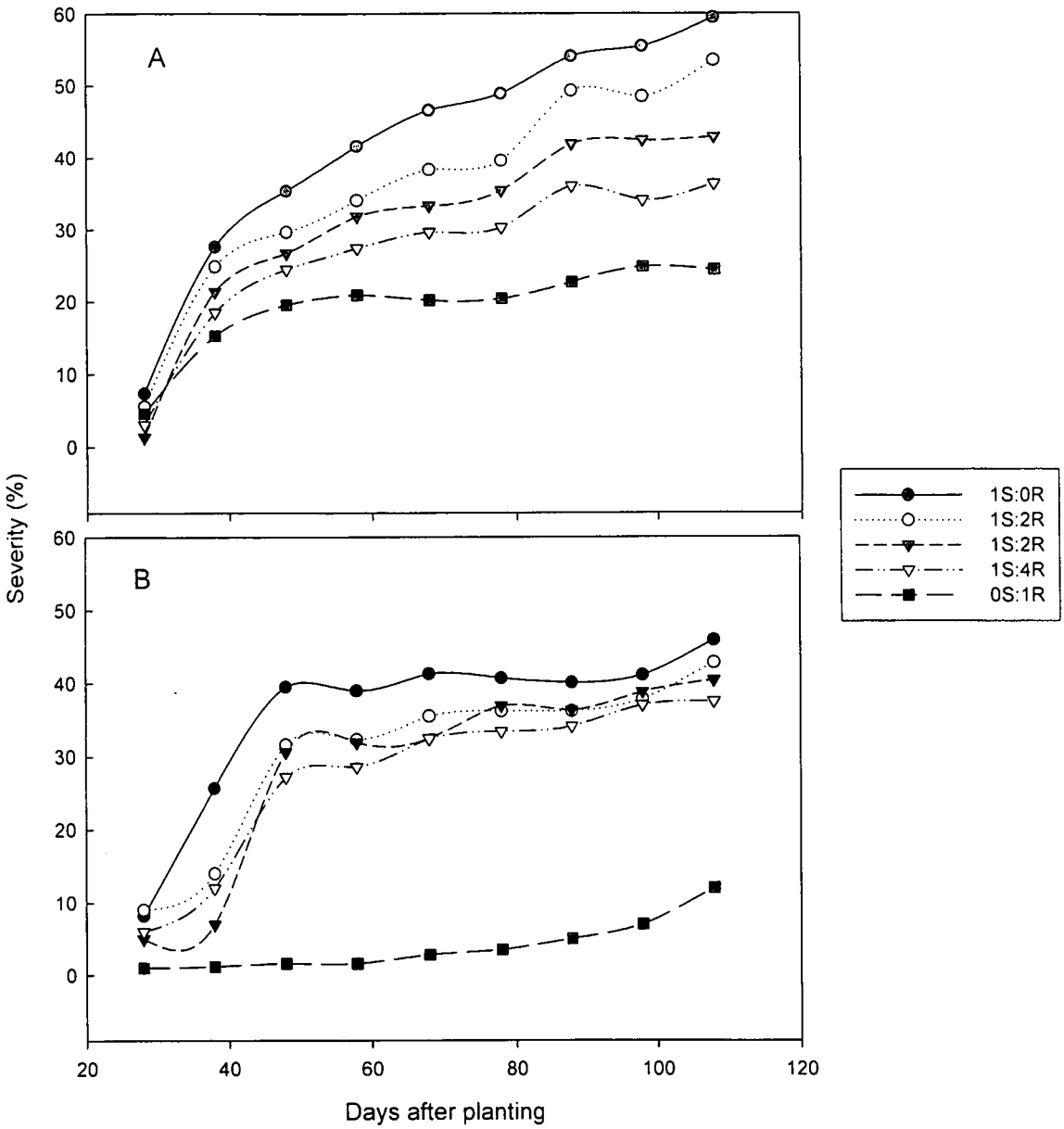


Fig 2. Progress curves of anthracnose as measured at Ambo (A) and Bako (B) in different bean cultivar mixtures. Treatments were: 1S:0R, 1S:1R, 1S:2R, 1S:4R and 0S:1R. (S = susceptible and R = resistant).

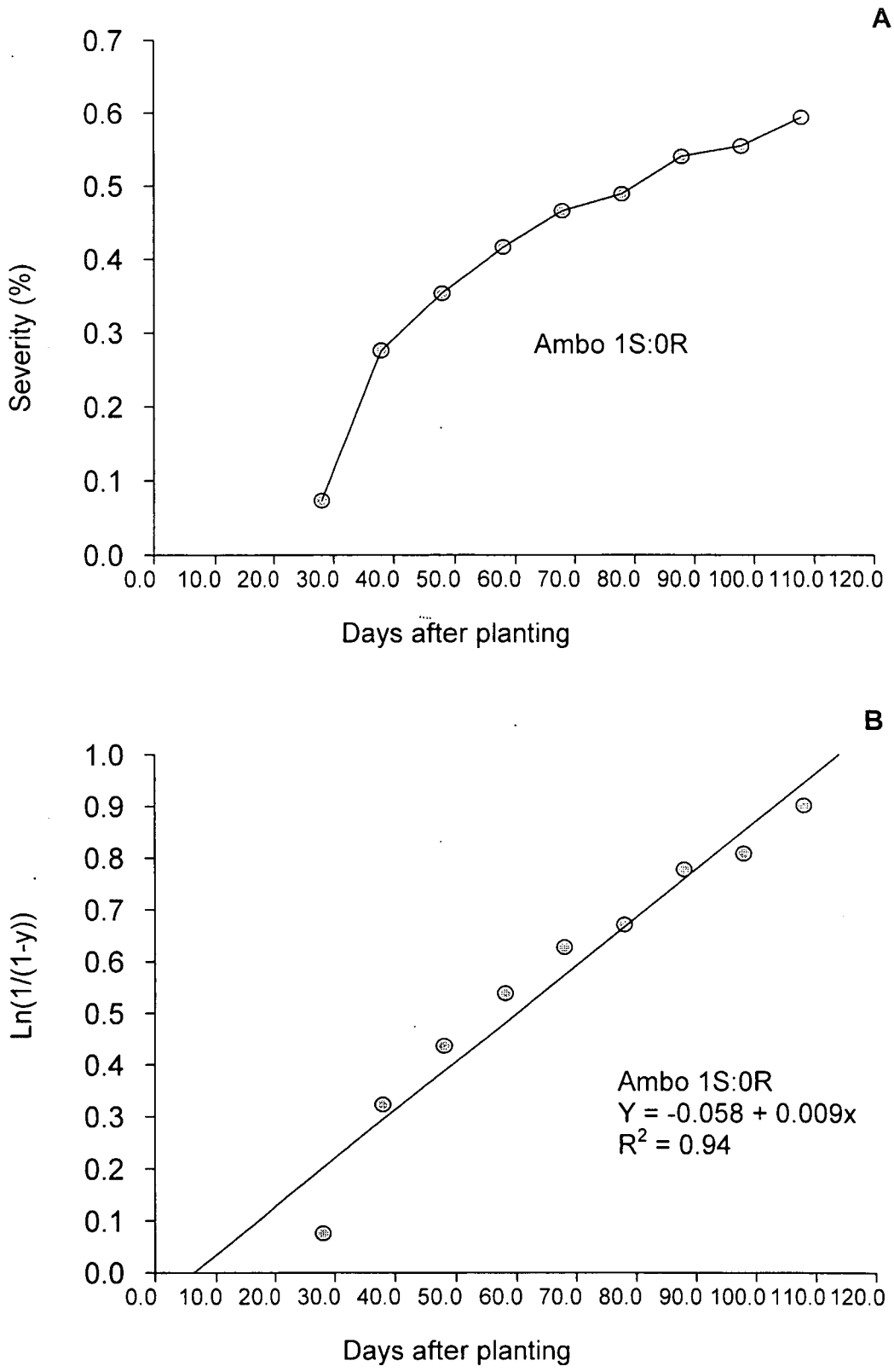
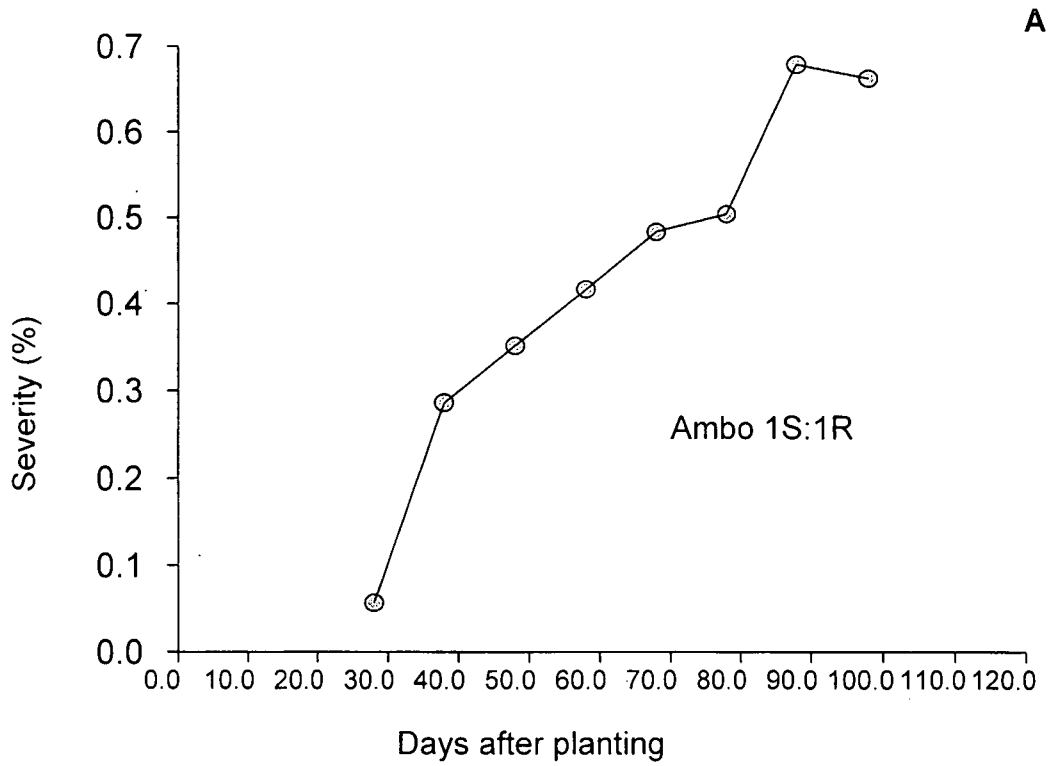


Fig 3. Disease progress curves (actual [A] and transformed [B] values) of anthracnose in different bean mixtures in the field at Ambo in 2001. To linearize the curve a monomolecular model (ln (1/(1-y)) was applied.


A
Severity (%)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Ambo 1S:1R
0.0 10.0 20.0 30.0 40.0 50.0 60.0 70.0 80.0 90.0 100.0 110.0 120.0
Days after planting

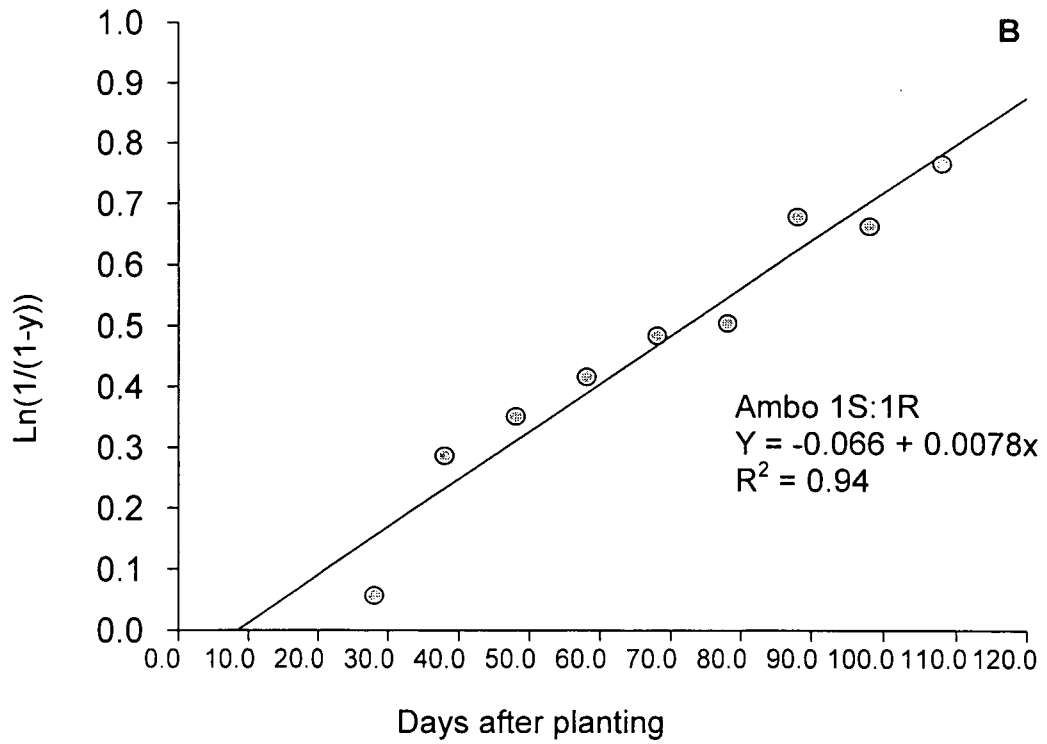
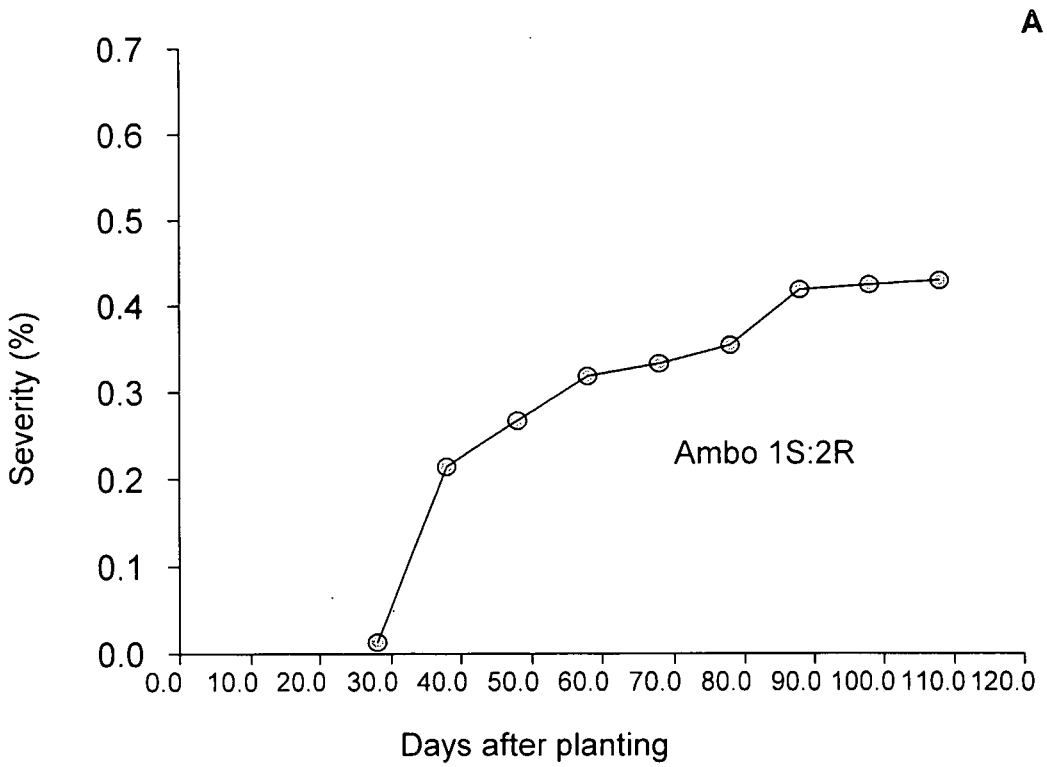

B
Ln(1/(1-y))
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Ambo 1S:1R
Y = -0.066 + 0.0078x
R2 = 0.94
0.0 10.0 20.0 30.0 40.0 50.0 60.0 70.0 80.0 90.0 100.0 110.0 120.0
Days after planting

Fig . 3. cont'd


A
Severity (%)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.0 10.0 20.0 30.0 40.0 50.0 60.0 70.0 80.0 90.0 100.0 110.0 120.0
Days after planting
Ambo 1S:2R

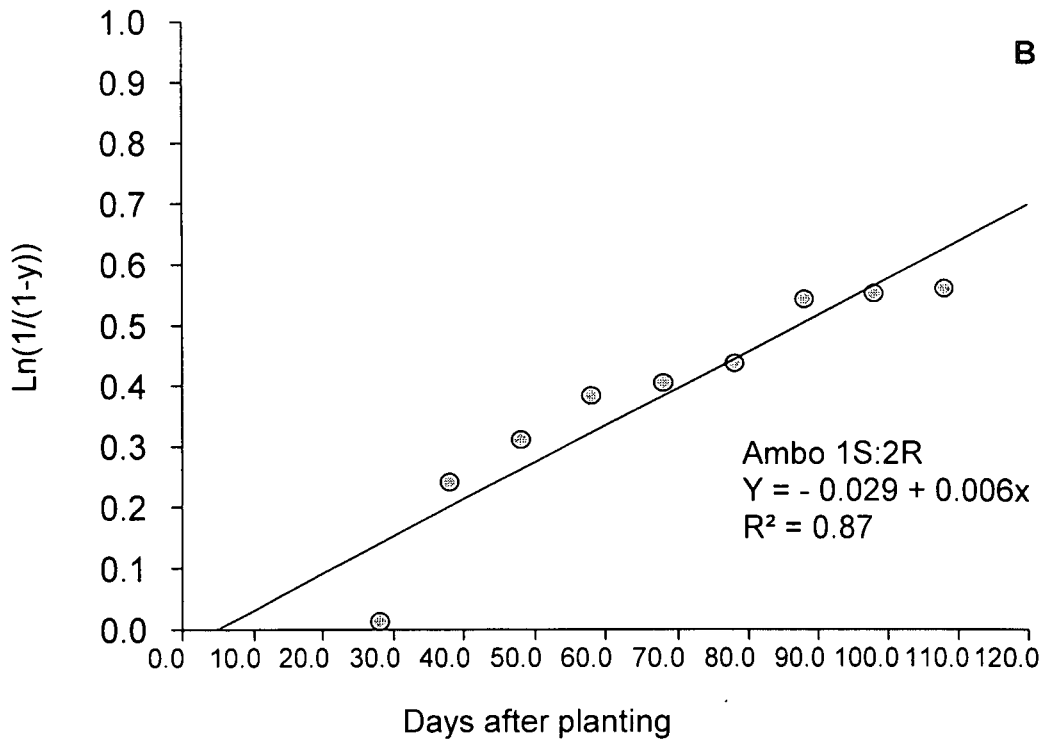
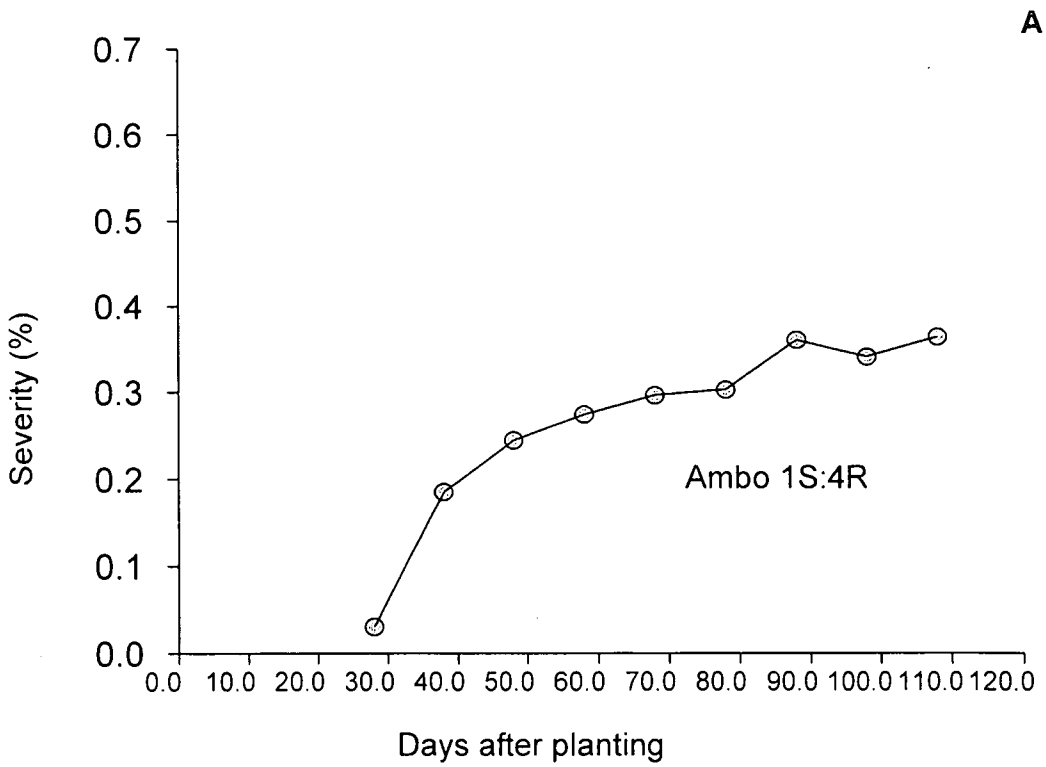

B
Ln(1/(1-y))
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.0 10.0 20.0 30.0 40.0 50.0 60.0 70.0 80.0 90.0 100.0 110.0 120.0
Days after planting
Ambo 1S:2R
Y = - 0.029 + 0.006x
R² = 0.87

Fig . 3. cont'd


A
Severity (%)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Ambo 1S:4R
0.0 10.0 20.0 30.0 40.0 50.0 60.0 70.0 80.0 90.0 100.0 110.0 120.0
Days after planting

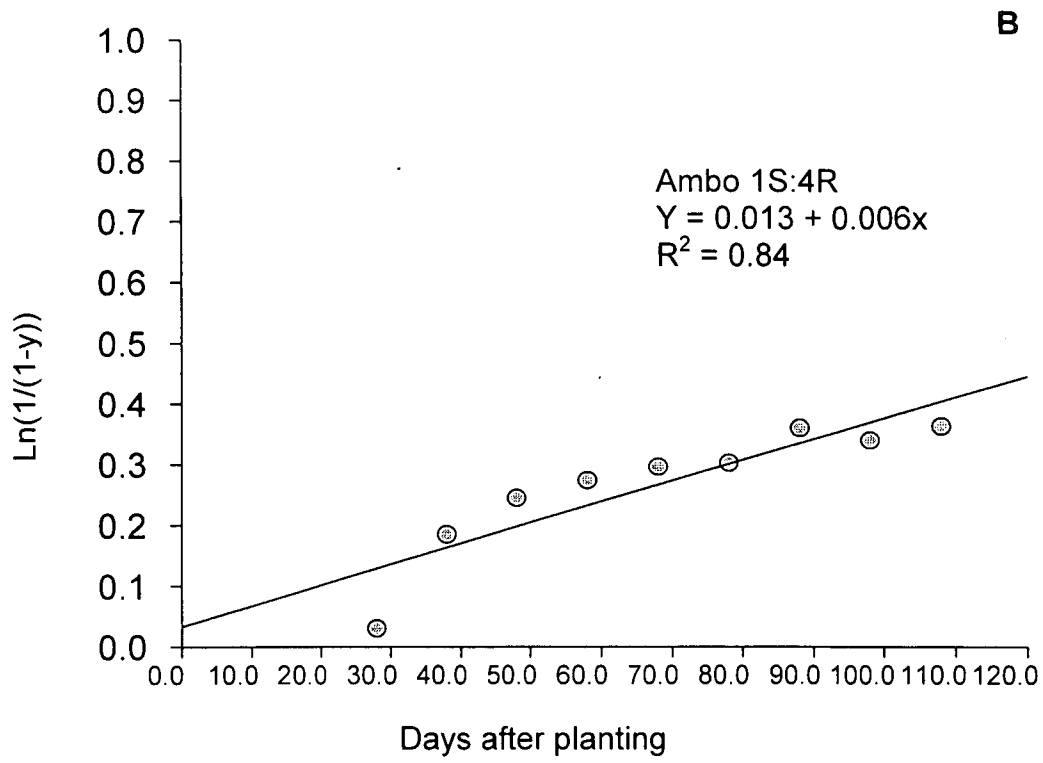
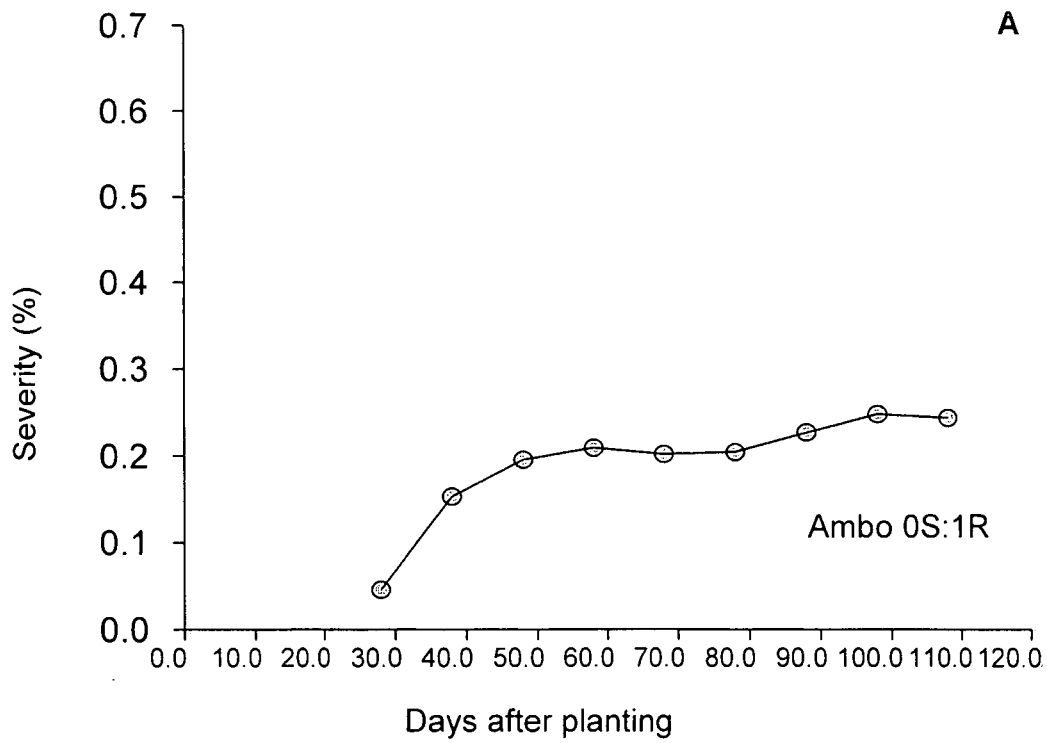

B
Ln(1/(1-y))
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Ambo 1S:4R
Y = 0.013 + 0.006x
R2 = 0.84
0.0 10.0 20.0 30.0 40.0 50.0 60.0 70.0 80.0 90.0 100.0 110.0 120.0
Days after planting

Fig . 3. cont'd


A
Severity (%)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Ambo 0S:1R
0.0 10.0 20.0 30.0 40.0 50.0 60.0 70.0 80.0 90.0 100.0 110.0 120.0
Days after planting

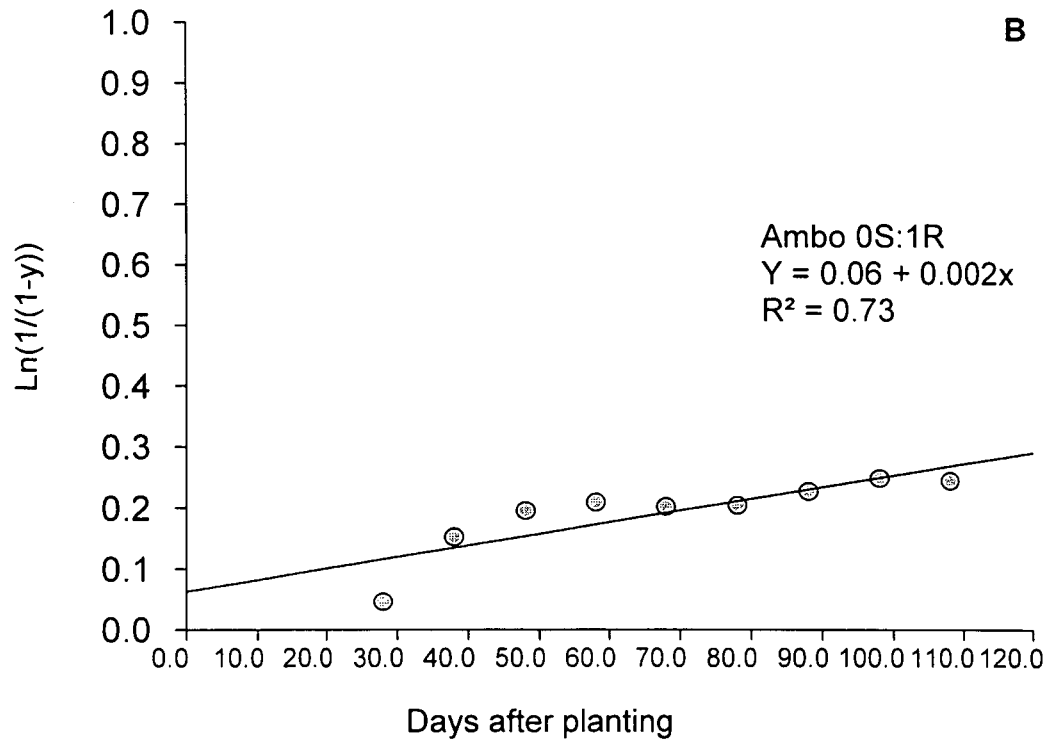

B
Ln(1/(1-y))
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Ambo 0S:1R
Y = 0.06 + 0.002x
R² = 0.73
0.0 10.0 20.0 30.0 40.0 50.0 60.0 70.0 80.0 90.0 100.0 110.0 120.0
Days after planting

Fig . 3. cont'd

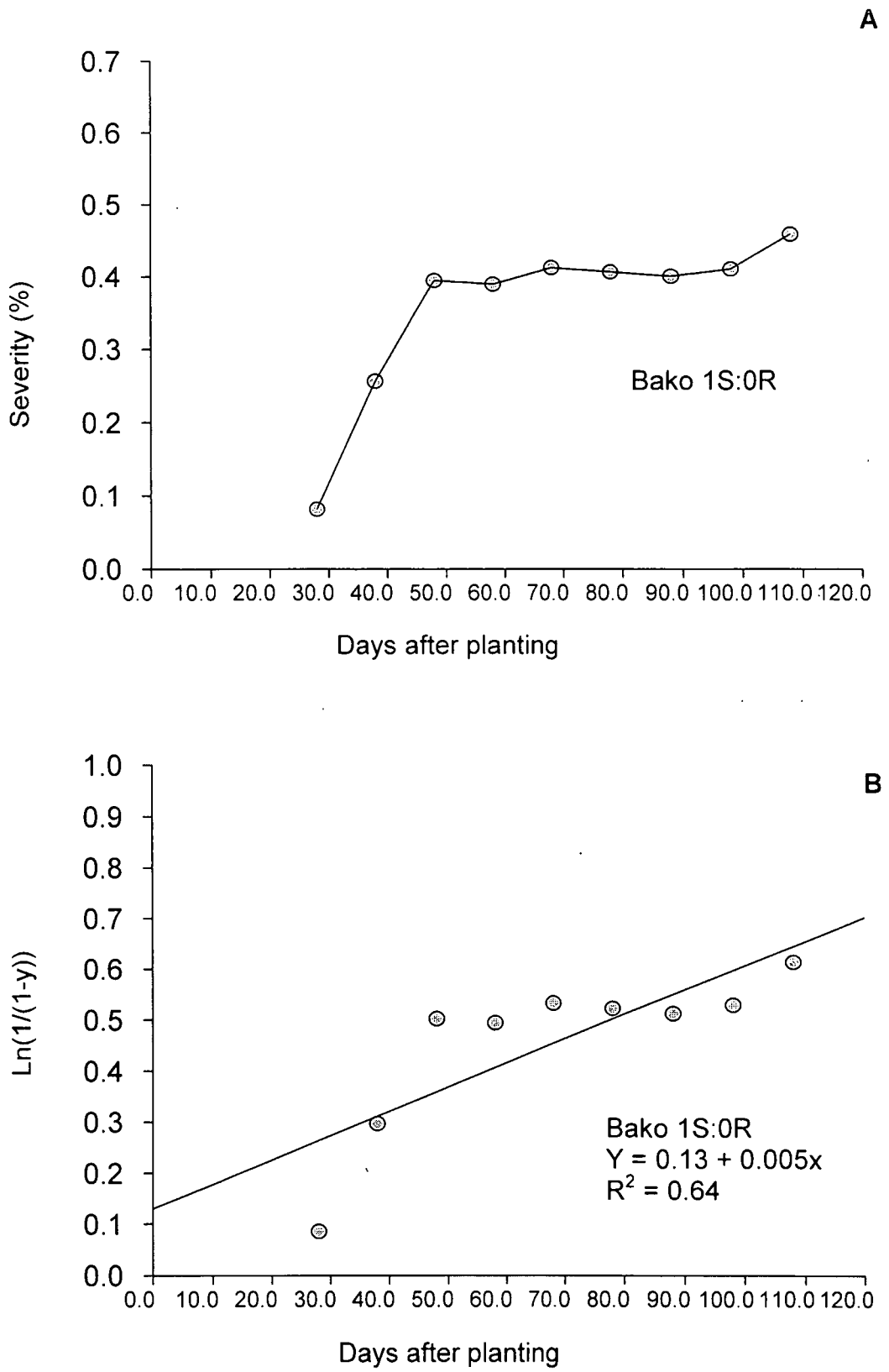
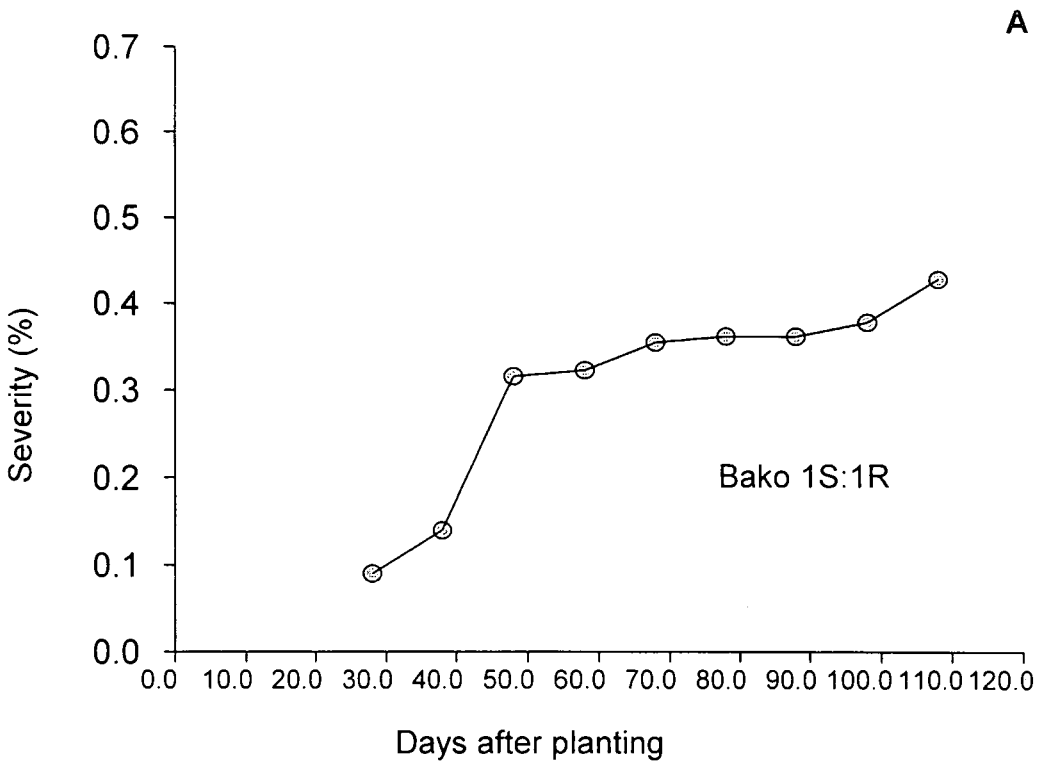


Fig 4. Disease progress curves (actual [A] and transformed [B] values) of anthracnose in different bean mixtures in the field at Bako in 2001. To linearize the curve a monomolecular model (ln (1/(1-y)) was applied.


A
Severity (%)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Bako 1S:1R
0.0 10.0 20.0 30.0 40.0 50.0 60.0 70.0 80.0 90.0 100.0 110.0 120.0
Days after planting

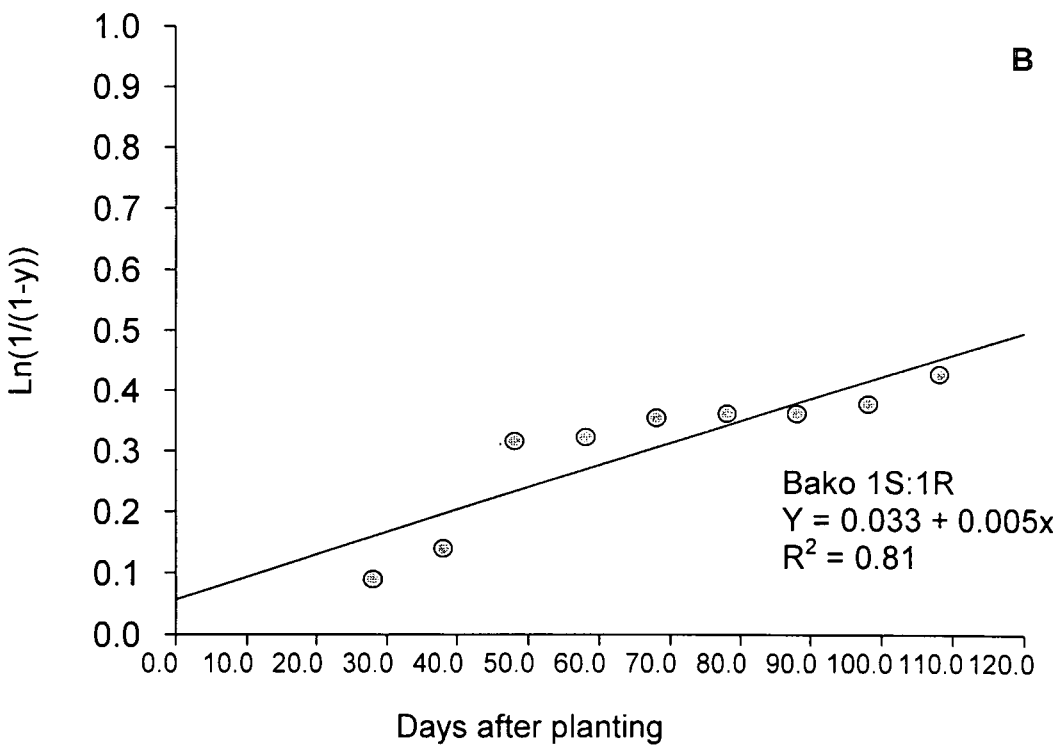
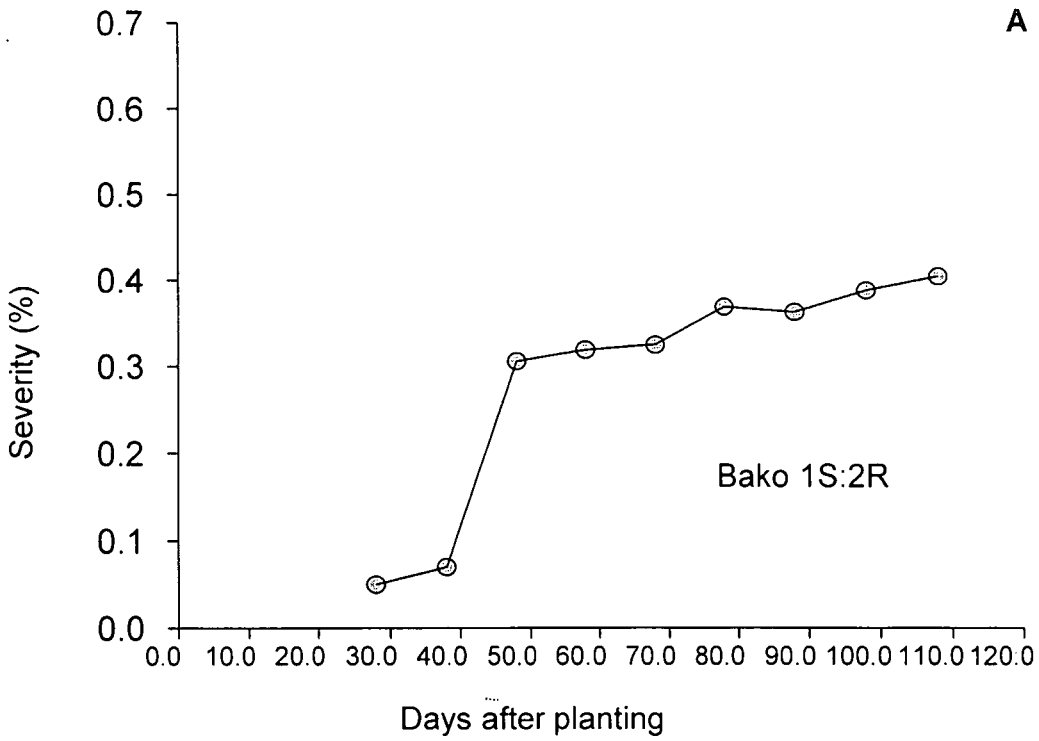

B
Ln(1/(1-y))
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Bako 1S:1R
Y = 0.033 + 0.005x
R2 = 0.81
0.0 10.0 20.0 30.0 40.0 50.0 60.0 70.0 80.0 90.0 100.0 110.0 120.0
Days after planting

Fig . 4. cont'd


A
Severity (%)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Bako 1S:2R
0.0 10.0 20.0 30.0 40.0 50.0 60.0 70.0 80.0 90.0 100.0 110.0 120.0
Days after planting

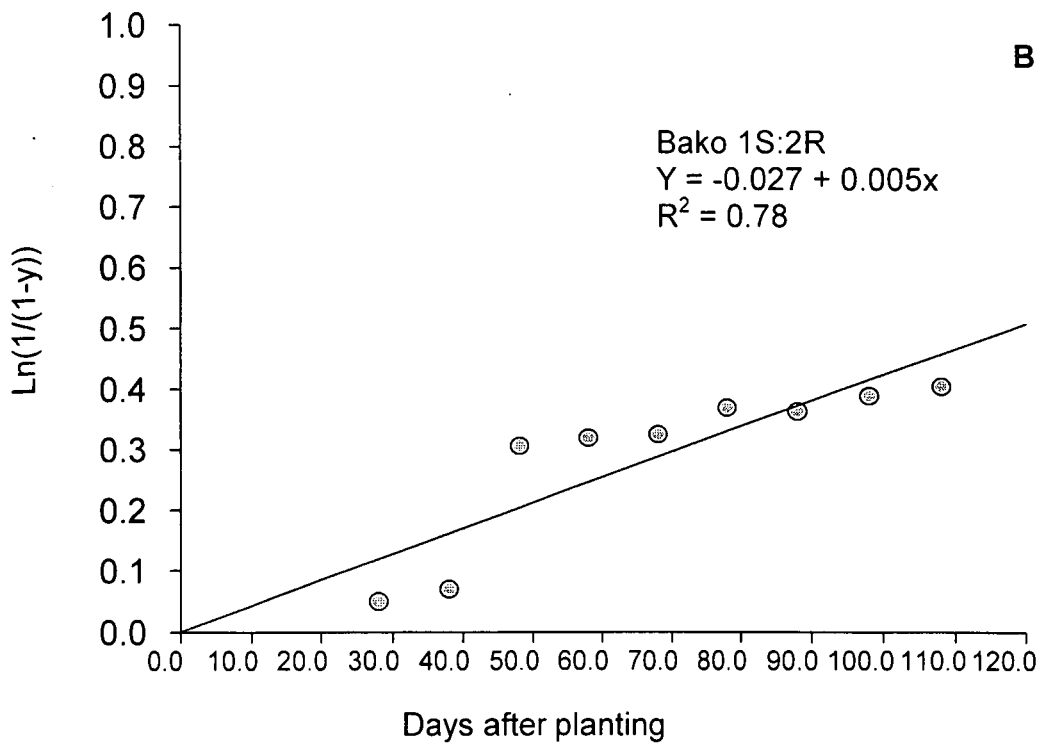
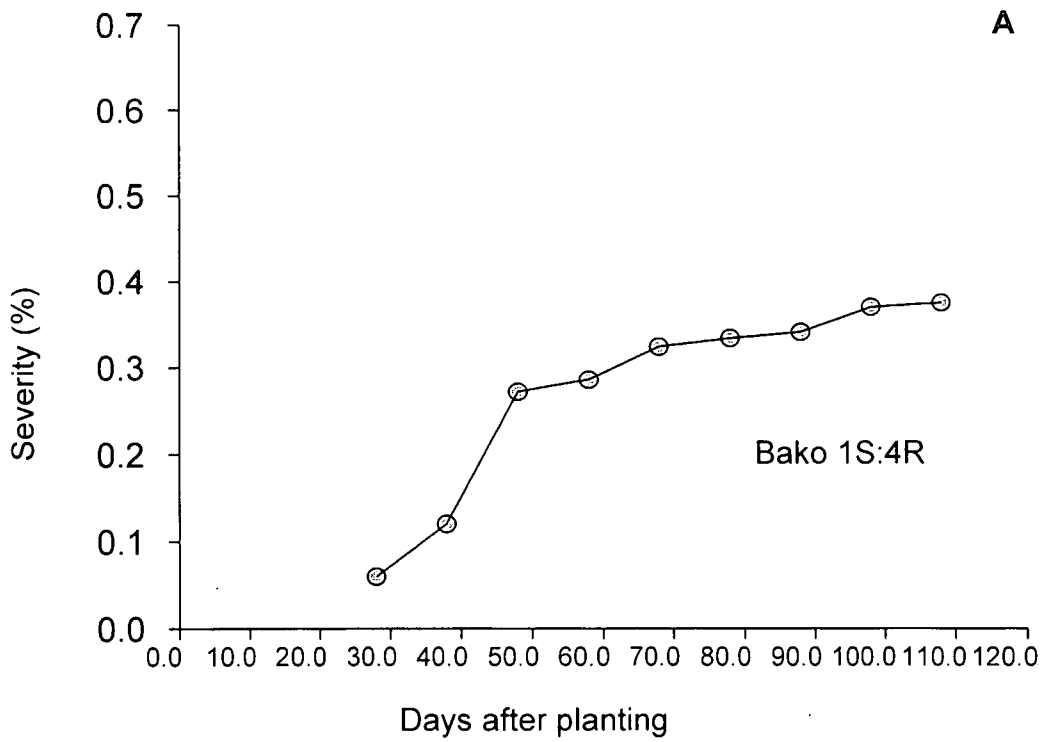

B
Ln(1/(1-y))
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Bako 1S:2R
Y = -0.027 + 0.005x
R2 = 0.78
0.0 10.0 20.0 30.0 40.0 50.0 60.0 70.0 80.0 90.0 100.0 110.0 120.0
Days after planting

Fig . 4. cont'd


A
Severity (%)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Bako 1S:4R
0.0 10.0 20.0 30.0 40.0 50.0 60.0 70.0 80.0 90.0 100.0 110.0 120.0
Days after planting

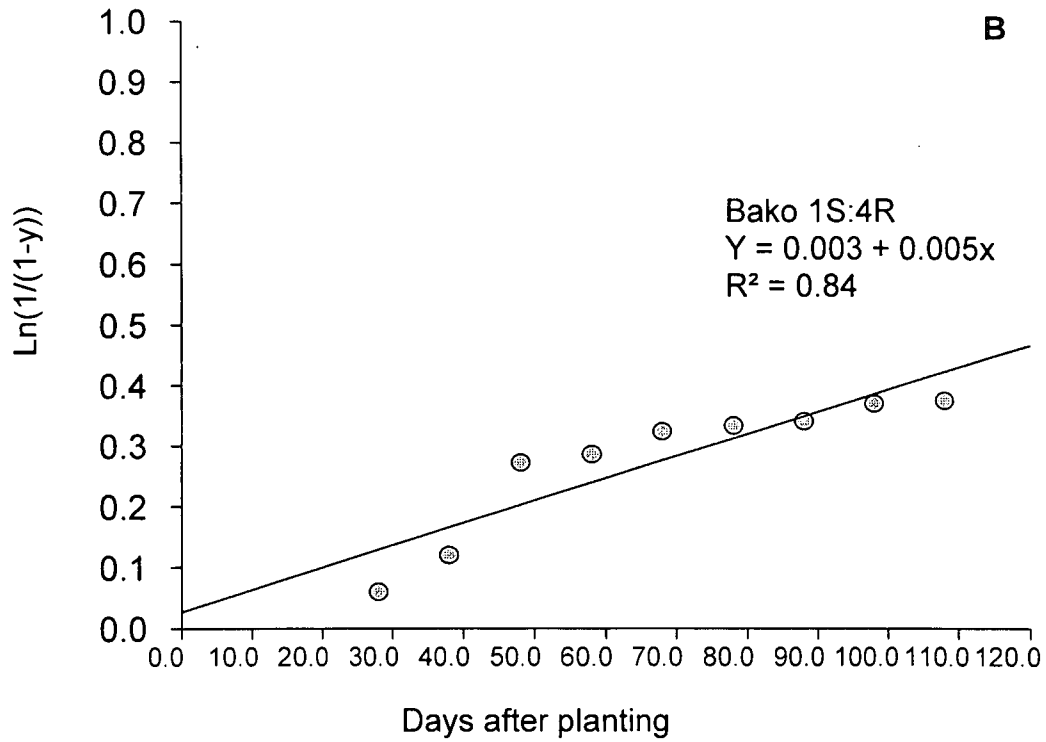
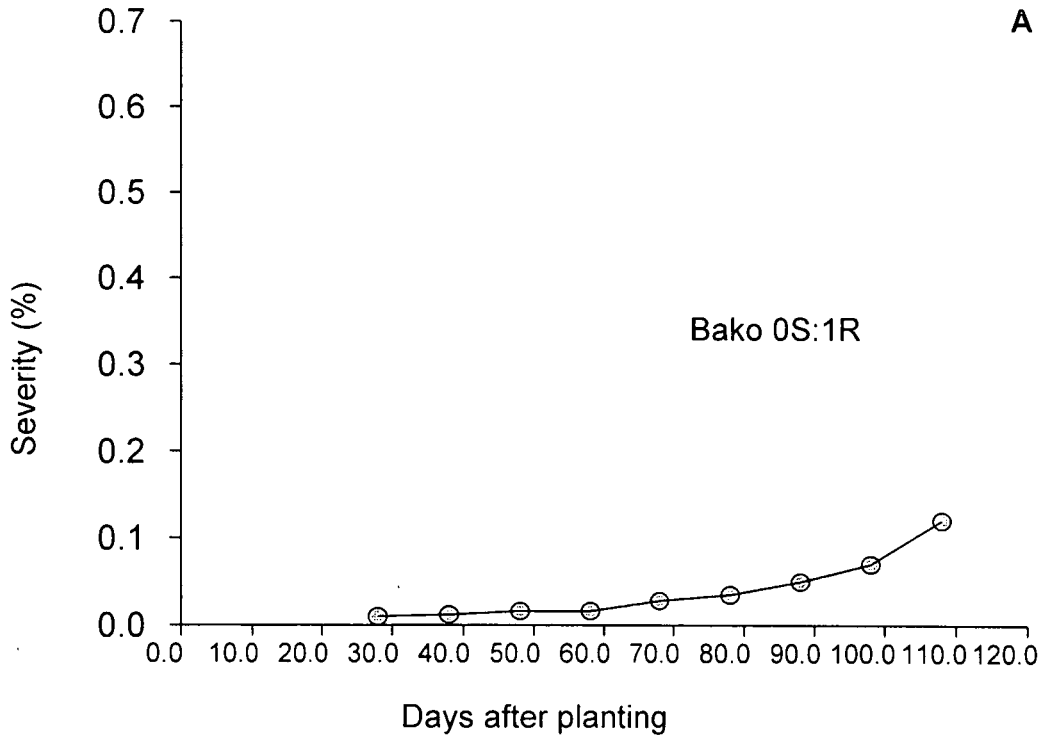

B
Ln(1/(1-y))
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Bako 1S:4R
Y = 0.003 + 0.005x
R² = 0.84
0.0 10.0 20.0 30.0 40.0 50.0 60.0 70.0 80.0 90.0 100.0 110.0 120.0
Days after planting

Fig . 4. cont'd


A
Severity (%)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Bako 0S:1R
0.0 10.0 20.0 30.0 40.0 50.0 60.0 70.0 80.0 90.0 100.0 110.0 120.0
Days after planting

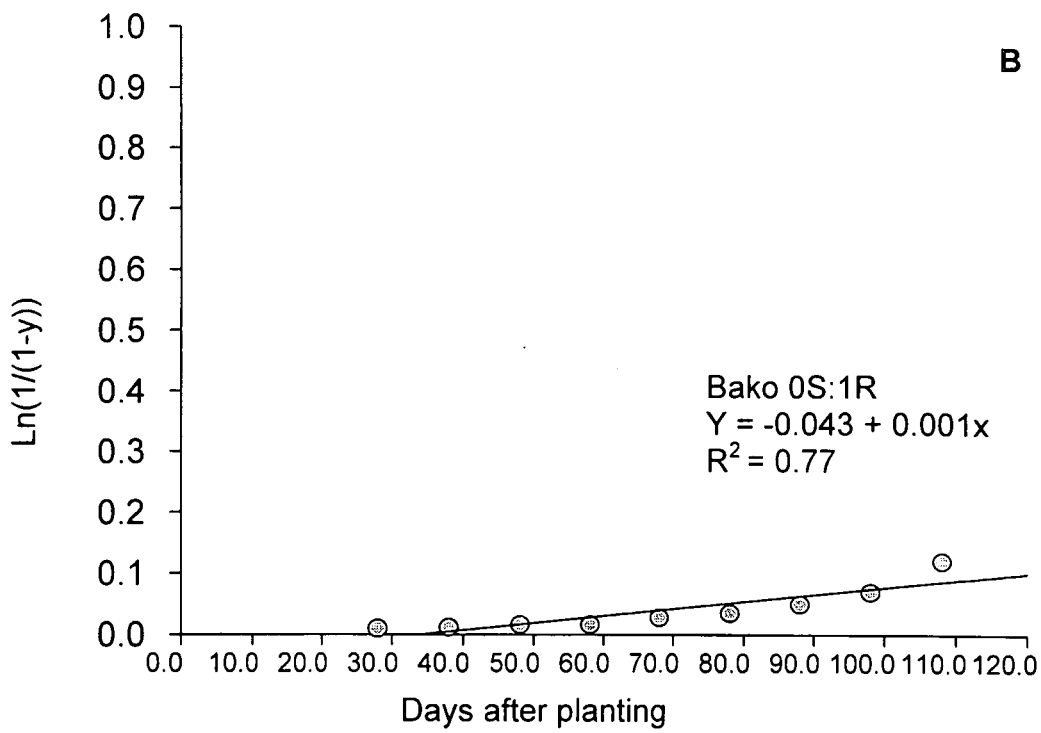
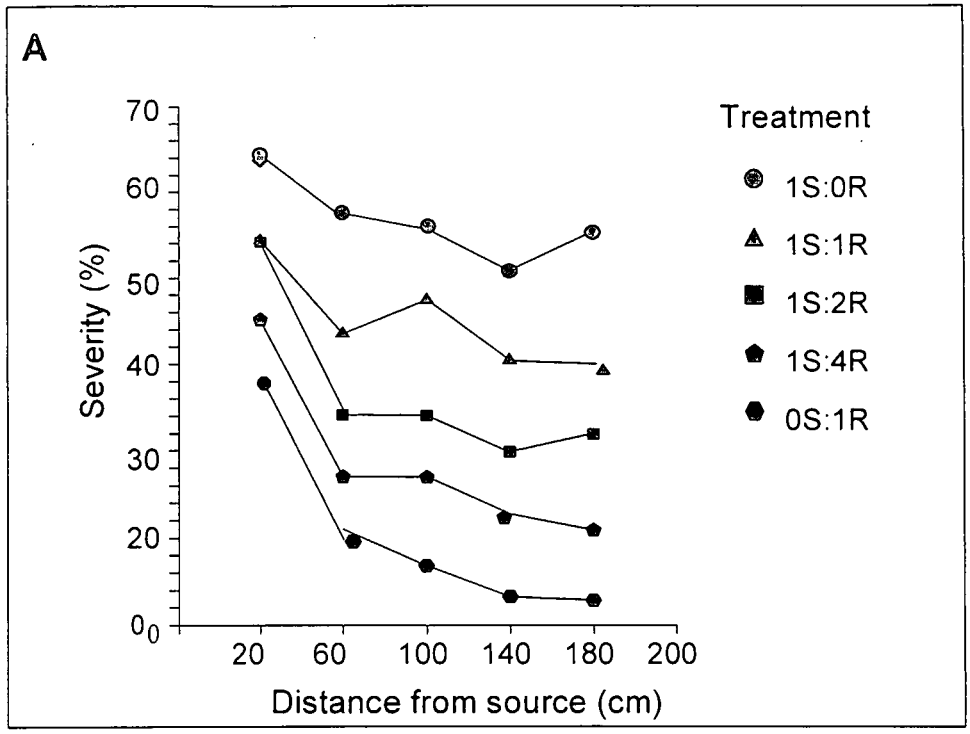

B
Ln(1/(1-y))
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Bako 0S:1R
Y = -0.043 + 0.001x
R2 = 0.77
0.0 10.0 20.0 30.0 40.0 50.0 60.0 70.0 80.0 90.0 100.0 110.0 120.0
Days after planting

Fig . 4. cont'd



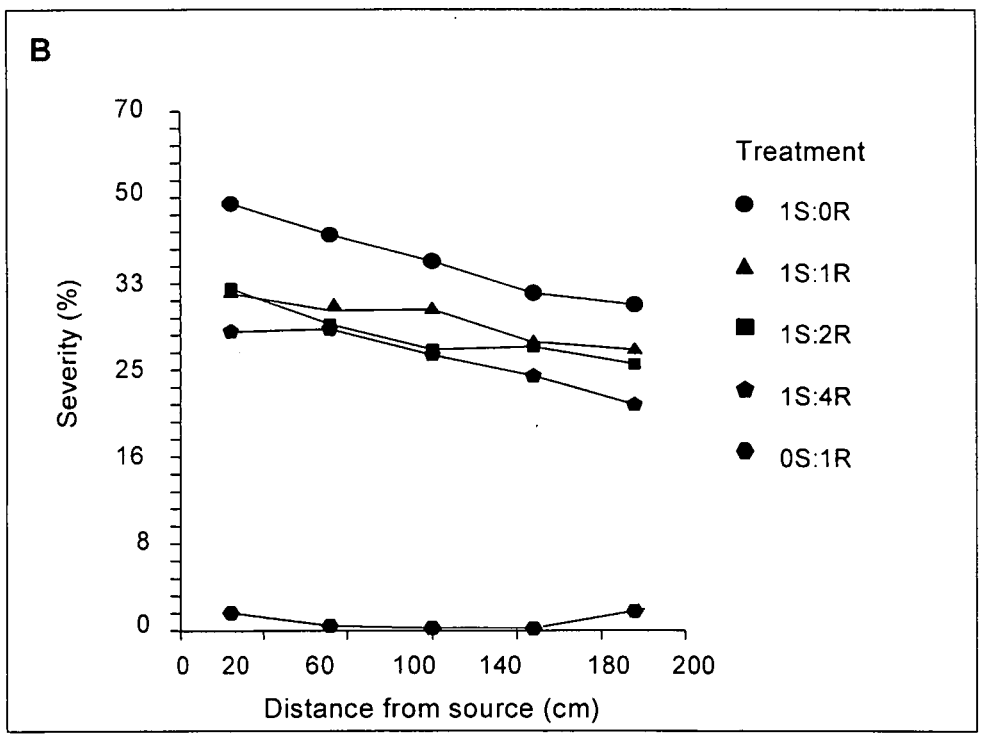


Fig. 5 Disease gradients for anthracnose spread from an inoculum focus in mixtures of susceptible (S) and resistant (R) common bean plots at Ambo (A) and Bako (B) in Ethiopia , 2001.

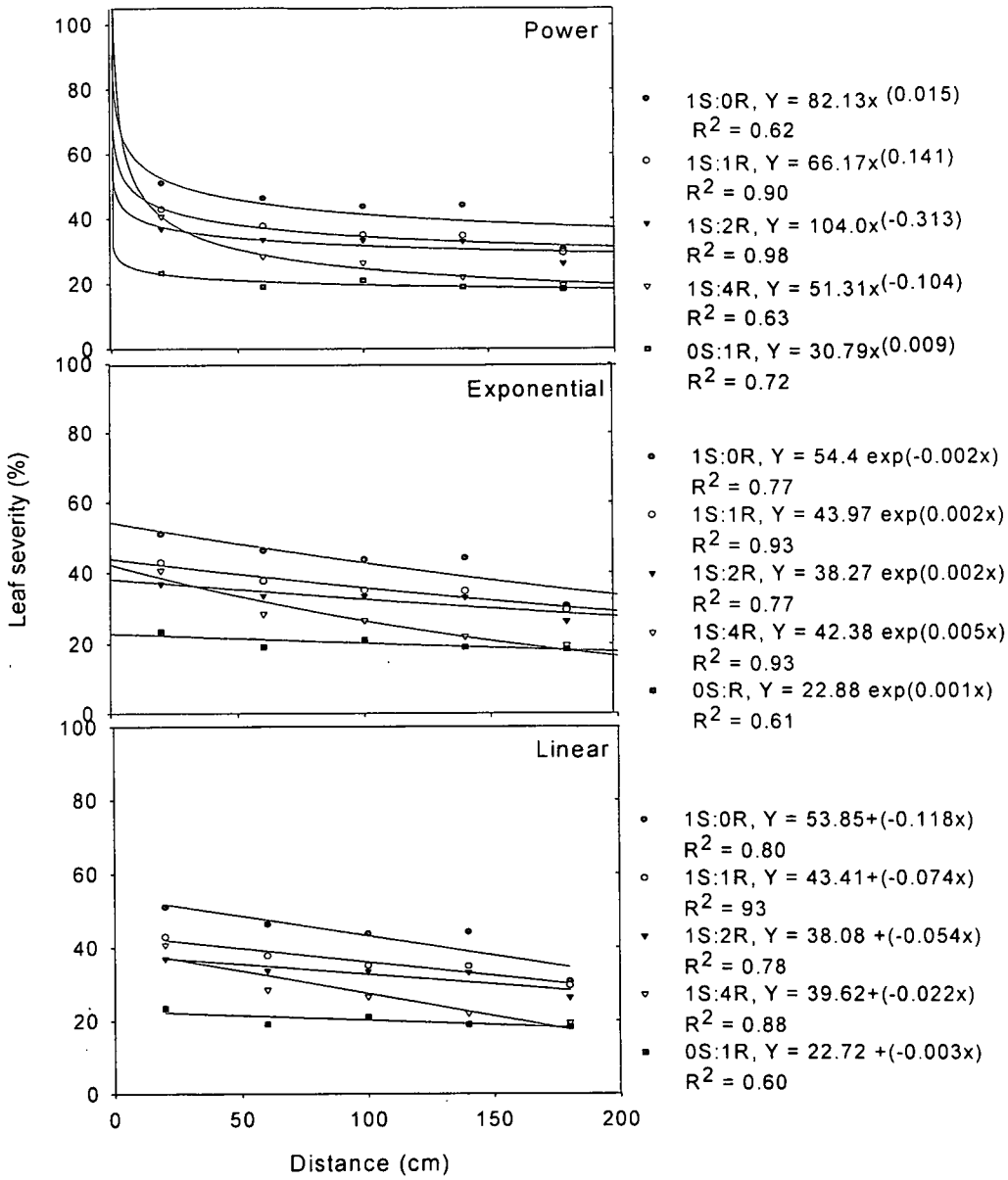


Fig 6. Disease gradients for anthracnose spread from an inoculum focus in mixtures of susceptible (S) and resistant (R) common bean plots at Ambo after fitting the Power, Exponential and Linear models.

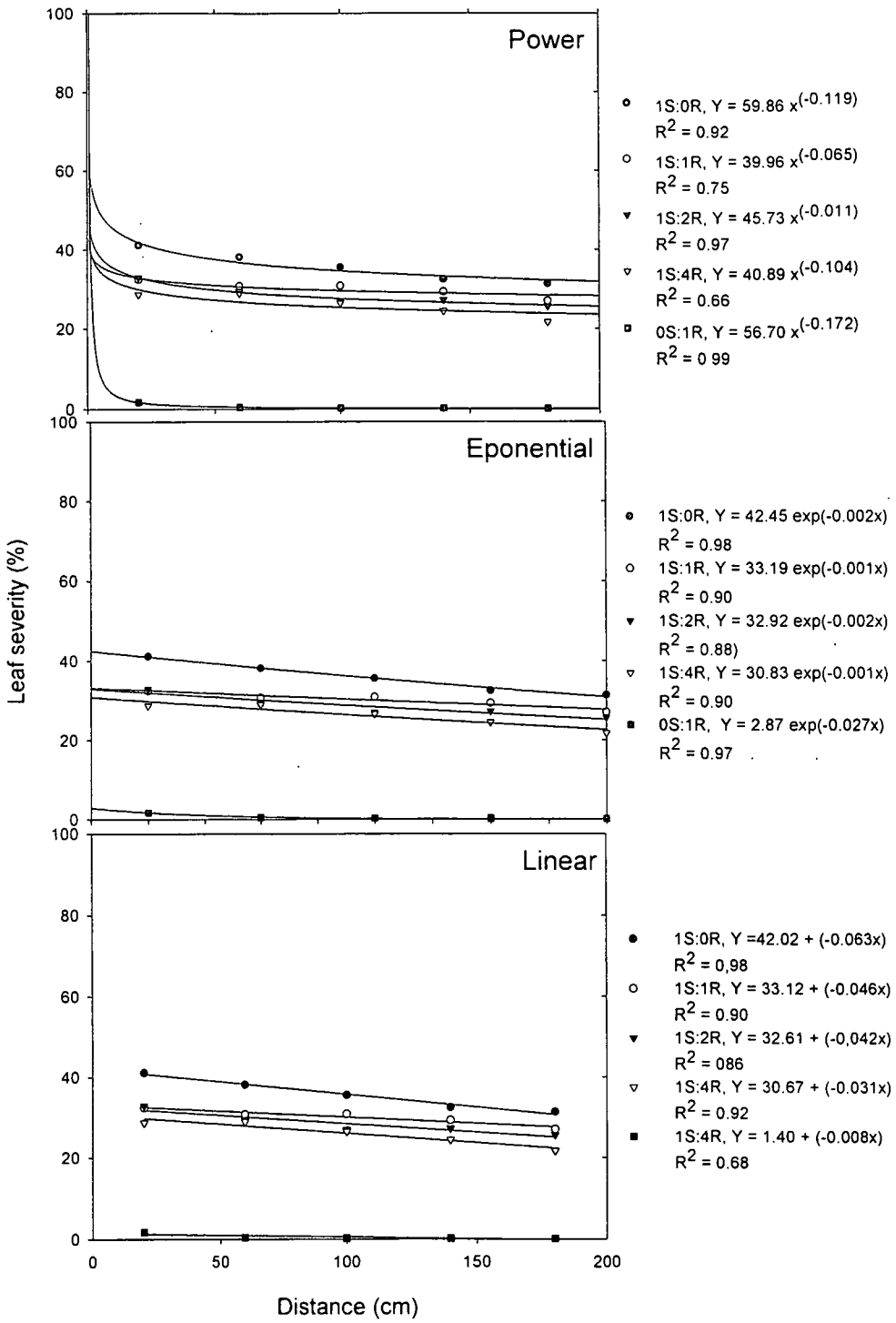


Fig 7. Disease gradients for anthracnose spread from an inoculum focus in mixtures of susceptible (S) and resistant (R) common bean plots at Bako after fitting the Power, Exponential and Linear models.

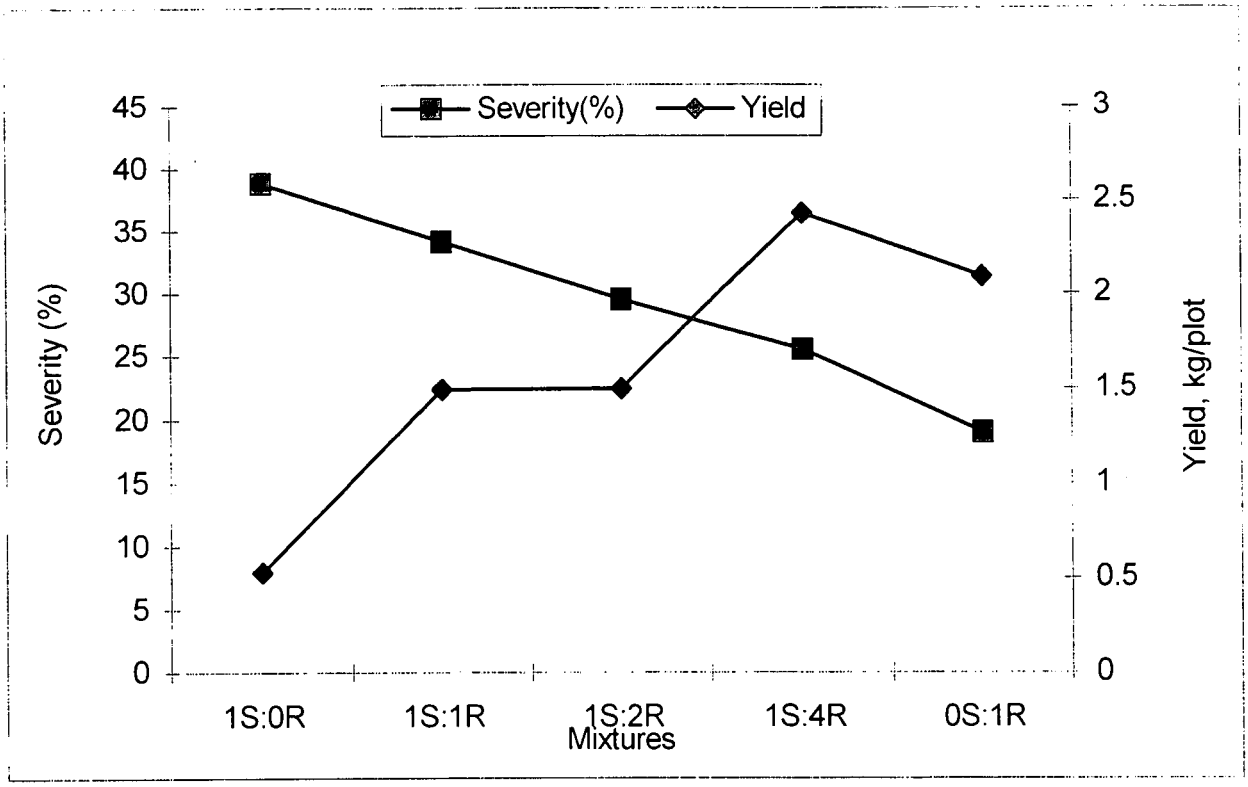


Fig 8. The relationship between anthracnose severity (Y_1-axis)and yield of common bean (Y_2-axis) for different ratios of susceptible and resistant cultivars at Ambo.

Chapter 6

Effect of seed treatment and foliar application of fungicides on the control of bean anthracnose

ABSTRACT

Field experiments were conducted at Ambo and Bako (Ethiopia) to assess the efficacy of fungicides to control anthracnose (*Colletotrichum lindemuthianum*) in bean (*Phaseolus vulgaris*). Three fungicides were used: benomyl 500 g a.i./kg WP (at a rate of 2g/kg as seed dressing and 1kg/750 l as foliar spray), mancozeb 800 g a.i./kg WP (at a rate of 200g a.i./100 l as foliar spray) and difenoconazole 250 ml a.i./l EC (at a rate of 87.5 g a.i./ha foliar spray). Eight treatment combinations were studied: benomyl seed treatment, benomyl seed treatment + benomyl foliar spray, benomyl seed treatment + mancozeb foliar spray, benomyl seed treatment + difenoconazole foliar spray, mancozeb foliar spray, difenoconazole foliar spray, benomyl foliar spray and an untreated control. Foliar sprays were applied three times at 20 days intervals commencing seven days after inoculation. Compared with other treatments, seed treated with benomyl followed by difenoconazole, or difenoconazole alone, reduced anthracnose severity by 62% and 64% at Ambo, and by 31% and 25% at Bako, respectively. The seed treatment plus difenoconazole spray resulted in higher bean yields at Ambo (2.23 kg/plot) and Bako (2.04 kg/plot). Although it is unlikely that chemical control will be practised by small-scale farmers in Ethiopia, this study provided information on yield loss due to anthracnose as well as which compounds are effective in controlling the disease.

Keywords: Anthracnose, fungicide, *Phaseolus vulgaris*

INTRODUCTION

Anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. & Magn.) Briosi and Cav is one of the most devastating seed borne diseases of common bean (*Phaseolus vulgaris* L.). Foliar infection also results from crop residues and secondary inoculum originating from other infected plants. Anthracnose affects all aerial parts of the plant and total yield losses are possible when contaminated seed is planted and favourable conditions exist (Schwartz, 1991).

In subsistence farming systems in Africa farmers retain their seed for planting the next season. When infected, these seeds serve as a primary source of inoculum for disease development. Hence, disease intensity proportionally increases as infected seeds increase. Although use of clean seed is an appropriate option to control anthracnose, there is little effort in this regard. In most African countries structured seed production and certification schemes do not exist and there is little opportunity for obtaining disease-free seed (Fredrica and Teri, 1985).

The use of seed treatments is an important disease control tactic in general and in particular for anthracnose in developed countries (Berger and Wolf, 1974; Smith and Black, 1993; Wilson *et al.*, 1993; Freeman, *et al.*, 1997). However, seed treatment alone could be inefficient and often requires follow-up applications of protectant or systemic foliar fungicides (Yourman *et al.*, 2000; Koch, 1996). Furthermore, chemical disease control should form part of an integrated disease management system including host resistance, cultural practices and manipulation of environmental conditions (Schwartz *et al.*, 1982).

Various control strategies have been advocated in an attempt to reduce losses caused by anthracnose (Zaumeyer and Thomas, 1957; Chaves, 1980; Ferraz, 1980). However, grower acceptance and utilization of fungicides are not always possible especially in the case of subsistence farmers in developing countries. These growers possess few land holdings and resources and often are unable to readily obtain or adopt the recommended practices for their region (Schwartz *et al.*, 1982). At present no information is available on the chemical control of bean anthracnose under Ethiopian conditions. This study was therefore conducted to

investigate fungicides as a seed treatment, foliar spray or in combination in the control of *C. lindemuthianum* on common bean.

MATERIALS AND METHODS

Field experiments

Field studies were carried out at the Ambo and Bako research centers, Ethiopia in 2001 (see Chapter 5 for descriptions). The anthracnose-susceptible common bean variety Mexican 142 was used to compare fungicide treatments. Plots consisted of 10 4-m-rows, spaced 40 cm apart. Intra-row spacing was 10 cm. Treatments were arranged in a randomized complete block with six replications.

Treatments

The susceptible bean variety Mexican 142 was used for this study. Seed treatment and foliar fungicide applications studied were:

- benomyl (500 g a.i./kg WP) applied as a seed dressing at a rate of 2 g/kg seed.
- benomyl seed dressing (as above) followed by benomyl foliar spray at a rate of 1 kg/ha.
- benomyl seed dressing (as above) followed by a mancozeb (800 g a.i./kg WP) foliar spray at a rate of 200g a.i./ha.
- benomyl seed dressing (as above) followed by difenoconazole (250 ml a.i./L EC) foliar spray at a rate of 87.5 g a.i./ha
- mancozeb foliar application (as above)
- difenoconazole foliar application (as above)
- benomyl foliar application (as above)
- control (untreated)

Foliar fungicides were applied three times at 20-day-intervals, starting 14 days after planting.

Inoculation

Plots were inoculated with mixed isolates of *C. lindemuthianum* collected from different bean growing areas in Ethiopia (see Chapter 4, Material and Methods). The isolates were prepared by harvesting conidia from 7-day-old cultures multiplied on sterilized bean leaves embedded on potato dextrose agar medium. A spore suspension of (1.2×10^6 spores/ml) was sprayed at the trifoliate leaf stage using a knapsack sprayer.

Anthracnose assessment

Twenty randomly selected plants from the central four rows were tagged for disease and yield assessment. Disease severity on foliage was rated nine times at 10 day intervals on a scale of 1-9 scale (Schoonhoven and Pastor-Corrales, 1987), where 1=1-10%, 2=11-20%, 3=21-30%, 4=31-40%, 5=41-50%, 6=51-60%, 7=61-70%, 8=71-80%, 9= >81%. Incidence was evaluated by rating the proportion of infected plants.

1. No visible disease symptoms,

3. Presence of very few and small lesions, mostly on the primary vein of the lower leaf surface.

5. Presence of several small lesions on the petiole or on the primary and secondary veins of the lower leaf.

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 on the petioles.

9. Severe necrosis on 25% or more of the plant tissues is evident as a result of lesions on the leaf, petioles, stems, branches, and even on the growing point, which often results in the death of most plant tissue.

Yield assessment

Yield and 100 seed weight were determined on the 20 tagged plants per plot.

Data analysis

To investigate the effect of locations and treatments on disease severity, yield and 100 seed weight, combined analyses of variance were conducted using

Agrobase (2000). Mean comparisons between treatments were carried out following the Fisher's LSD procedure at 5% level of probability.

RESULTS

The analysis of variance showed that fungicide treatments had a significant effect on disease severity and incidence, as well as on plot yield and 100 seed weight (Table 1). Furthermore, all variables measured were significantly influenced by location and an interaction between treatments and location occurred for all except yield/plot. At Ambo disease severity was significantly lower in all fungicide treated plots as compared to the untreated control (Table 2). Here incidence was similarly reduced, except for the seed treatment followed by a mancozeb application. Difenoconazole provided the best protection at Ambo, reducing severity by 63.8% and incidence by 67.5%. At Bako difenoconazole alone (24.9% reduction in severity), or in combination with benomyl (30.4% reduction), reduced disease significantly (Table 2).

With regard to yield measurements, all treatments significantly improved yield and 100 seed weight at Ambo. At Bako, none of the fungicide treatments had any significant yield improvement, whereas only three treatments increased 100 seed weight (Table 2).

DISCUSSION

This study revealed that the application of difenoconazole as a foliar spray, or a benomyl seed treatment followed by foliar applications of difenoconazole, reduced leaf disease severity, incidence and increased yield per plot and 100 seed weight. At both sites the benomyl seed dressing followed by mancozeb as well as mancozeb foliar spray alone, were found least effective in controlling anthracnose. Results suggest that seed treatment alone will not guarantee meaningful control of anthtracnose. This is supported by the fact that secondary infection during the crop growing period occurs when seed dressings are not effective any more (Ntahimpera *et al.*, 1996). However, benomyl as seed treatment has an important role in reducing initial inoculum and protecting

seedlings from seed and soil borne infection. If benomyl is to be used more regularly in anthracnose control in Ethiopia, a fungicide resistance management strategy needs to be implemented. Several workers (Griffee, 1973; Cook and Pereira, 1976; Okioga, 1976) reported the occurrence of tolerance to benzimidazole-related compounds in *C. lindemuthianum*, *C. coffeanum* and *C. musae*. Fungicide mixtures or alterations should be developed to avoid the anticipated build-up of resistance in the Ethiopian population of *C. lindemuthianum*, should chemical control become a common practice.

Difenoconazole was shown to be effective in controlling bean anthracnose in the present study. This efficacy was due to the protective and systemic mode of action (Freeman *et al.*, 1997) whereby primary and secondary infection levels are reduced. Although other compounds such as benomyl has the same mode of action it was not as effective as difenoconazole in present study. Foliar application of mancozeb with or without benomyl seed treatment was less effective but comparatively better than the control in both locations. A zineb formulation (Dithane Z-78) was also reported to be least effective in controlling *C. lindemuthianum* (Sindhan and Bose, 1981) and stem rot of rice (*Sclerotium oryzae*) (Sharma and Mehrotra, 1985).

All fungicide applications increased yield both at Ambo and Bako regardless of differences in reducing the level of anthracnose severity and incidence. However, yield improvements at Bako could not be statistically confirmed. Seed treatment and foliar application with benomyl and difenoconazole increased yield by 328%, and 172% at Ambo and Bako, respectively. Foliar application with difenoconazole alone increased yield by 287% and 115% at Ambo and Bako. At Ambo, all fungicide applications increased 100 seed weight the latter ranging from 13.97 to 15.12 g compared to the control 9.38 g. At Bako, 100 seed weights varied from 12.46 to 14.54 g as compared to 11.60 g of the untreated control. A yield increase of 105% was recorded by Sindhan and Bose (1981) after applying benomyl to control bean anthracnose.

Detailed information on the efficacy and feasibility of chemical control of bean anthracnose in Ethiopia has hitherto not available. It should not be assumed that

chemical control can not be the primary disease control strategy for bean anthracnose. The use of fungicides by Ethiopian farmers is impractical due to small land tenure and the high cost of chemical compounds and application equipment. Furthermore, fungicides are often not readily available to small-scale farmers in Ethiopia. However, the present findings suggest that seed treatment followed by a foliar application of a suitable compound such as difenoconazole, should effectively control bean anthracnose. In combination with other anthracnose management strategies such as host resistance, mixtures of susceptible and resistant cultivars, sanitation and other cultural practices, fungicide applications, where appropriate, should therefore enhance the overall efficiency of bean production in Ethiopia.

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Table 1. Combined analysis of variance for disease severity, incidence, yield per plot and 100 seed weight. Eight treatments were tested for the control of anthracnose in the common bean variety Mexican 142 at two localities in Ethiopia in 2001

Variable	Source of variation	Mean square	F-value
Severity	Treatment (T)	1166.945	21.95**
	Location (L)	835.676	15.72**
	Treatment X Location (T X L)	424.52	7.98**
	Replication (R)	102.57	1.93**
	Error (E)	53.17	
Incidence	Treatment (T)	3785.719	37.00**
	Location (L)	14292.788	139.69**
	Treatment X Location (T X L)	448.750	4.39**
	Replication (R)	103.981	1.02^{ns}
	Error (E)	102.318	
Yield per pot	Treatment (T)	1202895.061	2.88*
	Location (L)	3263806.260	7.81**
	Treatment X Location (T X L)	792446.599	1.90^{ns}
	Replication (R)	1633698.584	3.91**
	Error (E)	418100.633	
100 seed weight	Treatment (T)	17.458	21.41**
	Location (L)	22.533	27.63**
	Treatment X Location (T X L)	7.560	9.27**
	Replication (R)	1.752	2.15^{ns}
	Error (E)	0.815	

* and ** represent significance differences at 5 and 1% probability levels, respectively.
^{ns} = no significant differences.

Table 2. Mean anthracnose severity and incidence, yield per 20 plants and 100 seed weight after eight fungicide treatments at two locations (Ambo and Bako) in Ethiopia in 2001

	Severity		Incidence		Yield/20plants (kg)		100 seed weight (g)	
Treatment[a]	Ambo	Bako	Ambo	Bako	Ambo	Bako	Ambo	Bako
1	45.62	42.38	62.00	82.87	1.58	1.71	14.27	12.46
2	33.60	38.63	37.07	81.04	2.10	1.95	14.30	14.54
3	52.83	40.8	77.78	87.77	1.32	1.88	13.97	12.49
4	27.34	31.07	25.27	55.08	2.23	2.04	14.88	12.46
5	52.55	40.43	69.20	86.06	1.40	1.98	13.72	12.31
6	26.6	33.53	26.63	56.22	2.01	1.94	15.12	12.56
7	44.18	37.58	46.75	81.47	1.28	2.14	14.30	13.78
8	73.57	44.65	82.00	91.42	0.52	1.74	9.38	11.60
LSD	3.71	8.30	8.45	11.28	0.66	0.52	0.85	0.91

[a] 1= benomyl seed treatment; 2= benomyl seed treatment+benomyl spray; 3= benomyl seed treatment + mancozeb spray; 4= benomyl seed treatment + difenoconazole spray; 5= mancozeb spray; 6= difenoconazole spray; 7= benomyl spray; 8= untreated control.

Chapter 7

Reaction of common bean cultivars to *Colletotrichum lindemuthianum* in Ethiopia

ABSTRACT

Anthracnose is one of the most important diseases of beans in Ethiopia. The causal agent, *Colletotrichum lindemuthianum,* is seedborne and widely distributed in bean production areas. The aim of the present study was to determine the reactions of common bean (*Phaseolus vulgaris*) cultivars to different isolates of *C. lindemuthianum* under field and greenhouse conditions, hence identifying sources of resistance for future breeding work. A total of 200 common bean cultivars were evaluated with a mixture of eight races of *C. lindemuthianum* in the field at Ambo and Bako in 2001 and also tested against eight single races in a greenhouse. Considering the cultivar G 2333 as the resistant control, 54 entries (27%) had lower disease ratings at both sites. Cultivars GLP X 1132, A 482, A 193 G 2333, HAL 5, A 483, G 7 and AB 136 were resistant at both locations and showed high levels of resistance to the individual anthracnose races in the greenhouse. Variation in disease did not explain variation in yield. Furthermore, cultivars had variable expression for growth habit, maturity, seed colour and seed size. This study showed that *P. vulgaris* genotypes resistant to Ethiopian races of *C. lindemuthianum* exist and that breeding efforts should be directed towards incorporating durable resistance in local cultivars.

Keywords: *Colletotrichum lindemuthianum*, *Phaseolus vulgaris*, cultivar resistance, AUDPC.

INTRODUCTION

Bean (*Phaseolus vulgaris* L.) is an important and cheap source of protein, natural fibre and calories in eastern and southern Africa and tropical America. It is an important food and cash crop in Africa, particularly in the eastern (Kenya, Uganda, Tanzania and Ethiopia), southern (Zambia, Zimbabwe and South Africa) and Great Lakes (Rwanda, Burundi, and Zaire) regions of the continent (CIAT, 1981; Beebe and Pastor-Corrales, 1991). Ethiopia ranks fifth after Uganda, Tanzania, Burundi and Rwanda in bean production in Eastern Africa (http://www.ciat.cgiar.org/beans/worldstat2/). In Africa, crop yields of slightly more than 500 kg/ha are commonly attained, which is significantly less than yields achieved for most commercial common bean varieties. These low yields result from the combined effect of constraints such as adverse climate, low soil fertility, and damage by insects and plant diseases.

In Ethiopia, production of beans is concentrated mainly in the south and south-eastern regions of the rift valley. It is normally grown from sea level to about 2800 m. Under these conditions the crop is well adapted to altitudes ranging between 700 m and 2000 m and is grown in irrigated and rain-fed conditions. This wide ecological adaptation is associated with several diseases in Ehtiopia, all known to be major factors in reducing bean yields, estimated at 600-700 kg/ha (Ayele, 1990; Habtu *et al.*, 1996).

Bean anthracnose, one of the most widespread and severe diseases of the common bean is caused by the fungus *Colletotrichum lindemuthianum* Sacc. and Magnus Lams. Scrib. (Baxter *et al.,* 1985; Pastor-Corrales and Tu, 1989). In previous surveys anthracnose has been found to be severe at Ambo (08°58'N, 037°26'E) and Arsi-Negelle (07°00'N, 038°35'E), intermediate at Awassa (06°48'N, 037°43'E), Bako (09°06'N, 37°09'E), Didessa (09°14'N, 36°47'E), Jimma (07°28'N, 035°37'E), Metu (07°02'N, 35°63'E), and slight to absent at Melkassa (08°23'N', 039°19'E) and Kobo (12°04'N, 39°37'E) (Habtu *et al.*, 1996; Beshir, 1997).

Anthracnose usually fails to develop in relatively cool and wet regions of the tropics and is endemic in southern and central Brazil, Peru, Ecuador, Colombia, Costa-Rica, Nicaragua, Honduras, Guatemala, Mexico, Central and East Africa (Gepts *et al.*, 1986). The pathogen is seedborne, and planting infected seed usually results in poor germination and infected seedlings. Most aerial parts of the plant, especially pods, seeds, leaf petioles, and lower surfaces of leaflets, including veins, are infected. When susceptible cultivars are grown in the presence of *C. lindemuthianum*, yield losses up to 100% can be expected in regions with cool to moderate temperatures (13°-26° C) and abundant moisture (Chaves, 1980; Koch *et al.*, 1989). Reduced pod and seed quality adds to the economic losses associated with this disease (Guzman *et al.*, 1979; Shao and Teri, 1985).

Many races of *C. lindemuthianum* exist, resulting in differences in cultivar resistance over time and space (Tu, 1988; Campbell and Madden, 1990; Tu, 1992). Resistance mechanisms have been identified in the common bean (Pastor-Corrales *et al.*, 1994; 1995; Prusky *et al.*, 2000), and high levels of genetic resistance, controlled by a single or few major genes, have been reported (Balardin *et al.*, 1997).

At the time of the study by Schwartz *et al.* (1992) information about variation in common bean and its races and gene pools was not readily available. Since then, CIAT's genetic resources unit has developed new common bean accessions and considerable information has been accumulated on the origin, domestication and evolution of common bean (Gepts *et al.*, 1986; Gepts, 1988; Gepts and Debouck, 1991; Singh *et al.*, 1991; Singh, 1992). Similarly, much more is known about the virulence diversity of *C. lindemuthianum* in Latin America, and on its likely co-evolution with the cultivated common bean (CIAT, 1992). The virulence phenotype of more than 600 isolates of *C. lindemuthianum* from 12 Latin American countries has been characterized. Based on virulence, these isolates were grouped into gene pools analogous to those found on the cultivated common bean (Mualet *et al.*, 1981; Singh *et al.*, 1991; CIAT, 1992).

Of the various control strategies attempted, including cultural, chemical, and plant host resistance, the latter was found to be most practical when pursued as the principal tactic in an integrated disease management strategy (Baker and Drury, 1981).

The present study was prompted by the observed susceptibility of cultivars previously resistant to the pathogen in Ethiopia, and a need to obtain meaningful data on anthracnose resistance in local germplasm. Specific objectives were, firstly, to learn how the common bean cultivars vary in their reaction to representative isolates of the Ethiopian *C. lindemuthianum* population, and secondly, to identify possible sources of resistance in those areas of Ethiopia where anthracnose losses are significant.

MATERIALS AND METHODS

Germplasm and sites

A total of 200 accessions (81 breeding lines from Melkassa Agricultural Research Centre, Ethiopia and 119 lines from the CIAT germplasm bank) were used for the study. Mexican 142 (susceptible) and G 2333 (resistant), were included for comparison. Seeds were planted in the experimental plots at Ambo (alt. 2225 m) and Bako (alt. 1665 m) in 2001, both research centres of the Ethiopian Agricultural Research Organization.

Isolates and inoculum preparation

During the 2000/2001 cropping season anthracnose-infected bean stems, leaves, pods, and seeds were collected from a wide range of cultivars grown in the major bean growing zones of the central rift valley (East Shoa and South Shoa), Eastern Ethiopia (Hararghe highlands), Southern Ethiopia (Sidama and Gamo-Gofa) and Western Ethiopia (Kaffa, Wollega, Bako, Ambo, Illubabur and Gojjam). Inoculum of each isolate was obtained from single spore cultures grown and maintained as fungus-colonized filter paper for long-term storage. The pathogenicity and racial identity of each isolate was confirmed by the phenotypic reaction displayed by the anthracnose differential set. The numerical system used to identify races was based on the sum of the binary values

assigned to each of the 12 differential cultivars on which the specific race was pathogenic. Monosporic cultures of each isolate were grown and maintained on potato-dextrose agar in glass petri dishes, incubated at 22°-25° C in darkness (Balardin and Kelly, 1996). Conidia from sporulating colonies were harvested in sterile water and used to inoculate common bean accessions in the field and greenhouse at a concentration of 1.2×10^6 spores/ml.

A mixture of races of *C. lindemuthianum* was used for disease assessment, viz. 65, 73, 128, 296, 511, 589, 961 and 1027. According to reactions on the differential set, races 65, 73, 128 and 961 are virulent to bean varieties belonging to the Middle-American gene pool, whereas races 296, 511, 589 and 1027 have virulence to those of Middle-American and Andean descent only.

Field experiment

Design. The experiments were conducted during the main crop-growing season (June–October) at both sites. The soil type is a black vertisol at Ambo and a clay-loam at Bako. In the year prior to the experiment, the fields used for the experiment were planted with wheat and maize at Ambo and Bako, respectively. The treatments (single row plots of 3 m) were arranged in a randomised complete block design with three replications. Rows were spaced 0.6 m apart. The susceptible variety Mexican 142 was planted as a control after every five rows with-in and surrounding the nursery (Fig. 1).

Inoculation. Following the CIAT (1988) method, bean plants at the trifoliate leaf stage were inoculated using a knapsack sprayer. Inoculation was conducted three times at 10-day intervals.

Anthracnose assessment. Five randomly selected plants were tagged for disease and yield assessment. Nine assessments of disease severity were done at 10-day intervals. Disease severity was monitored using the 1-9 scale (Schoonhoven and Pastor-Corrales, 1987), where 1= 1-10%, 2= 11-20 %, 3= 21-30%, 4= 31-40%, 5= 41-50%, 6= 51-60%, 7= 61-70%, 8= 71-80%, 9= >81%. Incidence was evaluated by rating the proportion of infected plants.

1. No visible disease symptoms.

3. Presence of very few and small lesions, mostly on the primary vein of the lower leaf surface.

5. Presence of several small lesions on the petiole or on the primary and secondary veins of the lower leaf.

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 on the petioles.

9. Severe necrosis on 25% or more of the plant tissues are evident as a result of lesions on the leaf, petioles, stems, branches, and even on the growing point, which often results in death of most plant tissue.

Yield measurements

The yield of the five-tagged plants and 100 seed weight were calculated for each entry. Where necessary, 100 seed weight was determined by extrapolation.

Greenhouse experiment

Design. Five seeds of each cultivar were planted per 15-cm-diameter plastic pot, filled with a 3:1 mixture of soil and compost, and raised in a greenhouse at Ambo. All treatments were replicated three times and pots were arranged in a randomised complete block design.

Inoculation. Different sets of the 200 cultivars and lines were individually inoculated with races 65, 73, 128, 296, 511, 589, 961 and 1027 of the pathogen. For abundant production of spores, each isolate was grown separately at 19°-21°C on previously sterilized young bean leaves placed on potato-dextrose agar in petri dishes. Fourteen-day-old seedlings were sprayed with a conidial suspension of the race mixture. Inoculated seedlings were kept for four days in a dew chamber at 21° to 23° C with 95-100% relative humidity.

Assessment. Seedlings were evaluated three times starting 10 days after inoculation, using the 1-9 scale as described for the field experiment (Schoonhoven and Pastor-Corrales, 1987).

Data analysis

Data from each location were analysed separately. Analysis of variance using the Number Crunching Statistical System (NCSS) (Jerry, 1997) was performed on yield data and area under the disease progress curve (AUDPC) as calculated from severity ratings (Campbell and Madden, 1990). In addition, the relationship between disease and yield, and between sites for both AUDPC and yield, were determined using linear regression analysis.

RESULTS

Differences in response among cultivars to the *C. lindemuthianum* mixture race were observed at Ambo and Bako (Table 1) in terms of AUDPC, yield and seed weight. Cultivars which showed AUDPC lower than the control G 2333, were considered resistant. Overall AUDPC ranged from 57 to 3106 (median 1068, mean 1257) at Ambo, and at Bako from 51 to 3493 (median 1250, mean 1383). The statistical analysis showed that AUDPC, calculated for severity scores, as well as yield varied significantly among cultivars at both locations (Tables 2 and 3). Cultivars showed significance difference for seed weight at Ambo only (Table 3).

At Ambo cultivars within the AUDPC range of 57 to 742 (= score of G 2333), with yield >40 g per five plants and high seed weights, were Calima, AFR 579, AFR 630, RAB 484, G 19070, G 85190, DRK 66, ICA 15541, RAZ 54, G 20858, G 06040, G-7, G 6130, G 2599 and S X WW 2-3. Similarly, at Bako resistant cultivars within an AUDPC range of 51 to 784 (= score of G 2333) were A 643, A 193, G 3608, A 588, Planco Patzicia, TRA-3, G 02618 and AFR 630.

Under greenhouse conditions cultivars GLP X 1132, A 482, A 193, G 2333, HAL 5, A 483 and G 7 showed resistance to all races (Table 4). Races 511 and 128 were the most virulent in terms of number of cultivars attacked.

At Ambo, yield of the five tagged plants ranged from 1.2 to 76.6 g (median 26.9 g, mean 27.3 g). Mexican 142, the susceptible control, yielded 18.4 g and 14.8 g for 100 seed weight. For those entries considered resistant yield varied from 1.2 to 54.6 g (median 31.6 g) and 100 seed weight from 30.9 to 57.8 g (Table 1). At Bako, mean yield of the five plants ranged from 3 g to 39.6 g (median 16.7 g, mean 16.6 g), with those regarded as resistant from 6.0 to 30.7 g (median 17.6 g) and 100 seed weight from 0.6 to 7.9 g (median 3.3 g). Corresponding values for the susceptible control were 10.3 g (five plant yield) and 0.8 g (100 seed weight) (Table 1).

Regression analysis showed that variation in AUDPC did not explain variation in yield (Fig. 2). The R^2 values determined for Ambo and Bako were 0.075 and 0.06, respectively. The 200 entries responded similarly across locations for anthracnose (R^2=0.97) (Fig. 3). However, yield differed significantly between Ambo and Bako (R^2=0.005) (Fig. 4).

Discussion

Differences in response to anthracnose among bean cultivars were clearly detected under field conditions at Ambo and Bako. Of the 200 cultivars assessed 54 were resistant in the field at both locations. Of all the cultivars tested against different *C. lindemuthianum* races in the greenhouse, 42 were resistant to the race 511. According to the response of the differential cultivars (see chapter 4) this race is virulent to nine of the 12 differentials, including the genes *Co-1, Co-2, Co-3* and *Co-4*.

Based on AUDPC, cultivars GLP X 1132, A 482, A 193, G 2333, HAL 5, A 483, G-7 and AB 136 were highly resistant to the isolate mixture in both field experiments and to the individual races as confirmed in the greenhouse

Previously, G 2333 and AB 136 have been reported to be resistant to almost all known European, American and Brazilian races (Balardin *et al.*, 1990; Kelly *et al.*, 1994; Pastor-Corrales *et al.*, 1994; Alzate-Marin *et al.*, 2001). In addition, AB 136 was resistant to Indian races but susceptible to races of 1473 and 1572 of Middle-American origin (Pastor-Corrales *et al.*, 1994). Alzate-Marin *et al.* (2001) reported that AB 136 contains two genes for anthracnose resistance. One of these, *Co-6*, could be followed in segregating progeny using the molecular marker $OPAZ20_{950C}$. The second gene behaved recessively and the designation *Co-8* was proposed (Alzate-Marin *et al.*, 2001). The resistance in G 2333 has also been well characterised and Young *et al.* (1998) reported that this cultivar contains two dominant genes for anthracnose resistance, viz. $Co\text{-}4^2$ and *Co-7*. In the present study, some variation was observed for AB 136, which showed an intermediate response to races 511, 73 and 1027. However, none of the Ethiopian races attacked cultivar G 2333.

With respect to yield at Ambo, resistant cultivars such as Calima, AFR 579, AFR 630, RAB 484, G 19070, G 85190, DRK 66, ICA 15541, RAZ 54, G 20858, G 06040, G-7, G 6130, G 2599 and S X WW 2-3 appeared superior to others. At Bako, A 643, A 193, G 3608, A 588, Planco PATZICIA, TRA-3, G 02618 and AFR 630 combined anthracnose resistance and higher yields.

From a disease assessment point of view, cultivars responded similarly at both sites. As these localities are known for anthracnose occurrence, specifically in the more humid Bako region, entries should perform even better in environments with a lower disease risk. Consistency in rating was also evident, which is essential if multilocation trials are conducted to identify highly resistant cultivars. The advantage of screening resistant cultivars at more than one site increases the opportunity to select for a broad range of anthracnose resistance. If the field results are then confirmed by multi-race testing in the greenhouse, the breeder could be confident of working with useful resistance.

Considering the potential of the two sites, genotypes yielded significantly better at Ambo than at Bako. It is likely that other diseases such as angular leaf spot, rust

and floury leaf spot, which occurred to a certain extent at Bako, may have influenced plant productivity. The lack of a significant relationship between disease and yield most probably resulted from the small sample size, and the fact that many genotypes were not agronomically adapted to the test environments. It will thus be meaningful to conduct more detailed yield experiments with the cultivars identified as resistant and potentially high-yielding in this study. Such studies should also include quality assessments.

To summarize, this study confirmed that current Ethiopian varieties under production (Bayota, Ayenew, Gofta) showed either a resistant or intermediate response to all races. Furthermore, the resistant cultivars identified should support the Ethiopian national bean improvement gene pool for developing new cultivars especially for areas where anthracnose is economically important.

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Table 1. Reaction of 200 common bean cultivars evaluated for anthracnose resistance under field conditions at Ambo and Bako, Ethiopia in 2001

				Ambo			Bako		
Cultivar	Growth habit[a, b]	Origin[c]	Colour	AUDPC	Five plant yield (g)	100 seed weight (g)	AUDPC	Five plant yield (g)	100 seed weight (g)
A 193	II	MA	red	737	22.1	48.5	308	32.1	5.0
A 197	II	A	cream (yellow)	523	32.1	43.9	480	40.8	4.0
A 320	II (III)	MA	black (yellow)	1137	27.8	21.3	675	25.9	4.1
A 445	III	MA	cream (yellow)	1960	30.3	16.9	2498	13.7	3.3
A 475	II (I)	A (MA)	red (purple mottled)	1191	18.4	30.2	1412	26.2	2.7
A 482	II	MA	cream (pink)	367	33.8	34.9	862	17.2	2.8
A 483	II (III)	MA	red purple mottled	398	23.7	27.8	895	22.6	3.4
A 493	II	A (MA)	red (white)	1538	9.3	18.9	637	27.6	3.8
A 585	II (II B)	A (MA	white (cream)	1314	35.2	27.0	2675	26.5	3.6
A 588	III	A (MA)	cream	888	20.9	18.2	541	34.2	5.1
A 613	III (IIB)	MA	white (cream)	1007	26.5	24.2	1250	22.7	6.7
A 643	II (II B)	MA	cream	878	31.5	20.4	162	36.0	6.1
AAPEEP 12553	II	MA	cream	952	11.8	17.6	2182	17.1	2.3
AB 136	IV	MA	red	717	21.4	14.5	872	26.9	2.5
ACV 17	III (IVB)	MA (A)	white (red)	700	23.3	31.0	1146	16.0	3.6
ACV 46	III (III B)	MA	red	821	36.1	19.5	2028	17.6	2.5
AFR 550	II (I)	MA (A)	Cream	1011	34.7	48.2	939	37.2	3.2

[a] growth habit classification: I=determinate, II=indeterminate, bush habit, erect stem, III=indeterminate bush habit with weak main stem and prostrate branches and IV= indeterminate strong climbing habit.

[b] items in brackets indicate character description listed by Hidalgo *et al.* (1992) and Rodriguez *et al.* (1994). Variation from the lists could be attributed by lack of homogeneity of the studied entries for growth habit and seed colour.

[c] Origin: MA=Middle America, A=Andean.

... Continued Table 1.

				Ambo			Bako		
Cultivar	Growth habit[a, b]	Origin	Colour	AUDPC	Five plant yield (g)	100 seed weight (g)	AUDPC	Five plant yield (g)	100 seed weight (g)
AFR 579	II (I)	MA (A)	red	128	42.5	41.9	2236	15.1	2.8
AFR 585	II (I)	A	cream	402	31.1	45.6	376	42.8	4.2
AFR 630	II (IIB)	A	red	194	52.0	39.7	823	32.9	5.4
AFR 684	II	MA	cream	1340	34.5	26.9	1306	23.3	2.8
AFR 907	I	MA	red	1513	37.1	42.5	1123	16.5	1.4
ANCASH 66	II	MA	cream	582	20.7	32.7	1497	25.8	4.0
ARA 21	II	MA (A)	white (brown)	1568	23.1	15.9	1140	20.6	4.3
Atndaba	II	MA	cream	1392	23.8	24.4	2052	24.3	3.7
Awash 1	III	MA	white	2261	19.8	12.3	2291	20.3	0.9
Awash melka	II	MA	white	2845	15.5	16.1	2492	21.3	3.2
Ayenew	II	A	white	782	42.9	37.7	249	37.1	4.8
BAT 496 ANT	III	MA	cream	1015	26.0	20.4	1523	24.5	3.1
BAT 841	II (III A)	MA	cream	867	30.8	20.7	1462	18.6	2.7
Bayoto	IV	MA	white	477	13.3	15.7	1526	20.9	3.8
Beshbesh	III	MA	cream	2698	11.5	15.9	2244	17.5	1.7
Bico de Duro 1445	II	MA	cream	750	8.7	9.8	1140	16.1	2.5
Black dessie	II	MA	black	2242	42.1	15.8	1399	25.4	4.2
BLANCO	IV	MA	red (white)	893	37.5	26.2	1063	21.7	2.6
Brown Speckled	II	A	cream	778	29.7	39.9	1180	38.9	4.0
Cahpainten 12-3	I	A	cream	484	15.1	18.6	325	40.6	3.6
CAL 27	II (I)	A	red (purple)	522	31.6	52.6	831	41.7	4.1
Calima	II (I)	A	red	57	45.0	47.2	440	34.9	3.0

... continued Table 1.

Cultivar	Growth habit[a, b]	Origin	Colour	Ambo			Bako		
				AUDPC	Five plant yield (g)	100 seed weight (g)	AUDPC	Five plant yield (g)	100 seed weight (g)
CEL 60970	III	MA	cream	1375	28.6	16.1	2596	16.1	2.8
Chile	III	A	cream	580	10.8	18.1	1141	22.3	4.2
CIFAAC 87110	II	MA	red	995	28.0	38.5	448	29.4	2.5
Coco ala crème	I	A	white	769	20.3	25.1	1343	28.5	2.7
CTC 1	II (III)	MA	cream (gray)	1450	70.7	29.4	1868	23.6	3.5
DICTA 105	II	MA	red	1288	39.8	19.4	943	30.9	3.5
DOR 517	II	MA	red	2189	26.9	16.9	333	33.1	3.5
DOR 523	II	MA	red	2084	26.7	16.3	1946	22.4	3.3
DOR 527	II	MA	red	2493	17.0	16.8	2506	20.0	3.0
DOR 567	II	MA	white	2137	28.5	16.8	2087	17.1	5.3
DOR 711	II	MA	cream	2030	22.0	19.5	1522	21.6	3.3
DOR 764	II	MA	white	2099	21.6	34.7	1669	19.6	4.4
DOR 811	III	MA	red	1826	22.2	17.9	2033	17.0	3.1
DRK 51	I	MA (A)	red	637	35.5	61.0	1759	40.2	4.4
DRK 66	II	A	red	393	41.1	57.5	561	39.2	3.5
E 530	II	MA	cream	3097	18.0	18.2	1240	16.4	4.4
EAP 4	II	MA	red	2628	29.2	18.2	3011	16.0	2.1
Ecuador 1056	IV	MA	cream	1061	19.3	31.0	1143	21.5	1.9
Ecuador 299	III	A (MA)	ed	590	17.4	28.6	1211	14.6	1.8
EMP 212	II (II A)	MA	white	1427	26.0	15.4	1677	20.3	3.9
EMP 220	II (II A)	MA	red (cream)	1847	32.7	19.4	1446	26.3	4.2
EMP 233	II (II A)	MA	red (white)	2062	35.8	17.1	912	39.7	3.3
EMP 263	II (I)	MA (A)	cream	1846	23.4	17.6	1388	19.4	3.9
EMP 311	III (II A)	MA	white	2391	30.0	17.1	2399	17.8	4.2

... continued Table 1.

Cultivar	Growth habit[a, b]	Origin	Colour	Ambo			Bako		
				AUDPC	Five plant yield (g)	100 seed weight (g)	AUDPC	Five plant yield (g)	100 seed weight (g)
EMP 312	I (II B)	MA	white	2893	8.4	16.8	3057	18.0	4.1
EMP 385	II	MA	cream	870	19.8	15.5	3461	11.9	0.9
EMP 386	II	MA	red	3097	7.6	17.9	2713	14.9	2.0
ENP 236	II	MA	cream	1907	39.6	21.4	1677	20.7	3.3
ENT 3	II	MA	cream	1882	25.6	10.8	1711	22.5	3.0
FAO- 54-500	II	MA	cream	2096	33.2	21.7	2338	15.2	3.5
FEB 147	II (II A)	MA	cream	2578	15.1	15.4	3199	14.8	1.8
FEB 183	III	MA	cream	2048	36.0	15.4	1580	16.4	3.4
Flor de brl	III	MA	pink	866	27.2	18.9	663	21.2	2.9
G 02333	III	MA	red	649	31.0	18.9	698	11.5	1.2
G 02338	IV	MA	cream	604	33.2	24.4	1333	24.5	3.5
G 02618	II	A	yellow	859	42.6	30.5	752	38.0	5.8
G 02618	I	MA	red	1129	30.1	23.2	2201	35.8	4.0
G 02641	IV	MA	cream	2191	16.0	14.2	657	23.0	4.8
G 03367	IV	MA	cream	750	24.9	18.9	681	15.2	1.2
G 03991	IV	MA	cream	750	22.8	16.9	1388	15.6	0.6
G 04032	IV	MA	cream	1143	12.9	18.1	1479	25.7	3.0
G 05653	III	A (MA)	red	790	31.7	26.8	1059	25.0	2.6
G 05971	III	MA	black	911	16.4	18.6	592	21.9	3.9
G 06040	III	MA	black	459	40.8	18.3	342	19.7	2.8
G 06436	III	MA	black	1323	28.3	20.9	1531	17.3	4.9
G 07199	II	A	red	525	25.5	33.1	592	27.0	4.5
G 07303	III	MA	cream	1011	26.8	20.8	1620	19.6	3.5
G 0984 ANT	III	MA	black	1101	49.3	26.7	3183	11.7	1.8
G 11060	IV	MA	cream (brown)	2893	8.4	16.8	1386	13.2	1.9

... continued Table 1.

Cultivar	Growth habit[a, b]	Origin	Colour	Ambo			Bako		
				AUDPC	Five plant yield (g)	100 seed weight (g)	AUDPC	Five plant yield (g)	100 seed weight (g)
G 11635	III	MA	red	788	36.0	18.0	1715	16.7	2.8
G 11680	II (III)	MA	red	1292	14.4	20.7	560	21.4	3.1
G 13071	III	MA	red	2203	25.5	17.4	92	19.3	2.8
G 13609	II (IV)	MA	yellow	2905	31.6	44.0	3177	24.9	3.4
G 13613	II (IV)	MA	red	1373	33.1	22.9	981	26.3	3.7
G 13627	III (IV)	MA	red	898	25.5	27.5	356	31.6	3.3
G 13630	III	MA	pink (red)	1150	31.6	20.2	1134	21.4	2.7
G 14936	II (IV)	MA	cream	2250	36.1	18.2	1034	22.6	5.0
G 17426	II	MA	white	1341	11.6	13.4	2171	11.9	2.2
G 18094	IV	A	cream	701	31.8	22.4	360	29.7	3.9
G 18117	III (IV)	MA	cream	520	21.8	16.7	408	16.4	2.2
G 18211	II (III)	A	cream	1447	45.4	19.8	1131	25.5	4.3
G 18264	II (I)	A	red (purple)	729	27.0	33.7	557	24.1	2.2
G 18549	III (II)	MA	cream	1022	29.4	17.8	1381	23.9	1.7
G 18566	I (III)	MA	pink	1570	33.0	17.0	2079	18.6	3.8
G 18689	II (III)	MA	cream	1237	27.5	18.0	647	26.7	2.9
G 19012	II (III)	MA	white (cream)	2802	20.6	28.5	1709	16.7	2.6
G 19070	II (IV)	MA	cream	269	40.9	20.5	2662	32.0	6.3
G 19096-B	II (IV)	A	cream	786	35.1	25.6	1509	21.0	3.1
G 19153-A	III	MA	black	932	76.6	18.9	1765	23.0	1.9
G 19833	II (III)	A	yellow	661	25.6	26.9	388	20.1	3.9
G 20858	III (IV)	MA	red (cream)	448	43.1	29.1	801	20.8	1.6
G 21703	II (III)	MA	pink	1638	23.2	16.2	152	28.1	2.1
G 22024	II	MA	cream	1037	25.7	26.8	1640	18.3	2.6
G 22779	III (I)	MA	cream (white)	678	21.8	25.1	746	27.8	3.7

... continued Table 1.

Cultivar	Growth habit[a, b]	Origin	Colour	Ambo			Bako		
				AUDPC	Five plant yield (g)	100 seed weight (g)	AUDPC	Five plant yield (g)	100 seed weight (g)
G 2333	IV (IV)	A (MA)	red	742	31.8	18.6	903	34.0	5.5
G 2599	III	MA	black	656	51.8	20.1	285	22.1	2.5
G 2887	III	MA	cream	712	26.7	21.0	1231	22.2	3.8
G 3369	I (IV)	MA	pink (yellow)	1334	29.8	16.7	2540	16.8	3.1
G 3488	III	MA	cream	622	20.9	18.4	1545	19.3	2.7
G 3608	III	MA	black (white)	873	42.5	20.3	399	22.6	5.6
G 5150	III	MA	cream	530	29.6	17.9	1349	16.2	2.8
G 564	III	MA	red (brown)	1869	25.5	17.7	1690	20.8	2.0
G 6	II (III)	MA	red	2321	17.1	20.4	444	17.8	2.9
G 6130	III	MA	black	645	42.3	22.7	277	42.5	3.0
G 7	II (IV)	A	red (white)	389	41.3	17.8	98	19.4	3.1
G 85190	II	MA	black	1076	37.2	19.8	988	22.3	5.7
G 877	III	MA	pink	662	27.8	18.2	2230	17.7	4.6
G 9636	II	MA	cream (black)	1190	27.6	16.8	2342	15.4	4.0
G 9807	III	MA	cream	537	22.3	18.3	2331	15.1	1.6
Gentry 21161 Azufrado	III	MA	yellow	500	45.4	57.8	514	16.7	4.1
Gentry N. 12142	IV	A	red	1116	27.0	17.3	1204	20.9	1.4
GLPX 1132	II	MA	cream	536	35.3	15.5	784	20.2	4.2
Gofta	II	MA	cream	584	12.8	31.6	986	24.0	3.7
H2 Mulat Hind	II	MA	cream	1181	38.3	27.4	988	27.6	4.3
HAL 5	II	A (MA)	white	1355	29.4	16.9	786	28.9	2.3
IAPAR MD 160	II	A	white	322	1.2	30.9	1261	18.7	2.0
IAPAR RA I 54	II	MA	cream	990	25.2	24.8	390	23.0	2.5
ICA 15541	II	A	red	1228	18.9	18.8	857	36.8	4.9
II CORAMA	III	MA	red	422	46.6	43.3	1993	20.5	4.3

... continued Table 1.

Cultivar	Growth habit[a, b]	Origin	Colour	Ambo			Bako		
				AUDPC	Five plant yield (g)	100 seed weight (g)	AUDPC	Five plant yield (g)	100 seed weight (g)
Imuna	I	A	white	868	23.9	21.7	638	27.3	3.5
K 2	II	A	red	1272	15.8	21.1	1109	31.4	4.4
Kaboon	I	A	white	1383	8.2	25.1	989	46.8	2.1
Kisozi 4508	II	MA	black	841	24.7	17.8	154	21.3	4.1
MAM 48	II	MA	cream	3107	7.8	14.5	3022	19.7	2.5
Melke	II	MA	red	393	36.2	42.2	1247	31.7	3.9
Mexican 142	II	MA	white	2586	18.4	14.8	3493	10.3	0.8
Mexican 222	I	A	white	1759	15.6	15.6	1523	25.2	3.0
MEXICO 80	II	MA	red	1479	28.6	16.7	1891	23.5	2.3
Michelite	IV (II)	A (MA)	white	1484	14.2	15.6	1031	24.8	5.7
MX 2500-19	II	MA	cream	2546	19.6	22.7	2504	18.9	1.9
Nicaragua 35	III	MA	red	1630	35.6	24.6	2704	16.6	2.7
NZBR	II	MA	white	784	40.1	16.5	1896	23.5	4.3
NZBR 2-11	II	MA	white	1173	33.9	13.6	1209	17.6	3.0
NZBR 2-5	II	MA	cream	1351	34.2	13.4	1045	21.3	4.1
NZBR 2-8	II	MA	red	853	30.8	14.4	2483	19.1	4.1
Oaxaca 104	II	MA	black	982	19.6	18.8	1315	18.2	7.9
OTZKAL									
TSATK	II	MA	cream	1116	16.9	13.6	965	21.6	3.8
PAN 164	II (II A)	MA	white	2718	11.6	14.5	2639	21.6	1.9
PAN 182	II (II B)	MA	white	2241	41.7	13.4	2203	14.8	3.2
PEF 7	II (III)	A	cream	649	35.0	42.2	323	36.3	3.6
Perry Marrow	III	MA	white	1110	12.3	39.4	1864	32.7	2.5
PI 207 262	III	MA	cream	1083	18.5	16.3	641	18.5	4.0
Planco patzicia	III	MA	white	1116	18.9	20.6	723	26.0	5.0
Plor Bancadchi	I	MA	cream	1189	25.1	21.9	1673	14.8	2.2

... continued Table 1.

Cultivar	Growth habit[a, b]	Origin	Colour	Ambo			Bako		
				AUDPC	Five plant yield (g)	100 seed weight (g)	AUDPC	Five plant yield (g)	100 seed weight (g)
Princor	I	MA	white	599	9.0	8.3	444	23.0	3.4
PUM XI 659	II	MA	cream	894	40.2	23.5	2128	21.1	3.4
PVA 1076	I	A	pink	300	38.5	45.1	1107	37.7	4.1
PVAD 1003	II	MA	pink	1183	25.7	23.3	616	24.1	4.9
PVAD 1184	II	A	red	546	29.1	33.3	51	37.9	3.3
PVAD 791	II	A	red	987	15.6	29.7	210	32.3	2.9
RAB 401	II (II B)	MA	red	1650	20.3	18.3	2251	22.6	4.2
RAB 404	II (II B)	MA	red	1596	30.7	17.8	2392	18.0	4.2
RAB 484	II (IIB)	MA	red	195	54.6	50.0	1675	20.6	2.6
RAP 94	II	MA	red	924	22.7	18.7	2113	22.2	2.5
RAZ 19	II (III)	MA	white	2615	13.7	17.1	2878	12.5	0.7
RAZ 2	II (II/III)	MA	white	927	15.6	17.8	1883	15.5	1.7
RAZ 54	II	MA	red	439	42.0	18.0	1115	18.0	3.0
Red wolaita	III	MA	red	2224	27.2	16.5	2238	18.5	1.5
ROAC/Hungo de yoro	III	MA	red	1104	17.8	16.9	375	12.3	2.1
STTT 165-72	II	MA	white	2397	35.7	13.9	1549	18.2	4.1
SUG 9	II	A	cream	941	16.1	18.8	1933	21.1	4.2
SXWW 13	II	MA	cream	706	44.9	19.1	1237	19.9	2.8
SXWW 2-3	II	MA	cream	1007	34.7	21.7	1953	21.9	2.9
TAR-3	II (III)	MA	cream	655	27.8	21.8	418	25.4	4.2
TO	III	MA	cream	955	38.2	26.3	738	27.0	5.1
TRA 03	II	MA	cream	912	36.6	24.3	1299	24.8	3.9
Tu	III	MA	black	1429	9.9	15.3	2094	21.2	2.7
Ty 3396-1	II	MA	cream	1820	47.5	25.5	2090	14.1	3.4
TY 3396-7	III	MA	cream	2765	14.4	18.4	2699	18.6	2.8

... continued Table 1.

Cultivar	Growth habit[a, b]	Origin	Colour	Ambo			Bako		
				AUDPC	Five plant yield (g)	100 seed weight (g)	AUDPC	Five plant yield (g)	100 seed weight (g)
UTT 24-131	II	MA	white	2957	10.5	14.0	3089	11.8	3.2
UTT 27 -24	II	MA	red	3060	9.0	13.8	2062	19.1	3.3
V 83028	IV	MA	cream	1280	32.1	21.4	1780	16.1	2.7
VCA 81007	III (IV A)	MA	white	2137	32.8	23.9	1893	20.8	4.1
VRA 81035	IV (IVA)	MA	cream (yellow)	962	23.9	19.6	1059	20.6	3.7
VRB 81069	II (III B)	MA	red	1313	26.7	19.8	292	31.0	2.6
Widusa	I	A	white	762	14.9	17.0	185	26.0	3.6
XAN 307	II	MA	white	2964	9.7	16.2	2346	17.8	3.9
XAN 317	II	MA	red	1164	44.6	24.6	1241	15.6	3.8
ZAA 5	II (I)	A	red (purple)	732	37.0	33.0	296	26.3	3.5
ZEBRA	III	MA	cream	1910	47.7	25.0	1566	24.9	2.9
LSD at 5%				531.35	19	7.3	1180.5	11	2.5

Table 2. Analysis of variance for area under disease progress curve (AUDPC) in bean cultivars screened for anthracnose resistance at two locations (Ambo and Bako) in Ethiopia in 2001

		AUDPC					
		Ambo			Bako		
Source	df [a]	Mean square	F	P[b]	Mean square	F	P[b]
Cultivar	199	1587934.4	10.2	0.0001*	1933209.5	2.5	0.0001*
Replication	2	12185925.8	78.6	0.0001*	39209.8	0.05	0.95
Error	398	155092.2					

[a] Degrees of freedom.
[b] Significantly different at 5% level of probability.

Table 3. Analysis of variance for yield and 100 seed weight of bean cultivars screened for anthracnose resistance at two locations (Ambo and Bako) in Ethiopia in 2001

Character	Sources	df [a]	Ambo			Bako		
			Mean square	F	P[b]	Mean square	F	P[b]
Yield (g/5plants)	Cultivar	199	397.4	2.01	0.0001*	159.5	2.4	0.0001*
	Replication	2	410.6	2.08	0.1268	33.9	0.51	0.5998
	Error	398						
Seed weight (g)	Cultivar	199	281.1	9.5	0.0001*	4.0	1.18	0.0798
	Replication	2	18.9	0.64	0.5280	19.7	5.79	0.0033*
	Error	398						

[a] Degrees of freedom.
[b] Significantly different at 5% level of probability.

Table 4. Reaction of a selection of common bean cultivars tested for anthracnose resistance under greenhouse conditions at Ambo, Ethiopia in 2001

Resistant cultivars[a]	Origin[b]	Reaction of cultivars to *C. lindemuthianum* races under greenhouse conditions									
		Colour	Mix[c]	65	511	73	128	296	1027	589	961
GLP x 1132	A	cream	R	R	R	R	R	R	R	R	R
A 482	MA	Cream	R	R	R	R	R	R	R	R	R
A 193	MA	Red	R	R	R	R	R	R	R	R	R
G2333 [d]	A	Red	R	R	R	R	R	R	R	R	R
HAL 5	A	White	R	R	R	R	R	R	R	R	R
A 483	MA	Red	R	R	R	R	R	R	R	R	R
G-7	A	Red	R	R	R	R	R	R	R	R	R
Bayoto	MA	White	R	R	R	R	R	R	R	R	R
G 07199	A	Red mottled	R	R	R	R	R	S	R	R	R
Ecuador 299	A	Black	S	R	R	R	R	R	R	R	R
DRK 66	A	Red	R	R	R	R	R	R	R	R	S
H2 Mulat Hind	MA	Cream	S	R	R	R	R	S	R	R	R
Widusa	A	White	R	R	R	R	R	R	R	R	R
Calima	A	Red	R	R	R	R	R	S	R	R	R
Ayenew	A	White	R	R	R	R	R	R	R	S	S
AB 136	MA	Red	R	R	S	R	R	R	R	R	R
ACV 17	MA	White	S	R	R	R	R	R	S	R	S
G 06040	MA	Black	R	R	R	R	S	S	R	R	R
G 22779	MA	Cream	R	R	R	R	R	R	R	S	S
G 18117	MA	Cream	R	R	R	R	S	S	R	S	R
G 20858	MA	Red	R	R	R	R	R	R	S	R	S
G 6130	MA	Black	R	R	R	R	R	S	R	R	S
G 2599	MA	Black	R	R	R	R	R	R	R	R	S
G 18094	A	Cream	R	R	R	R	R	S	R	S	S
Bico de Duro 1445	MA	Cream	S	R	R	R	R	S	R	R	R
CAL 27	A	Red	R	S	R	R	R	S	R	R	S
Cahpainten 12-3	A	Cream	R	R	R	R	S	R	R	R	S
Brown speckled	A	Cream	S	S	R	R	S	R	R	R	R
RAZ 54	MA	Red	R	S	R	R	R	S	R	S	S
AFR 585	A	Cream	R	R	R	R	S	S	R	S	R
PVAD 1184	A	Red mottled	R	R	R	R	S	S	R	R	S
G 19833	A	Yellow	R	S	R	R	R	S	R	S	S
REF 7	A	Cream	R	S	R	R	R	S	R	R	S
PVA 1076	A	Red	S	R	R	R	R	S	R	R	S
AFR 630	A	Red	R	R	R	R	R	R	R	S	S
ZAA 5	A	Red mottled	R	R	R	R	S	S	R	R	S
To	MA	Cream	R	R	R	R	S	R	R	R	S
Gofta	MA	Cream	S	R	S	S	S	R	R	R	R
G 03367	MA	Cream	R	R	R	R	S	S	R	R	R
Mexican 142[e]	MA	white	S	S	S	S	S	S	S	S	S

[a] Some of the 200 cultivars tested under greenhouse conditions at Ambo.
[b] MA : Middle-American (small seeded) , A : Andean (large seeded).
[c] Mix : mixture of isolates of *C. lindemuthianum.*
[d] = Resistant control;
[e] = Susceptible control; R = resistant; S = susceptible.

Fig 1. General view of the field experiment showing defoliation due to anthracnose on the spreader rows.

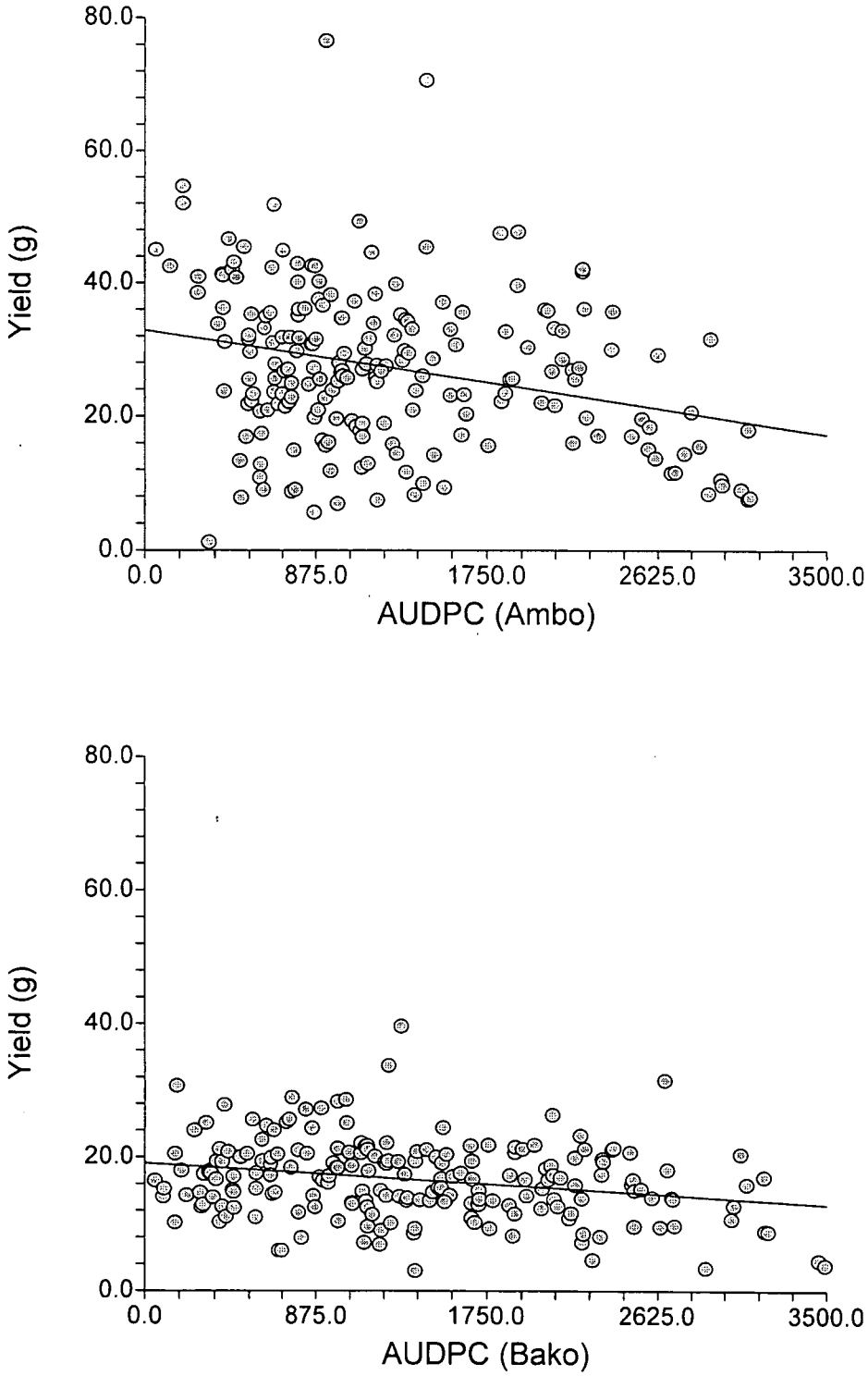

Fig. 2. The relationship between anthracnose as quantified by area under the disease progress curve (AUDPC) and the mean yield of five tagged plants for each of 200 common bean entries at Ambo (top) and Bako (bottom) in 2001. The R^2 values for the linear relationship between disease and yield were 0.075 and 0.060 at Ambo and Bako, respectively.

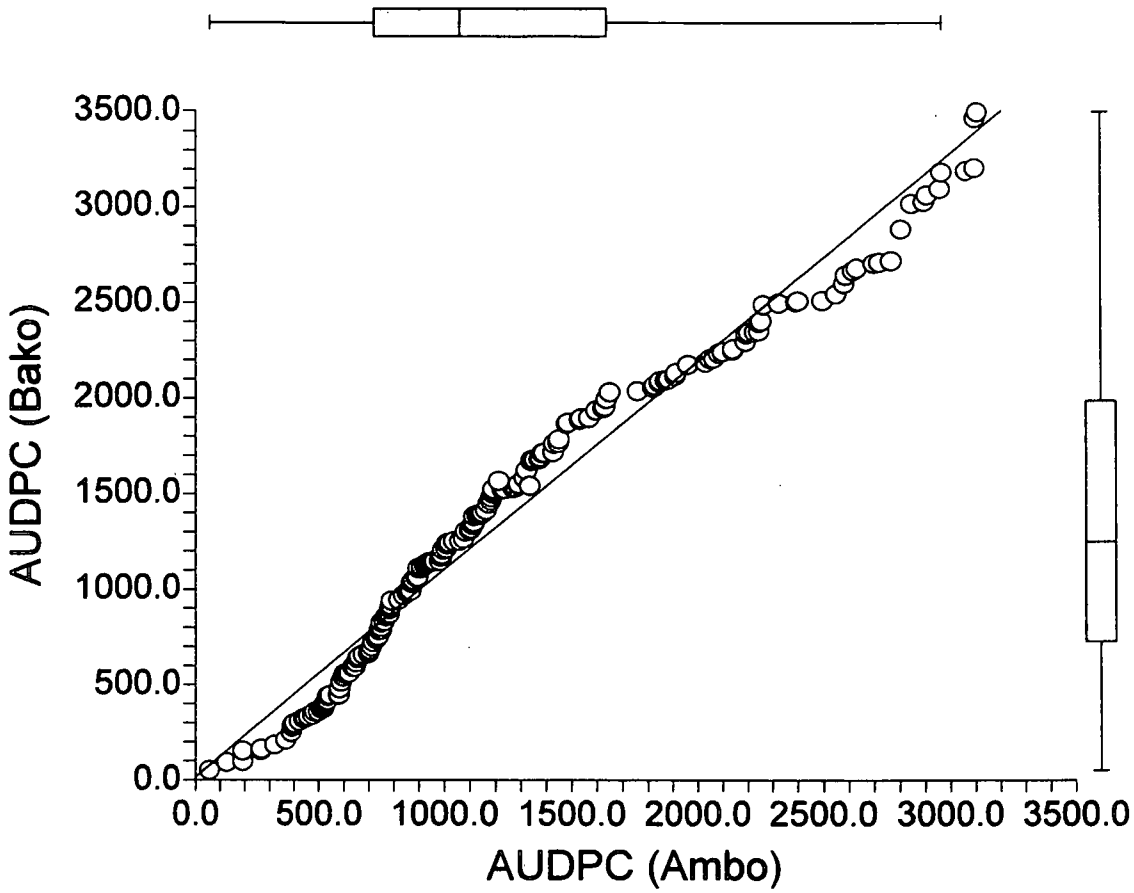

Fig. 3. Relationship between area under the disease progress curves determined at Ambo and Bako sites for anthracnose development on 200 common bean entries (R^2= 0.97). The length of the box plots (top and right) indicate the interquartile range (middle 50% of the data) for Ambo and Bako respectively, and the centre line of each box the median.

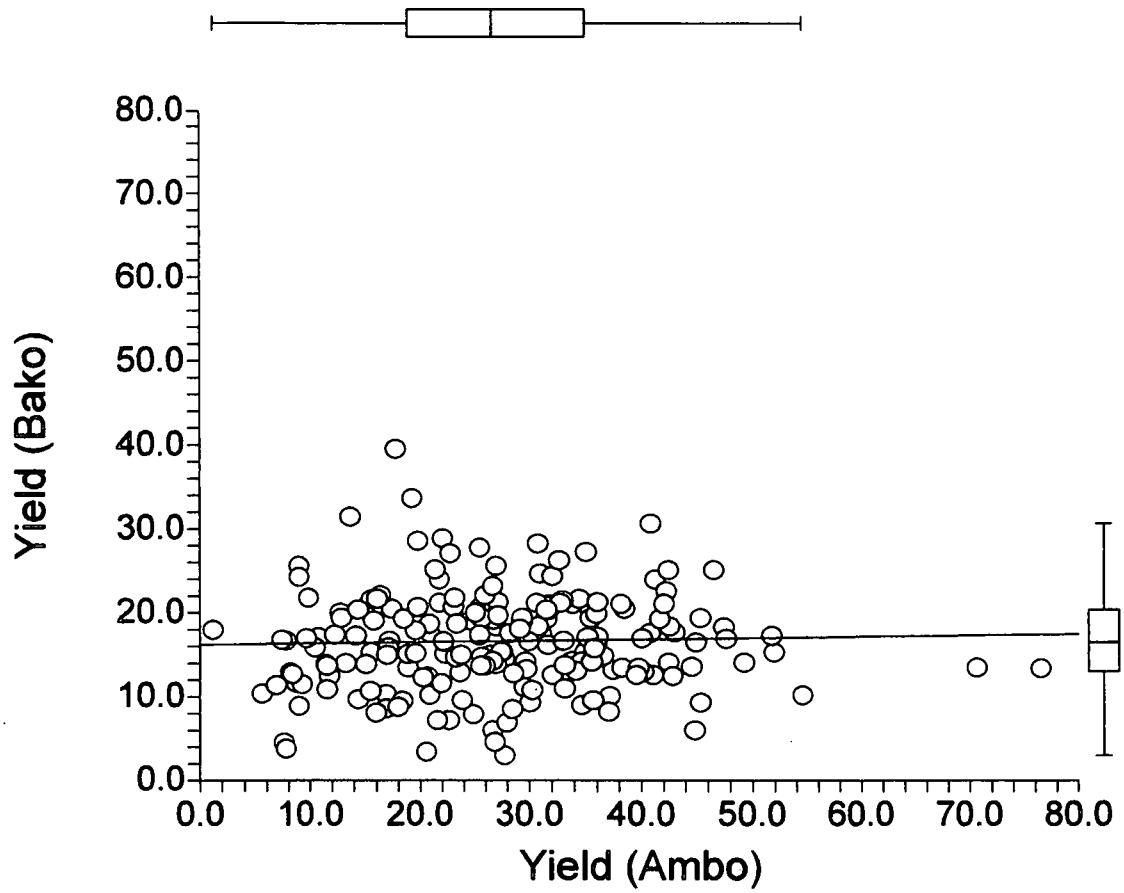

Fig. 4. Relationship between grain yield (g) of five plants for each of 200 common bean entries as determined at Ambo and Bako sites in anthracnose resistance assessment trials (R^2= 0.001). The length of the box plots (top and right) indicate the interquartile range (middle 50% of the data) for Ambo and Bako respectively, and the centre line of each box the median.

Chapter 8

Summary

Common bean (*Phaseolus vulgaris* L.) is an important food legume for millions of Ethiopian people. However, diseases are major constraints in bean production with anthracnose, caused by *Colletotrichum lindemuthianum* (Sacc. & Magn.) Briosi and Cav. (teleomorph = *Glomerella cingulata* (Stonem.), being of particular concern. Anthracnose is the most important disease of bean in Ethiopia and can cause complete yield losses on susceptible cultivars when contaminated seed are planted. The impact of epidemics is further enhanced when favourable environmental conditions prevail. The main objective of this study was to provide information that can be used as part of an integrated control program for anthracnose in Ethiopia.

Field surveys were conducted in the foremost bean growing areas of Ethiopia to acquire information on the geographical distribution of anthracnose. Similar to bean rust, anthracnose is favoured by high moisture and relative humidity. In surveys of the different agro-ecological zones (AEZ) anthracnose was found severe at Ambo (08°58'N, 037°26'E), Meki (08°05'N, 038°51'E), Arsi-Negele (07°00'N, 038°35'E), Areka () and Bako (09°06'N, 37°09'E), intermediate at Awassa (06°52'N, 038°27'E), Jimma (07°28'N, 035°37'E), and Metu (07°02'N, 35°63'E), and slight to absent at Melkassa (08°23'N', 039°19'E). Other diseases observed included bacterial blight, angular and floury leaf spot and rust.

The success rate in breeding for resistance depends to a large extent on the degree of variability in a pathogen. Using the standard differential cultivar set and binomial system proposed by CIAT (Centro Internacional de Agricultutra Tropical), eight races of *C. lindemuthianum,* viz. 65, 73 128, 296, 511, 589, 961, and 1027, were identified from Ethiopia and compared to races 3, 6, 81, 323, 390 and 593 from Southern Africa. Races 128 and 511 constituted more than 50% of all isolates characterised. Considering the differential cultivars, G 2333 was resistant to all races in Ethiopia, and AB 136, G 2333, Kaboon, Cornell 49-242 to all races in Southern Africa.

Field studies were conducted at Ambo and Bako (Ethiopia) to evaluate resistant and susceptible cultivar mixtures as a means of anthracnose control. Results showed that disease incidence and severity were consistently lower in mixtures containing 50%, 67% and 80% of the resistant component at both locations. Disease progress curves and disease gradients indicated that disease development and spread were slower or reduced in all treatments that contained an increased proportion of the resistant cultivar. It was thus found that anthracnose could be controlled by growing cultivar mixtures containing at least 60% of a resistant cultivar. In an assessment of the efficacy of chemical control of anthracnose, a benomyl seed dressing followed by a foliar difenoconazole spray, or difenoconazole application alone, showed the most promising results.

Finally, greenhouse and multilocation field experiments were carried out to identify bean genotypes as possible sources of resistance to Ethiopian isolates of *C. lindemuthianum*. The study established that cultivars Widusa, GLP X 1132, A 482, A 193, G-7, HAL 5 and G 2333 were valuable sources of anthracnose resistance.

Opsomming

Droëbone (*Phaseolus vulgaris* L.) is 'n belangrike voedselgewas vir miljoene Ethiopiërs. Siektes speel egter 'n belangrike rol in droëboonproduksie en veral antraknose, veroorsaak deur *Colletotrichum lindemuthianum* (Sacc. & Magn.) Briosi and Cav. (teleomorf = *Glomerella cingulata* (Stonem.), verdien spesiale vermelding. Antraknose word beskou as die belangrikste siekte van droëbone in Ethiopië en kan totale oesverliese in vatbare kultivars tot gevolg hê wanneer besmette saad geplant word. Die impak van epidemies word verder vergroot indien gunstige omgewingstoestande voorkom. Die doelwit van hierdie studie was om inligting in te samel wat kan bydra tot 'n antraknose-beheerprogram in Ethiopië.

Veldopnames is gedoen in die belangrikste produksie-areas in Ethiopië ten einde inligting te bekom oor die geografiese verspreiding van antraknose. Soortgelyk aan roes word antraknose bevoordeel deur hoë vog en relatiewe humiditeit. In opnames van die verskillende agro-ekologiese gebiede is gevind dat antraknose in ernstige graad voorkom by Ambo (08°58'N, 037°26'E), Meki (08°05'N, 038°51'E), Arsi-Negele (07°00'N, 038°35'E), Areka (06°48'N, 037°43'E) en Bako (09°06'N, 37°09'E), terwyl intermediêre vlakke by Awassa (06°52'N, 038°27'E), Jimma (07°28'N, 035°37'E), en Metu (07°02'N, 35°63'E), en slegs enkele tekens van die siekte by Melkassa (08°23'N', 039°19'E) aangetref is. Ander siektes waargeneem het bakteriese skroei, hoekige blaarvlek, *Ramularia*-vlek en roes ingesluit.

Sukses in weerstandsteling hang tot 'n groot mate af van die hoeveelheid variasie in 'n patogeen. Met behulp van die staandaardstel differensiërende lyne en binomiale nomenklatuursisteem van CIAT (Centro Internacional de Agricultutra Tropical), is agt rasse van *C. lindemuthianum,* nl. 65, 73 128, 296, 511, 589, 961, en 1027, in Ethiopië geïdentifiseer in vergelyking met rasse 3, 6, 81, 323, 390 en 593 in Suidelike Afrika. Rasse 128 en 511 het meer as 50% van alle isolate beslaan. Met in ag neming van die differensieerders was G 2333

bestand teen alle Ethiopiese isolate en AB 136, G 2333, Kaboon en Cornell 49-242 teen alle isolate uit Suidelike Afrika.

Veldproewe is by Ambo en Bako (Ethiopië) uitgevoer om te bepaal tot watter mate kultivarmengsels antraknose kan beheer. Siektevoorkoms en ergheidsgraad was deurgaans laer in mengsels met 50%, 67% en 80% van 'n weerstandbiedende kultivar. Siektevorderingskurwes en siektegradiënte het aangetoon dat ontwikkeling en verspreiding stadiger en meer beperk was in alle behandelings met 'n proporsie bestandheid. Daar is tot die gevolgtrekking gekom dat antraknose beheer kan word in mengsels wat ten minste 60% van 'n bestande kultivar bevat. 'n Beraming van die doeltreffendheid van chemiese antraknose-beheer het aangedui dat 'n benomyl saadbehandeling, gevolg met 'n difenoconazole loofbespuiting, of laasgenoemde alleen, die beste beheer gegee het.

Laastens is glashuis en multilolaliteitsveldproewe gedoen om droëboongenotipes met genetiese weerstand teen Ethiopiese isolate van *C. lindemuthianum* te identifiseer. Die studie het die kultivars Widusa, GLP X 1132, A 482, A 193, G-7, HAL 5 as G 2333 as waardevolle bronne van antraknose-bestandheid uitgewys.