INTEGRATED MANAGEMENT OF CHARCOAL ROT IN COMMON BEAN IN KENYA

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CONTENTS

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Charcoal rot in common bean with special reference to Kenya

(*Keywords: Macrophomina phaseolina*, charcoal rot, beans, Kenya)

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Abstract. Charcoal rot caused by *Macrophomina phaseolina* (Deuteromycetes: Coelomycetes) is found throughout the tropics and subtropics and has a wide host range. Together with most of the legume crops, the common bean (*Phaseolus vulgaris*) is a good host for the fungus which causes a range of symptoms, depending on environmental conditions and age of the plant. In addition to charcoal rot, which is a stem or stalk rot disease, the pathogen also causes damping-off and seedling blight in beans. Charcoal rot in the mature plant is associated with senescence which is accelerated by water stress. The disease is most damaging in areas of unreliable rainfall and high temperature. In Kenya, beans are usually grown in mixed stands with maize, sorghum or millet. Population pressure has led to the cultivation of beans on land prone to drought. *M. phaseolina* is one of the most important pathogens affecting all the main crops of the farming systems in the semi-arid areas of eastern Kenya and resistance to charcoal rot is a priority if beans are to be increasingly grown in these areas. The paper reviews the literature on charcoal rot of beans and on other crops where similar work has not been reported specifically for beans.

1. Introduction

Charcoal rot of beans (*Phaseolus vulgaris*) is caused by *Macrophomina phaseolina* (Tassi) Goid. (Deuteromycetes: Coelomycetes). The pathogen is widely distributed in the tropics and sub-tropics and has a host range which includes more than 300 plant species (Cottingham, 1981). The fungus can cause seedling disease in legumes and other crop species and is responsible for stalk rots in cereal crops as well as root and stem diseases in numerous crop species. These diseases are known variously as charcoal rot, black rot and ashy stem, depending on the host. For the sake of consistency the disease will be referred to as charcoal rot in this review.

The fungus causes economic losses in areas with a hot climate where crops are exposed to periods of moisture stress (e.g., Dhingra and Chagas, 1981). It is quoted as the single most important pathogen of cowpeas, sorghum and mung bean in the Bay region of Somalia (Gray *et al.*, 1990) and is the main disease affecting legumes in the semi-arid areas of Nigeria (IITA, 1984). Charcoal rot is important on beans in the warmer areas of production in the USA (Hagedorn, 1991), Latin America (Diaz-Franco, 1984), India (Satischandra *et al.*, 1979), the Carribean (Echavez-Bedel and Beaver, 1987) and Africa (Emechebe and McDonald, 1979; CIAT, 1981).

In Kenya, increasing population pressure has led to the cultivation of food crops in eastern parts of the country, previously regarded as unsuitable for crop production owing to their unreliable rainfall. The semi-arid areas of Kenya, which include most of Eastern Province (Figure 1), contribute 35% of the country's total bean production (Gitu and Ngalyukia, 1989). With their continued cultivation in increasingly drier environments, there is a need to produce bean varieties with improved drought tolerance and resistance to *Macrophomina* (Muigai and Rono, 1990).

Having a worldwide distribution and wide host range, there is a large literature on *M. phaseolina*. The International Centre for Research in the Semi-Arid Tropics (ICRISAT) has published a bibliography of literature up to 1987 (Singh *et al.*, 1990) and the biology and pathogenicity of the fungus was reviewed by Dhingra and Sinclair (1978). The purpose of this paper was to review the literature concerning *M. phaseolina* on beans, in relation to conclusions drawn from work on other crops and to emphasize its importance in the semi-arid regions of eastern Kenya.

2. Symptoms

M. phaseolina causes seedling disease in bean as well as the more conspicuous ashy stem or charcoal rot symptom in older plants. Although they are manifestations of the same syndrome, the nature of the aetiological link between seedling disease and charcoal rot is not clear.

The first visible symptom on the seedling is a small dark brown lesion on the cotyledon which spreads down onto the hypocotyl (Figure 2) or, sometimes, the lesion may appear first at the base of the hypocotyl. Early infection results in pre-emergence damping-off or seedling mortality, soon after emergence (Figure 3). In established seedlings, lesions spread down the hypocotyl, girdle it and cause the plant to collapse. If the lesion develops late or the plant has some resistance, the hypocotyl is not completely girdled and the plant may recover from the infection. Plants which survive the first 2–3 weeks after sowing usually survive until flowering when the first symptoms of the charcoal rot phase of disease appear at the base of the stem as grey, necrotic areas, bearing numerous small black sclerotia. As senescence progresses, the infection spreads up the stem and sclerotia may eventually appear on the pods (Figure 4), leading to seed infection. Plants which develop charcoal rot symptoms relatively early in their growth exhibit signs of accelerated senescence, such as foliar chlorosis and fewer seeds reach maturity.

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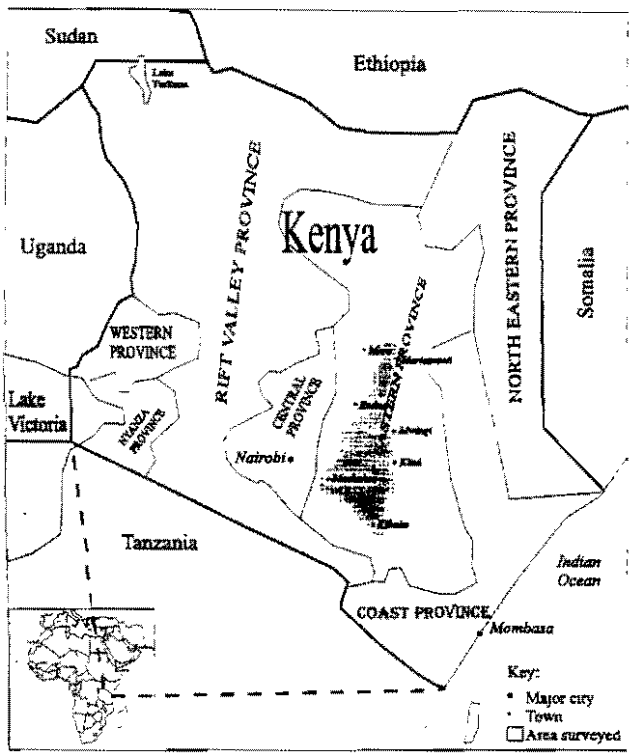

Figure 1. Sketch map of Kenya showing survey area where isolates of M. phaseolina were collected.

3. Morphology and cultural characteristics of the causal organism

Macrophomina phaseolina (synonym: *Macrophomina phaseoli*, *Rhizoctonia bataticola*, *Sclerotium bataticola*) can be readily isolated from infected host tissues on standard media. It has a high optimal temperature for mycelial growth of 32–34 C, covering a 9 cm Petri dish or potato dextrose agar (PDA) in 48 h.

Young hyphae are colourless, becoming light brown with age. Within a few days of growth on PDA, abundant sclerotia are produced which range from 100 to 300 μm in diameter. These are smooth or irregular in shape, black in colour and rich in oils (Holliday and Punithalingam, 1970, Dhingra and Sinclair, 1978). Sclerotia constitute the principal propagules for dissemination and are resistant to extremes of temperature and humidity. Within the infected host, sclerotia appear in large numbers when the tissues begin to senesce.

Figure 2. Infection originating from the cotyledons and spreading to the hypocotyl

Figure 3. Post emergence seedling disease in beans growing in soil inoculated with M. phaeolina.

Black, globose pycnidia are sometimes seen on the host and can be induced in culture (Watanabe, 1972). They are 100–200 μm in diameter and contain single-celled fusiform conidia (Dhingra and Sinclair, 1978).

4. Variation in the causal organism

Macrophomina phaseolina is a plurivorous pathogen and isolates from one plant species are often found to infect a range of other hosts (Holliday and Punithalingam, 1970). Lee *et al.* (1986) for instance, found that isolates from bean, cotton

Figure 4 Charcoal rot in mature field-grown beans often extends to the pods resulting in infection of the seed

and groundnut were all pathogenic on cotton. Similarly, Diourte (1987) reported that isolates from sorghum, groundnut, bean and cotton all cross-inoculated each of the other hosts although there was a trend towards greater virulence on the host from which each strain was initially isolated. However, there is some evidence that certain isolates exhibit a degree of host specificity. Isolates causing ashy stem of cowpea in Botswana also infected beans, but not sorghum (Burke *et al.*, 1986). Care must be exercised in interpreting this result as it is easier to induce symptoms on legumes, following artificial inoculation, than on the cereals which are so dependent on predisposition. Further support for the occurrence of host specificity is to be found in the work of Chohan and Kaur (1977) who found that three isolates of *M. phaseolina* which were highly aggressive on cotton, okra and blackgram, did not cause symptoms on maize or groundnut. Ahmed and Ahmed (1969) have attempted to separate isolates of the pathogen from jute into physiological races. Pearson *et al.* (1987) were able to distinguish isolates of the fungus from maize from those isolated from soybean on the basis of their growth characteristics on a medium supplemented with potassium chlorate.

Isolates of the fungus from different regions (Dinghra and Sinclair, 1973a), hosts (Buadgi and Hegde, 1985), the same host, or, even different parts of the same plant (Dhingra and Sinclair, 1973b), exhibit wide variation in cultural and morphological characters such as growth rate, sclerotial production, sclerotial size and ability to produce pycnidia (Watanabe, 1972, Chidambaram and Mathur, 1975). However, these differences have not been linked to host specialization or to aggressiveness.

5. Disease incidence and yield loss

Under warm, moist conditions, following a long dry season and in the presence of high inoculum levels of *M. phaseolina*, seedling mortality in legume crops can be high. For instance, in 1983 in southern Nigeria the fungus was responsible for 70% cowpea seedling mortality (IITA, 1984). However, seedling loss is not normally as high and the surviving plants, especially among the long season varieties, have some capacity to compensate for early stand loss. Loss estimates for Africa are not available but yield losses of up to 65% have occurred in bean crops grown in the USA (Zaumeyer and Thomas, 1957).

Although the disease has been known in Kenya for many years (J.M. Waller, personal communication) it has become more prevalent in recent years with the cultivation of land in progressively drier areas. *M. phaseolina* was observed on sorghum, causing extensive lodging in the crop produced on the short rains (Oct.–Jan.) near Makueni in Machakos District (see figure 1) (Waite *et al.*, 1984). Mukunya *et al.*, (1983) reported serious incidences of the disease at specific locations in the semi-arid area of Kenya. Seedling mortality of 10–14% has been observed in beans grown in experimental plots and farmers' fields in Machakos District (W. Songa, unpublished). The disease has since been observed all over eastern Kenya on many crops including beans (Stoetzer *et al.*, 1984). Table 1 shows a list of isolates collected in eastern Kenya by Songa (1995) and the crop species from which they

Table 1. Crop host and location in eastern Kenya from which isolates of M. phaseolina were collected during 1992 (Songa, 1995)

		Origin in Kenya	
Isolate	Host crop	Location	District
S3	*Phaseolus vulgaris*	Kangodi	Machakos
WS4	*P. vulgaris*	Katumani	Machakos
WS5	*Sorghum bicolor*	Katumani	Machakos
WS8	*P. vulgaris*	Mwingi	Kitui
WS10	*P. vulgaris*	Kiboko	Makueni
WS11	*P. vulgaris*	Kiboko	Makueni
WS12	*Zea mays*	Kampi-ya-Mawe	Makueni
WS14	*P. vulgaris*	Kampi-ya-Mawe	Makueni
WS16	*Pennisetum glaucum*	Ishiara	Embu
WS17	*P. vulgaris*	Masinga	Machakos
WS19	*S. bicolor*	Chuakiriga	Embu
WS20	*Zea mays*	Marimanti	Meru
WS21	*S. bicolor*	Marimanti	Meru
WS22	*S. bicolor*	Marimanti	Meru
WS24	*P. glaucum*	Marimanti	Meru
WS25	*P. glaucum*	Siakago	Embu
WS28	*P. vulgaris*	Siakago	Embu
WS34	*P. vulgaris*	Siakago	Embu
WS35	*P. vulgaris*	Kibwezi	Makueni

were isolated. Differences in seed weight of 35–50% commonly occur between inoculated and non-inoculated plots in trials conducted to screen bean germplasm for resistance to *M. phaseolina* at Kiboko Experimental Station in eastern Kenya (W. Songa, unpublished). A recent report records charcoal rot as an important cause of yield loss in beans grown in the semi-arid areas of Kenya with losses often exceeding 300 kg ha^{-1} (Wortmann and Allen, 1994).

6. Epidemiology and disease development

The pathogen survives in the soil in crop residues in the form of sclerotia and the initial infection derives either from soil-borne inoculum or from the planting of infected seed (Singh and Singh, 1982). The pathogen is known to be seed-borne in a number of hosts including soybean (Gangopadhyay *et al.*, 1970) and common beans (Abawi and Pastor-Corrales, 1990a) and can be carried within the seed coat (Dhingra and Sinclair, 1978). The fungus was detected in 30% of cowpea seed samples collected from markets in northern Nigeria (Emechebe and McDonald, 1979).

Dry conditions favour survival of sclerotia in the soil but mycelial growth and infection require moist conditions and are favoured by a temperature above 27 °C (Hagedorn, 1991). Provided there is sufficient soil moisture for fungal growth and high inoculum levels, seedling losses increase with increasing temperature up to 45 °C. While seedling loss in beans is dependent on high inoculum potential, adequate soil moisture and temperatures above 27 °C, symptom expression in the mature plant is not related directly to soil inoculum but is dependent on the physiological predisposition of the host (Songa, 1995). Predisposition is triggered by flowering and subsequent onset of senescence, provided this occurs at high temperature (above 32 °C). Moisture stress at flowering seems to be an important predisposing criterion, at least in sorghum (Edmunds, 1964). It is still uncertain whether this is due directly to the physiological effects of moisture stress,

or, because drought conditions lead to early senescence. In beans, plant mortality increased from 9% under ideal soil moisture conditions to 64% after 18 days of water deficit (Magalhaes *et al.*, 1982).

The exact nature of the aetiological link between seedling infection and symptom expression in the mature plant is not fully understood. The evidence from work with cowpea (Bourne, 1992) is that infection of the root and crown region may occur at any time during the life of the plant. The pathogen appears to invade the base of the stem, then remains dormant until the plant becomes predisposed to infection after the onset of flowering. Demooy and Burkeld (1990) reported that lesions on cowpea hypocotyl could remain dormant for up to 1 month before becoming activated by drought or senescence.

There is little reported work on the aetiology of *Macrophomina* infection in beans, more has been published on the subject with respect to other crops but some of the reports are contradictory. The apparent contradictions exist because of the failure to distinguish between infection and symptom expression or to distinguish between seedling disease and mature plant disease. Charcoal rot severity in cotton (Ghaffar and Erwin, 1969, Thakar, 1984) and in sorghum (Edmunds, 1964, Odvody and Dunkle, 1979) is inversely correlated with soil moisture levels. However, Bruton *et al.* (1987) showed that infection of cantaloup vines was favoured by wet conditions which led later to high levels of disease.

The stage of development at which a plant is most likely to exhibit symptoms of *Macrophomina* infection varies to some extent with host species. Legume crops tend to be more susceptible at the seedling stage than are the cereal crops. However, here again the distinction needs to be made between the optimum time for infection and the main period of symptom development which are often temporally separated. Norton (1958) considered that the pathogen can invade sorghum roots at any stage, but no further development occurs until the plant becomes drought stressed. The alternative view is that drought stress is a prerequisite for root penetration (Edmunds, 1964, Odvody and Dunkle, 1979). Some have argued that root invasion only occurs at physiological maturity, even if dry conditions prevail at an earlier stage of crop development. However, Cloud and Rupe (1994) found that sorghum roots were invaded by the fungus well before flowering, during a period of crop moisture stress. Young and Alcorn (1984) were also able to isolate *Macrophomina* from 1-month-old plants of *Euphorbia lathyrys* although symptoms did not become evident until 7 months later. In soybean, plant maturity was found to be the only factor affecting sclerotial production which was independent of moisture stress and temperature. The main factor required for severe disease was senescence which was enhanced by moisture stress. Flower removal has been shown to delay or prevent the appearance of charcoal rot symptoms (Wyllie and Calvert, 1969).

7. Association with other microorganisms

Macrophomina has a poor competitive saprophytic ability and is very responsive to changes in soil fungistasis. Reduction of bacterial populations has been correlated with increased sclerotial germination, while increased microbial activity can decrease sclerotial viability (Filho and Dhingra, 1980). Rhizobium strains indigenous to Pakistan were reported to inhibit growth of *M. phaseolina* in culture (Zaki and Ghaffar, 1987). *Aspergillus flavus*, a common soil-inhabiting fungus, has been reported to decrease infection by *M. phaseolina* in peanut kernels (Jackson, 1965).

Nematodes may also have some potential to increase susceptibility to *Macrophomina*, either through increased root invasion or by enhancing the effects of water stress. Al-Hazmi (1985) has demonstrated increased root rot of bean in the presence of root-knot nematode (*Meloidogyne incognita*). The lesion nematode *Pratylenchus zeae* and *M. phaseolina* act synergistically to reduce plant growth in sorghum (Bee-Rodriguez and Ayala, 1977). Norton (1958) reported similar results with *P. hexincisus*. Root-knot nematodes cause serious yield losses of beans in some parts of Kenya (Ngundo and Taylor, 1974) and lesion nematode is more or less ubiquitous in the country's maize-based cropping systems and the common bean is a good alternative host for *P. zeae* (Jones and Hillocks, 1995). The role of the nematode complex in eastern Kenya in predisposing beans to charcoal rot requires further research.

8. Control

8.1. *Cultural measures*

Crop management systems that reduce inoculum levels and conserve soil moisture are effective in reducing the incidence of stalk rot. Stalk rot incidence in sorghum was decreased from 39% to 11% in minimal tillage compared with conventional tillage (Doupnik *et al.*, 1975). Sowing date can also be manipulated in some areas to ensure that the crop is less likely to mature during periods of high temperature and unreliable rainfall. Intercropping has been reported as a method of reducing charcoal rot incidence. Disease incidence in cotton was decreased by intercropping with *Phaseolus aconitifolia* due to the soil cover provided and consequent lowering of soil temperature (Rajpurghit, 1983). In Kenya however, beans are usually grown as an intercrop (Njungunah *et al.*, 1981) with maize or sorghum, both of which are good hosts for the pathogen. Sorghum grown as a mixed crop has been reported to suffer less damage from charcoal rot than sole crop sorghum (Khune *et al.*, 1980). Crop rotation may be useful in decreasing disease incidence if a susceptible crop is grown after a less susceptible crop (e.g., Frankle *et al.*, 1988). A contrasting view was expressed by Short *et al.*, (1980) who found that viable sclerotia persist over a 2-year fallow period and their numbers increase rapidly when a susceptible host is grown. They conclude that rotation is unlikely to be effective as a control measure.

8.2. *Chemical treatment*

There are few reports of fungicides being used to control charcoal rot on beans. However, there has been some interest in their use for this purpose on soybean. In order of efficacy, benomyl, thiophanate methyl, thiram, thiabendazole, triforine and captan decreased viability of sclerotia in soil and

in soybean stem pieces (Ilyas *et al.*, 1976). Seedling infection was controlled best by benomyl and thiabendazole (Ilyas *et al.*, 1975). The use of fungicide applied to the seed may be effective in decreasing losses to *M. phaseolina* in crops which are particularly vulnerable at the seedling stage. In the case of jute for instance (Chattopadhyay *et al.*, 1981), significant control of seedling disease was obtained with carbendazim and pentachloronitrobenzene (PCNB). Abawi and Pastor-Corrales (1990b) state that seed treatment with benomyl or carboxin is effective in controlling charcoal rot of beans during the seedling stage. In India, carbendazim was not effective as a seed treatment to control root rot on beans but PCNB and captan gave some control (Satischandra *et al.*, 1979). Soil fumigation with methyl bromide decreased the number of viable sclerotia and controlled infection of pine seedlings (Watanabe *et al.*, 1970). However, in the case of sorghum, where the disease is more prevalent after flowering, PCNB did not control the disease in the field despite giving 87% inhibition of fungal growth *in vitro* (Anahosur *et al.*, 1983).

8.3. *Biological control*

The effect of organic soil amendments on population levels of *M. phaseolina*, depends on the carbon to nitrogen ratio of the residues. A decline in soil population of the pathogen followed the application of residues of various crops or of glucose–sodium nitrate. However, populations later increased in soil treated with wheat or corn straw but not in soil treated with glucose sodium nitrate or with nitrogen-enriched straw (Filho and Dhingra, 1980).

Treatment with *Trichoderma harzianum* decreased disease incidents by 37% in beans grown in pots and decreased charcoal rot of maize in the field by 28% (Elad *et al.*, 1986). Root rot of mung bean and other crops caused by *Macrophomina* was controlled after seed was dipped in a suspension of *Rhizobium* strains (Zaki and Ghaffar, 1987). Similar results were reported for soybean by Chakraborty and Purkayastha (1984) who concluded that the effect was due to the fungitoxic action of rhizobitoxine.

Soil solarization was found to decrease the population of viable sclerotia of *M. phaseolina* but many remained viable due to the high temperature tolerance of the fungus (Mihail and Alcorn, 1984).

8.4. *Host-plant resistance*

Resistance to *Macrophomina* has been identified in some bean varieties such as 'Negrito' (Dhingra and Sinclair, 1978). The bean improvement programme at CIAT (Centro Internacional de Agricultura Tropical) has developed methods to screen for resistance to charcoal rot (Abawi and Pastor-Corrales, 1986). Using these methods a number of lines with some resistance to the disease have been identified (Pastor-Corrales and Abawi, 1988). In some cases resistance is linked to drought tolerance (CIAT, 1983). Bean lines resistant to *Macrophomina* are available from the CIAT germplasm collection and are listed by Abawi and Pastor-Corrales (1990b). The lines A70 and A474 are particularly susceptible and are used as susceptible standards in screening tests. Avila *et al.* (1982) found some correlation between resistance and seed coat colour and recommended the use of black-seeded types on heavily infested soils.

9. Screening for resistance in Kenya

Recognizing the importance of *Phaseolus* bean in smallholder cropping systems in Kenya and the need for low cost disease control measures, much emphasis has been placed on breeding for disease resistance within the Kenyan National bean improvement programme, which is coordinated from Thika Research Station, north-east of Nairobi. Selection for resistance to charcoal rot is conducted from Katumani Dryland Research Station, near Machakos in the semi-arid zone of eastern Kenya. Evaluation of resistance is based on greenhouse and field screening in which plants are inoculated using colonized rice grains as the inoculum source (Abawi and Pastor-Corrales, 1986). Moderate levels of resistance have been identified in local and introduced genotypes. At Kiboko Experimental Station where high temperatures and low rainfall create excellent conditions for the development of charcoal rot, a large number of lines are being evaluated for resistance in adjacent inoculated and non-inoculated plots.

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Legume Hosts of *Macrophomina phaseolina* in Kenya and Effect of Crop Species on Soil Inoculum Levels

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With one figure

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Abstract

Studies were conducted in eastern Kenya to determine the common legume crop and weed hosts of *Macrophomina phaseolina* (Tassi) Goid., the inciter of charcoal rot disease. The effect of maize, sorghum, bean, and cowpea on the soil inoculum level was also investigated after field inoculation. All the legume crops and weeds tested were found to be infected by the pathogen after artificial inoculation. Common bean, soybean, cowpea were the most susceptible while pigeonpea, green gram, and hyacinth bean were moderately susceptible. Groundnut, chickpea, *Cassia* spp. and *Crotalaria* spp. were least susceptible after artificial inoculation. Monocropping of sorghum, maize, cowpea and common bean for three consecutive crop seasons increased *M. Phaseolina* soil inoculum in ascending order.

Zusammenfassung

Leguminosewirtspflanzen von *Macrophomina phaseolina* in Kenia und der Einfluß der angebauten Kulturpflanze auf das bodenbürtige Inokulumlevel

In Ostkenia wurden Versuche durchgeführt, um die am häufigsten vorkommenden Leguminosen-und Unkrautwirtspflanzen von *Macrophomina phaseolina* (Tassi) Gold, Erreger der charcoal rot Krankheit, zu ermitteln. Der Einfluß von Mais, Hirse, Bohnen und Kuherbsen auf das Inokulumlevel wurde auch in einem Feldversuch nach künstlicher Inokulation untersucht. Alle untersuchten Leguminosen und Unkrautspezies wurden durch das Pathogen nach künstlicher Inokulation befallen. Die Ackerbohne, die Sojabohne und die Kuherbse waren am anfälligsten, die Taubenerbse, die Mungobohne und Faselbohne wiesen dagegen eine mittlere Anfälligkeit auf. Die schwächste Anfälligkeit wurde nach einer künstlichen Inokulation bei den Erdnüssen, der Kichererbse, *Cassia* spp. und *Crotalaria* spp. beobachtet. Eine dreimalige Monokultur mit Hirse, Mais, Kuherbse und der Ackerbohne erhöhte das *M. phaseolina*-Inokulum in ansteigender Rangfolge.

Introduction

Macrophomina phaseolina, the charcoal rot disease pathogen, has a wide host range exceeding 300 plant species including legume and cereal crop plants (Reichert and Hellinger, 1947; Dhingra and Sinclair, 1977). In legumes, infection is evident from the seedling to mature plant stage while in cereals infection is usually observed after onset of anthesis. At the seedling stage infection usually occurs during moist conditions and at temperatures above 27 C (Hagedorn, 1991) while at the mature plant stage physiological predisposition of the host precedes infection. Predisposition may be triggered by drought stress(Anonymous, 1985; Schwartz, 1989), flowering and subsequent onset of senescence, provided this occurs at high temperature (above 32 C). In beans, plant mortality was found to increase from 9% under ideal soil moisture conditions to 64% after 18 days of water deficit (Magalhaes et al., 1982). Disease evaluation in legumes can be easily done at both seedling and mature plant stages.

The plurivorous nature of *M. phaseolina* enables this pathogen to survive on many alternative hosts in the absence of crop hosts. Although this nature of the pathogen limits the effectiveness of some cultural management methods such as crop rotation, the manipulation of cropping systems to reduce the inoculum level in soils is still promising (Singh et al., 1990; Francl et al., 1988). Crop species vary in their reaction to *M. phaseolina* depending on their inherent resistance. The more susceptible crop species succumb to the pathogen earlier and enable the pathogen to multiply on them and increase the levels of inoculum in the soil.

Phaseolus bean is one of the favoured legume crops grown in the maize-based cropping systems of eastern Kenya. It is, however, highly susceptible to *M. phaseolina*. The objectives of this study were to compare the reaction of common bean and other alternative legume crops to *M. phaseolina*. The effect of monocropping two legume and two cereal crops for three seasons on soil inoculum

levels of *Macrophomina phaseolina*, and charcoal rot disease incidence in a subsequent bean crop was also investigated.

Materials and Methods

Artificial inoculation

Legume crop and weed hosts of M. phaseolina

Nine legume crops and nine leguminous weeds common in charcoal rot disease 'hot spots' were used in the study. The legume crops were: common bean (*Phaseolus vulgaris* L.), cowpea (*Vigna unguiculata* (L.) Walp), green gram (*Vigna radiata* (L) Wilcz, Soybean (*Glycine max* (L.) Merr.), chickpea (*Cicer arietinum* L.), hyacinth bean (*Dolichos lab lab* L.), garden pea (*Pisum sativum* L. sens *ampl.*), pigeonpea (*Cajanus cajan* (L.) Millsp.) and ground nut (*Arachis hypogea* L.). The germination of the weed seeds was not uniform and only three species which had good germination were eventually used in inoculation tests. Two of the selected weeds were *Crotalaria* spp. and one was a *Cassia* spp. Of the two *Crotalaria* spp. one was early flowering and the other late.

The study of legume crop and weed hosts of *M. phaseolina* was carried out in a greenhouse maintained at 18–30 C. Five seeds of each legume crop and weed were planted per pot in four replications. Inoculation was done using rice grains colonized by *M. phaseolina* (Abawi and Pastor-Corrales, 1986; Songa, 1995) isolate WS 17 from common bean. A single plant was removed from each of the pots at 15, 30, and 60 days after emergence for determination of the presence of the fungus in the root, lower stem and upper stem. The procedure for isolation of the pathogen was as follows: nine pieces were cut from each of the plant parts and washed thoroughly in distilled water, sterilized in 0.5% NaOCl for 1 min and then rinsed in distilled water. The plant tissue pieces were then dried on blotting paper and plated on PDA. Three tissue pieces from each plant part were plated per Petri dish in three replications. Observations for growth of *M. phaseolina* were made after incubation at 33 C for 6 days.

Field experiment

Effect of crop species on the level of M. phaseolina *and disease incidence in beans*

The study of the effect of crop species on the level of *M. phaseolina* inoculum level in the soil was conducted at Kiboko research station in eastern Kenya. A mixture of bean, maize, cowpea and sorghum crop debris infested with *M. phaseolina*, were chopped and incorporated uniformly in the soil of the study field at the beginning of the experiment. This ensured some initial inoculum level in the field which was located in an area which had been left fallow for 2 years. Monocrops of maize, sorghum, cowpea and common bean were planted in plots of five by five m for three consecutive crop seasons following the recommended agronomic practices. After the first planting, the plots were subsequently prepared by hand to avoid moving soil from one plot to another. Occasionally, irrigation was given but only when wilting symptoms were apparent. At the end of each season, the crop debris of each plot were chopped and hand ploughed into the plot. The control plots were left fallow. There were four replications in a randomized block design.

In the fourth season all the plots were planted with common bean (cv. GLP 1004) to enable uniform evaluation of disease incidence in the plots. The disease level in the plots was assessed at 14 days after emergence and at harvest. Evaluations were carried out to monitor both the seedling and mature plant stages of the disease. The parameters taken were: number of plants germinated, number of seedlings infected, number of infected plants at harvest, total number of plants at harvest and yield per plot.

Isolation of M. phaseolina *from the plots at four depths*

The M. phaseolina sclerotia level in the soil was determined at the beginning of the experiment and every 3 months thereafter for a total period of 18 months. The isolation of *M. phaseolina* sclerotia from the soil was done by taking soil samples from depths (0–5, 5–10, 10–15 and 15–20 cm) in each plot. At each sampling date soil was taken from each plot and depth at four different points along the diagonals of the plot at random. The soil from similar depths in each plot was then bulked to constitute a sample for the plot. Isolation of *M. phaseolina* from the soil was done as described by Papavizas and Klag (1975).

Results

Artificial inoculation

Legume crop and weed hosts of M. phaseolina

The pathogen was isolated from the roots of common bean, cowpea, pigeonpea, green gram, hyacinth bean, soybean and the early flowering *Crotalaria* spp. 15 days after inoculation and in chickpea, groundnut, *Cassia* spp. and the late flowering *Crotalaria* spp. after 30 days (Table 1.1). Plants from which *M. phaseolina* was isolated from the stem after 60 days were those whose roots were already colonized at 15 days after inoculation. In chickpea, groundnut, *Cassia* spp., and the late flowering *Crotalaria* spp. the pathogen did not spread beyond the roots. The pathogen was found in the upper stem in common bean, soybean, hyacinth bean and pigeonpea. External disease symptoms were observed only in common bean, cowpea, soybean and greengram (Table 1).

Field experiment

Evaluation of level of M. phaseolina *in monocropped plots by isolation from the soil*

The level of sclerotia in the soil at the beginning of the experiment was found to be on average 11.2 sclerotia per g of soil. Significant differences ($P = 0.05$) were found between plots with different crop treatments in the level of sclerotia at each sampling (Fig. 1). There was a general tendency for the sclerotia level to increase in the soil from harvesting to the planting of the next crop. Cowpea and common bean plots had significantly ($P = 0.05$) higher average number of sclerotia per g of soil than sorghum and fallow control plots after three seasons of monocropping (Table 2). The sclerotia levels in the maize and sorghum plots were not significantly ($P = 0.05$) different

Table 1
Isolation of *M. phaseolina* from roots, lower stems, upper stems and symptoms on stem of some legume crops and weeds after artificial inoculation with isolate WS 17 from common bean

Legume	Plant part											
	Roots			Lower stem			Upper stem			Symptoms on stem		
	Days after emergence											
	15	30	60	15	30	60	15	30	60	15	30	60
Common bean	■	■	■	■	■	■	□	■	■	■	■	■
Cowpea	■	■	■	■	■	■	□	□	□	■	■	■
Pigeonpea	■	■	■	□	■	■	□	□	■	□	□	□
Green gram	■	■	■	□	□	■	□	□	□	□	□	■
Soybean	■	■	■	■	■	■	□	■	■	■	■	■
Hyacinth bean	■	■	■	■	■	■	□	□	■	□	□	□
Chickpea	□	■	■	□	□	□	□	□	□	□	□	□
Groundnut	□	■	■	□	□	□	□	□	□	□	□	□
Cassia sp.	□	■	■	□	□	■	□	□	□	□	□	□
Crotalaria spp. (late flowering)	□	■	■	□	□	□	□	□	□	□	□	□
Crotalaria spp. (early flowering)	■	■	■	□	■	■	□	□	□	□	□	□

■ *M. phaseolina* isolated; □ *M. phaseolina* not isolated

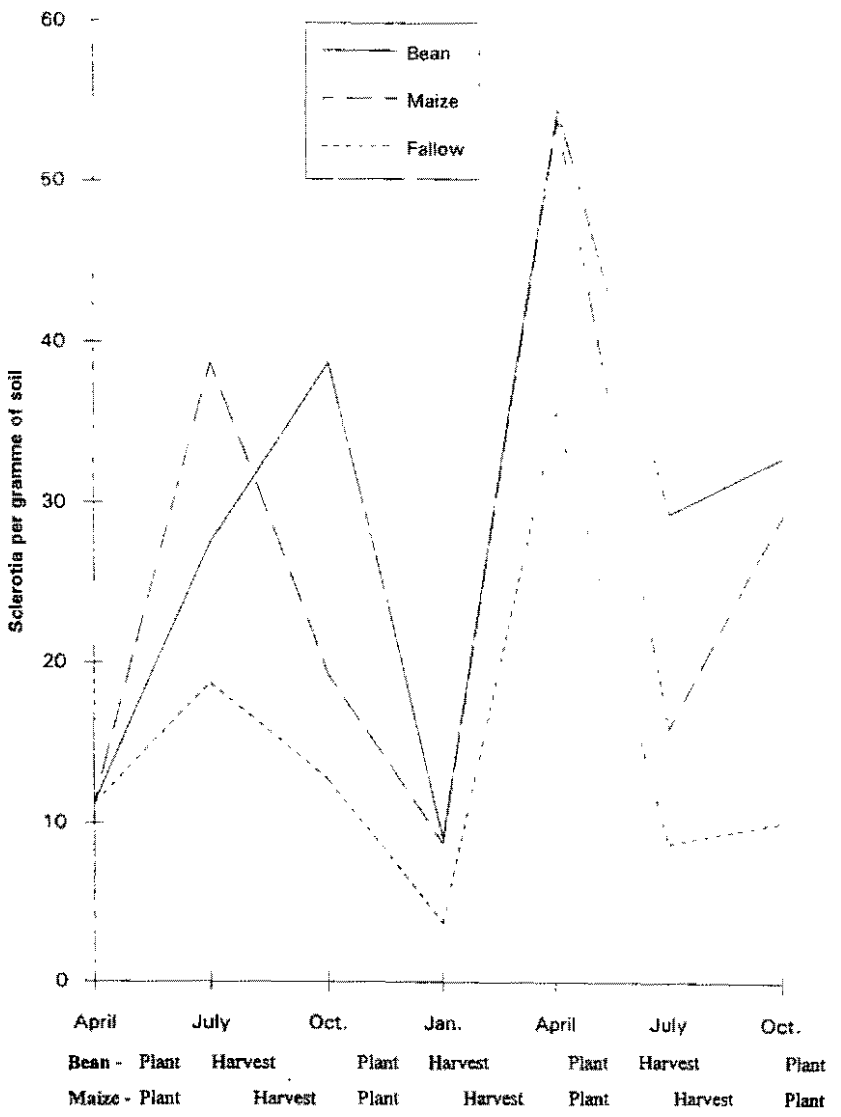

Fig. 1 Sclerotia level in plots monocropped with bean, maize, and fallow control at Kiboko in 1993/94

Table 2
Sclerotial level in field soil after monocropping cowpea, common bean, maize and sorghum for three seasons

Crop	Mean sclerotia level per gram soil
	Mean SD
Common bean	31.3[a] ± 14.9
Cowpea	29.3[a] ± 14.4
Maize	27.7[ab] ± 16.6
Sorghum	24.9[b] ± 11.2
Fallow (control)	15.0[c] ± 11.2
LSD	3.09

[a]Means with the same letter not significantly (P = 0.05) different.

but were significantly higher than in the control plots. There was no significant (P = 0.05) difference in the number of sclerotia isolated from different depths in the monocropped plots of the same crop species.

Evaluation of level of M. phaseolina *in plots using common bean*

The number of germinated bean plants in the different previously monocropped plots was not significantly different (P = 0.05). The plots previously monocropped with common bean had significantly (P = 0.05) higher bean seedling infection than those monocropped with maize, sorghum and fallow (Table 3). There was no significant difference (P = 0.05) in the number of infected bean seedlings in plots previously monocropped with common bean and cowpea but there was a significant difference between these plots and the fallow control plots. The number of infected plants at harvest did not significantly (P = 0.05) differ by monocropping. The number of plants at harvest was significantly (P = 0.05) lower in plots previously monocropped with common bean than with cowpea, maize, sorghum and fallow. However, there was no significant difference (P = 0.05) in the number of plants at harvest in plots monocropped with maize, sorghum and cowpea. A significant (P = 0.05) negative correlation ($r_s = -0.9$) was found between the number of infected bean seedlings and the number of plants at harvest. Yield was significantly different (P = 0.05) between common bean and fallow and was positively corelated ($r_s = 0.9$) with the number of plants at harvest and negatively correlated ($r_s = -0.9$) with number of infected seedlings.

Discussion

Macrophomina phaseolina was isolated from the roots of cowpea, common bean, hyacinth bean, pigeonpea, green gram, and one weed of *Crotalaria* spp. 15 days after inoculation. For chickpea and groundnut this was the furthest the pathogen was able to penetrate the host. In common bean, soybean, cowpea and hyacinth bean, the pathogen was found in the lower stem 15 days after inoculation. These results imply that chickpea and groundnut were able to restrict further entry of the pathogen beyond the roots. The pathogen was found in the stem in hyacinth bean, pigeonpea and the early flowering *Crotalaria* spp. but no symptoms were observed, whereas, clear symptoms were manifested in soybean, common bean, and cowpea. If disease symptom expression is taken as a measure of the pathogen's successful colonization of the host, then soybean, common bean and cowpea were very susceptible. These crops would increase the inoculum of the pathogen in the soil and would be unsuitable for use in crop rotation for control of the pathogen.

Symptoms were not apparent on green gram until 60 days after inoculation. This implied a good level of resistance in this crop. The absence of symptoms in chickpea and pigeonpea suggests more resistance to the pathogen in these crops and would therefore, be suitable for crop rotation in infested fields. These crops are drought tolerant and would fit well in farming systems of areas where charcoal rot disease is prevalent. Pigeonpea was reported by Singh et al. (1990) to be susceptible to *M. phaseolina* in India. The results in this study do not agree with this observation but reiterate the minor importance of charcoal rot in pigeonpea in semi-arid eastern Kenya where most of the crop is cultivated (Songa et al., 1991). Sorghum grown in the same area is often severely infected (Waite et al., 1984) but no disease occurrence of importance has been observed or reported on pigeonpea.

The observation that the pathogen could exist in hosts without any symptoms agrees with Edmunds (1964) who suggested that latent infection probably occurs in the crown of most sorghum plants. It would be worthwhile

Table 3
Effect of crop species on charcoal rot disease incidence on common bean after monocropping for three seasons

Monocropping	Crop species	Number of germinated bean seedlings plot	Number of infected bean seedlings plot	No. of infected bean plants plot at harvest	Number of bean plants plot at harvest	Yield of common bean plot (kg)
4 season	Common bean	438	71.2[a]	166.5[a]	320.0[c]	1.47[c]
	Cowpea	426	62.0[ab]	174.8[a]	346.0[b]	1.98[ab]
	Maize	422	51.7[b]	165.0[a]	368.3[ab]	2.21[b]
	Sorghum	419	55.5[b]	160.8[ab]	356.3[ab]	2.25[b]
	Fallow	427	46.0[c]	138.0[b]	378.0[a]	2.53[a]
3 season and fourth common bean	LSD	NS	15.11	23.48	24.55	0.71

Means with same letter not significantly different (P = 0.05).

to carry out more studies on common weed species to determine those weeds that may be susceptible and contributing to increase levels of inoculum in the soil.

Monocropping with common bean, cowpea, maize and sorghum increased charcoal rot disease incidence compared with fallow plots. The increase was more in the plots monocropped with common bean and cowpea. The number of infected seedlings, the number of infected plants at harvest and number of plants at harvest appear to be good parameters for indicating the level of inoculum in the soil. The significant negative correlation between the number of infected seedlings and the number of plants at harvest suggests that most infected seedlings do not survive to maturity. Common bean and cowpea mature earlier than maize or sorghum. This probably permits early formation of sclerotia on these crop residues. Wyllie and Calvert (1969) showed flowering and pod formation to be the conditioning factors for sclerotia formation. This may explain the observed tendency of sclerotial propagules to increase in the soil after harvesting, especially the susceptible legumes.

It was interesting to observe that the plots monocropped with legumes resulted in significantly ($P = 0.05$) more disease incidence than those monocropped with cereals. This was unexpected since maize and sorghum have more dry matter and provide more organic substrate for the pathogen's multiplication. It could be that due to their late maturity *M. phaseolina* is not able to colonize the maize and sorghum crop debris effectively due to the very dry conditions usually experienced at the end of the season. In addition the higher organic matter content from the crop debris in these plots may encourage other competitive micro-organisms like bacteria and actinomycetes which reduce colonization by *M. phaseolina* when soil moisture conditions improve (Ghaffar et al., 1969; Dhingra et al., 1976). Inoculum levels were increased by common bean, cowpea, maize and sorghum in that order. Francl et al. (1988) also reported that soybean-sorghum rotations were slightly better than soybean-corn rotations in lowering *M. phaseolina* in the soil. These results suggest that crop rotation among hosts of *M. phaseolina* may still be a good charcoal rot management option. Work on longer effects of crop species and population dynamics of the associated micro-organisms in the soil is required.

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Survival of *Macrophomina phaseolina* in bean seed and crop residue

(*Keywords: Macrophomina phaseolina*, charcoal rot, beans, seed infection, crop residue)

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Abstract. The survival of *Macrophomina phaseolina* in bean seed and crop residue was investigated. Studies on seed infection and survival of the pathogen were conducted in the laboratory. Sterilization of seed or storage for 6 months decreased the number of infected seeds by up to 50%. Seeds from plants with no disease symptoms were 2.5% infected while seeds from severely infected plants had up to 13.5% infection. Survival of the pathogen in crop residue was investigated in field soil in eastern Kenya. Up to 30% of sclerotia were found to be viable after 21 months in field soil. Soil moisture and temperature did not influence survival up to a depth of 20 cm.

1. Introduction

The most serious disease of common bean (*Phaseolus vulgaris* L.) in semi-arid eastern Kenya is charcoal rot (Songa and Hillocks, 1996a,b) caused by *Macrophomina phaseolina* (Tassi) Goid. Yield loss to charcoal rot has been estimated to be up to 300 kg/ha (Wortmann and Allen, 1994). The incidence of charcoal rot disease has increased in recent years because of increasing cultivation of marginal land due to population pressure (Songa and Hillocks, 1996b). Seedling mortality of 10–14% is common in experimental plots and farmers' fields (Songa and Rono, 1995). *M. phaseolina* is a soil-inhabiting fungus that grows poorly in soil and survives as sclerotia which provide the initial inoculum (Smith, 1969; Watanabe *et al.*, 1970). This pathogen is also seedborne (Andrus, 1938; Gangopadhyay *et al.*, 1970; Abawi and Pastor-Corrales, 1990). Since the level of seed infection depends on severity of plant infection, selection of seed from symptomless plants is expected to reduce the initial infection. Infected seeds are distorted, blemished and carry sclerotia and pycnidia of the pathogen. Five percent reduction in seed weight and as much as 60% reduction in emergence from diseased seed has been reported in susceptible varieties (Gangopadhyay *et al.*, 1970). Andrus (1938) found natural seed transmission of *M. phaseolina* in Henderson Lima bean seed to be approximately 85% in unsterilized seed and 57% in surface-sterilized seed, indicating that the fungus is found not only on the surface but also beneath the seed coat. Gangopadhyay *et al.* (1970) showed that 13–24% of soybean seed from infected plants harbour the fungus. Artificial infection of bean seed has been demonstrated and a higher percentage of seedling mortality obtained from such pre-infected seed than healthy seed planted in heavily infested soil (Andrus, 1938). The importance of cotyledons in primary infections by *M. phaseolina* was emphasized by Kendrick (1933) and Tompkins and Gardner (1935). With the pathogen established in the cotyledon, it is not important whether the infection of the seed comes after it is planted or before harvest, since it is likely that the infection of seed in the pod may occur through contact with infested soil. Severe infection early in the season results in more seed infection due to high inoculum levels on the plant and soil. Conditions favouring early infection, such as warm temperatures or low soil moisture, will encourage systemic seed infection, whereas moist conditions towards the end of the growing season would be expected to encourage pod infection. This natural seed infection may account for serious outbreaks of the disease occasionally observed in farmers' fields. The majority of small-scale farmers who produce common bean use their own seed from the previous crop for cultivation in the next season (Rono and Shakoor, 1990). The use of clean disease-free seed would reduce the initital inoculum and lower the severity of the disease. It would, therefore, be worthwhile to determine the extent of infection of seed from plants with varying disease seventy, the effect of storage on the viability of sclerotia and other sources of inoculum carry over.

Sclerotia are released in the soil by decaying stems and roots of infested plants. They are resistant to extremes of temperature and humidity (Dhingra and Sinclair, 1975). Soil temperature and moisture are important factors determining the saprophytic activity of *M. phaseolina* in soil (Dhingra and Chagas, 1981). High temperature enhances the saprophytic activity of *M. phaseolina* through reduced competition from other soil micro-organisms and growth in natural soils is reduced with increasing moisture (Norton, 1953). The reduction in activity at increased moisture levels has been attributed to increased bacterial activity which causes lysis of mycelium and inhibition of sclerotia germination (Bhattacharya and Samaddar, 1976). High soil moisture also reduces the survival of *M. phaseolina* sclerotia in soil (Dhingra and Sinclair, 1975). Cook *et al.* (1973) reported that *M. phaseolina* survived as sclerotia in corn and sorghum stalk residue for 18 and 16 months, respectively in Nebraska. In soybean-infested root and stem segments the population of sclerotia was found to be as high or higher after 2 years in Missouri (Short *et al.*, 1980). Survival of *M. phaseolina* sclerotia associated with bean residue has not been determined.

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In order, therefore, to develop effective control measures for charcoal rot disease based on cultural control practices, it is essential to determine the role of seed and crop debris in the survival of the pathogen. In fulfilment of this requirement, this study had the following objectives:

1. To determine the extent of common bean seed infection in relation to severity of plant infection.
2. To determine the extent of internal infection and surface infestation of seed.
3. To determine the effect of storage on viability of sclerotia.
4. To determine the survival of *M. phaseolina* sclerotia in bean residue at various depths.

2. Materials and methods

2.1. *Seed as source of inoculum*

Common bean genotype GLP 1004 was planted in a charcoal rot 'hot spot' at Kiboko in eastern Kenya. The trial was maintained following recommended production practices including fertilizer application, insect control and weeding. At harvest, the plants were harvested in three categories depending on the level of infection. The categories were:

(1) plants with no ashy stem symptoms,
(2) plants with ashy stem symptoms but pods without symtoms, and
(3) plants with ashy stem symptoms and pods bearing *M. phaseolina* sclerotia.

Seeds from each category of plants were tested for the presence of the pathogen without surface sterilization and after surface sterilization as described below. To test the effect of storage on the pathogen, enough seed was reserved and kept in Kilner jars at room temperature and the experiment was repeated 6 months later.

2.2. *Non-surface sterilized seed*

Fifty unsterilized seeds from each category were placed on moist filter paper placed on plastic trays previously cleaned and wiped with alcohol and covered. There were four replications in a completely randomized design. The trays were incubated at 33 C with a 12 h dL : 12 h D light regime. The trays were maintained moist by adding distilled water when they became dry. Observation for the presence of *M. phaseolina* was done using a binocular microscope from 3 to 7 days after incubation at 33 C. The number of seeds that produced colonies of *M. phaseolina* on the seed coat, characterized by numerous black sclerotia, were noted. Seeds with other fungal growth were transferred to PDA for identification. The experiment was repeated once.

2.3. *Surface sterilized seed*

The procedure here was as described above for unsterilized seed and the only difference was that the seed was surface sterilized by immersing in 1% NaOCl (10% dilution of commercially available solution diluted with distilled water) for 5–8 min and thoroughly rinsed in distilled water (Mihail and Alcorn, 1982). One percent NaOCl kills *M. phaseolina* mycelia that may be on the seeds and reduces significantly the viability of sclerotia (Papavizas and Klag, 1975).

2.4. *Survival in bean crop debris in field soil at different depths*

An experiment to determine the longevity of *M. phaseolina* in bean debris was conducted at Kiboko research station in eastern Kenya where charcoal rot disease is prevalent. The soil is a well drained sandy clay loam overlaying a sandy clay (table 1). About 500 g of bean debris (root and stem segments measuring 6–8 cm), heavily infested with sclerotia of *M. phaseolina* were placed in nylon net bags and buried in the soil at four depths (0–5, 5–10, 10–15, 15–20 cm). There were eight samples buried for each depth in three replications. A labelled wire peg was inserted at each point where a sample was buried to facilitate retrieval. The experimental design was a randomized complete block. The field plot was kept fallow and was maintained to keep the labelled wire pegs visible. Soil moisture and temperature measurements were taken as described below. The experiment commenced in April 1993 and ended in December 1994, covering a total period of 21 months.

2.5. *Determination of sclerotial viability*

The sclerotia viability at the beginning of the experiment was determined by plating 100 sclerotia from infected crop debris on selective medium PDA-DOPCNP (basal medium containing *p*-(dimethylamino) benzenediazo sodium sulphonate [DASS, Dexon], oxgall, and pentachloronitrobenzene [PCNB] at 50, 2,000 and 100 mg/l respectively) (Papavizas and Klag, 1975). The debris retrieved from the soil was air dried and ground using a coffee grinder. The debris was transferred to 177 μm and 44 μm sieves in tandem and wet sieved using distilled water. The contents of the 44 μm sieve were transferred to a beaker containing 0.5% NaOCl for 5–8 min and the contents of the 177 μm sieve were discarded. The contents of the beaker were transferred back to the 44 μm sieve and rinsed thoroughly with distilled water. The contents of the sieve containing sclerotia were carefully tansferred to a filter funnel fitted with filter paper using a wash bottle with distilled water. The sclerotia on the filter paper were picked out using a sterilized pin under a binocular microscope and plated on the selective medium. Observations for *M. phaseolina* colonies were made after 6 days' incubation at 32 C. Percentage germination was taken

Table 1. Percentage of sand, silt, and clay particles in different layers of the soil profile at Kiboko, Kenya (Kenya Soil Survey, unpublished data)

Depth (cm)	Sand	Silt	Clay
0–19	74	5	21
19–35	70	5	25
35–72	54	5	41
72–110	54	7	39

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SCREENING COMMON BEAN ACCESSIONS FOR RESISTANCE TO CHARCOAL ROT (*MACROPHOMINA PHASEOLINA*) IN EASTERN KENYA

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SUMMARY

Common bean (*Phaseolus vulgaris* L.) germplasm was screened for resistance to charcoal rot (*Macrophomina phaseolina*) under field conditions at Kiboko and Katumani, eastern Kenya. Of the 313 bean accessions evaluated, 50 lines were resistant and six were tolerant to *M. phaseolina*, the charcaol rot incidence was less than 25% and between 25% and 50% for the resistant and tolerant lines respectively. Yields ranged from 135 to 1051 kg ha^{-1} compared with 55 kg for the susceptible control A464. Time to maturity did not seem to influence or affect the susceptibility or resistance to *M. phaseolina* of the various bean accessions.

INTRODUCTION

Charcoal rot of common bean (*Phaseolus vulgaris*) is caused by *Macrophomina phaseolina* (Tassi) Goid. The pathogen is widely distributed in the tropics and has a wide host range (Cottingham, 1981). The fungus can cause disease in seedlings and mature legume plants and it is responsible for stalk and root rots in cereal crops. In hot areas prone to moisture stress the fungus causes economic losses (Dhingra and Chagas, 1981). Increasing population pressure has led to the cultivation of food crops in the eastern parts of Kenya previously regarded as unsuitable for crop production due to the unreliable rainfall (Gitu and Ngalyukia, 1989; Songa and Ronno, 1995; Songa and Hillocks, 1996). These areas experience severe charcoal rot incidence and sustainable management of *M. phaseolina* is vital if bean production is to increase in the region.

An integrated approach to the management of diseases and other pests is

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preferred because it relies on several methods for control. Host plant resistance, where available, usually assumes a central position in the integrated disease management approach. This is because it is easily adopted, requires few inputs and is therefore economically advantageous. Common bean is reported to have resistance to *M. phaseolina* (Pastor-Coralles and Abawi, 1988; Echavez-Bedel and Beaver, 1986; 1987). Pastor-Corrales and Abawi (1988) developed an international charcoal rot bean nursery of 40 accessions based on greenhouse and field data. However, resistance to disease may vary depending on the environment and the cultivar (Beebe and Pastor-Corrales, 1991). For this reason it is important for each country to screen germplasm and develop resistant varieties which are adapted to the environmental and climatic conditions that prevail within a given country. Screening for resistance to *M. phaseolina* was done at charcoal rot 'hot spots' at Kiboko and Katumani in eastern Kenya (Songa *et al.*, 1992; Songa and Hillocks, 1996). In addition, early maturing (drought escaping) genotypes were compared with late maturing genotypes for resistance to *M. phaseolina* under field conditions. The objectives of the work in this paper were therefore:

1. To screen common bean germplasm for resistance to *M. phaseolina.*
2. To develop a common bean charcoal rot nursery for use in bean improvement programmes in the semi-arid areas of eastern Kenya and other areas of eastern Africa.
3. To test the hypothesis that a drought escape mechanism may confer resistance to *M. phaseolina.*

MATERIALS AND METHODS

Screening common bean germplasm for resistance

Screening common bean germplasm for resistance to *M. phaseolina* was conducted at KARI centres, Kiboko (960 m asl) and Katumani (1575 m asl) in eastern Kenya. The inoculum used for field inoculation consisted of a mixture of isolates of *M. phaseolina* from eastern Kenya. It was prepared as follows: whole grain rice seeds in beakers covered with aluminium foil were sterilized by autoclaving with water in a one-to-one ratio (1 g rice seeds to 1 ml water) at 121 °C for 15 minutes and then cooled. A 0.8-cm disc, cut from a seven-day-old culture of *M. phaseolina* on potato dextrose agar (PDA) was transferred into each beaker containing rice seeds and incubated at 30–32 °C for 15 days. The fungus colonized the rice seeds within 14 days. The colonized rice seeds were used for inoculation at planting by placing three rice seeds per bean seed before covering with soil. This rate of inoculation was found suitable in preliminary experiments in this study (Songa, 1995).

A total of 313 common bean accessions consisting of local, improved and introduced germplasm were evaluated for their reaction to *M. phaseolina.* They were evaluated in batches over three seasons. Eighty, 101 and 162 accessions were evaluated during the long rains, March–July 1993, the short rains, October 1993

to January 1994, and the long rains, March–July 1994, respectively. Of the 313 accessions evaluated initially, 30 accessions were evaluated a second time. The susceptible line A 464 from CIAT was used as a control in each of the evaluation trials. Each accession was planted in two rows, each 2 m in length per replication. Each row was planted with 30 seeds. One of the two rows for each accession was inoculated and the other was left as a control. Inoculation was with *M. phaseolina*-colonized rice seeds as described earlier. The experimental design was a randomized complete block with three replications. The parameters measured were plant stand count at 14 days after emergence, number of infected seedlings, number of infected plants at harvest, total number of plants at harvest, grain yield (kg ha^{-1}) and percentage charcoal rot incidence. The accessions that performed at least three times better than the susceptible control, A 464, with respect to the number of infected plants at harvest, yield and good agronomic characteristics, were selected to constitute the charcoal rot nursery. Lines with good adaptability but marginal performance in the above mentioned parameters were re-evaluated.

Drought escape and resistance to M. phaseolina

Reactions of early- and late-maturing accessions to *M. phaseolina* were compared to determine if time to maturity influenced resistance to the pathogen. Field experiments were conducted at the KARI centres, Kiboko and Katumani. Ten early- and ten late-maturing accessions were used in the study and the early- and late-maturing lines reached 50% flowering between 25–37 and 38–45 days after emergence respectively. Each accession was planted in two rows (30 seeds per row) of which one was inoculated using rice seed colonized by the pathogen and the other left as the control. The early and late maturing accessions were planted alternately in a randomized complete block design in three replications. The susceptible line A 464, which is early maturing, was included as a control. The parameters measured were number of emerged plants, disease incidence at 14 days after emergence, number of days to 50% flowering, disease incidence and number of plants at harvest. Due to the difference in maturity the parameters taken at harvest had a time difference of ten days.

RESULTS

Screening common bean germplasm for resistance to M. phaseolina

The 313 accessions evaluated varied in their reactions to *M. phaseolina* at both the Kiboko and the Katumani screening sites. The disease incidence was usually higher at Kiboko than at Katumani (Table 1). Significant differences in emergence count, number of infected plants at harvest, stand count at harvest and seed yield were observed between accessions (Table 1 and 2). Charcoal rot incidence (%) and seed yield (kg ha^{-1}) were the most indicative of the overall response of common bean accessions to *M. phaseolina* (Table 2) Some accessions, for example V 8025 and BAT 1651, appeared susceptible at the end of the season, but the seed yield was still satisfactory. No significant difference in charcoal rot

Table 1. *Number of emerged plants and number of plants infected with* Macrophomina phaseolina *at harvest for twenty-four accessions of common bean flowering between thirty and thirty-five days and evaluated at Katumani and Kiboko during the long rains in 1994*†

Accession	Introduction	Origin	Katumani		Kiboko	
			Number of emerged plants	Number infected at harvest	Number of emerged plants	Number infected at harvest
GLP 819	20–2–3–35	Kenya	17.8	1.8	20.3	11.3
GLP 1032	Extender	USA	13.8	1.0	20.5	9.5
GLP 29	Porillo-1-H	Unknown	18.5	1.8	19.6	8.6
GLP 41	Diacol Nima × Verdon	Uganda	14.8	1.1	18.1	8.5
GLP 987	Panaminto Corriente	Unknown	16.0	0.3	21.3	8.1
GLP 990	Turrialba 1	USA	15.8	0.0	20.1	8.1
GLP 65	Dark Red Kidney	USDA	20.1	2.1	23.6	8.0
GLP 1102	Veracruz 1-A-6	Colombia	17.5	1.0	22.1	8.0
GLP 425	75-N2B-2829-5-1BK	Puerto Rico	19.5	0.8	20.1	8.0
K9/38B	–	Kenya	16.1	0.8	19.0	7.8
GLP 938	FF00012-3-1MF5	Colombia	17.6	0.8	18.5	7.6
GLP 1044	Lazy Housewife	Seychelles	17.6	1.8	22.5	7.6
GLP 988	San Pedro Pinda	Unknown	18.5	2.3	21.1	7.5
K23/45A	Unknown	Kenya	15.6	0.1	20.1	7.5
GLP 973	Mountainer White	Unknown	19.8	0.0	23.1	7.3
GLP 351	Red Haricot	Kenya	17.1	1.1	24.5	7.3
K13/26B	Unknown	Kenya	17.6	0.0	20.3	7.3
43338	Unknown	Kenya	19.6	2.8	21.6	7.3
GLP 1004	Mwezi Moja	Kenya	16.5	2.0	21.0	7.3
GLP 294	Rose Coco	Kenya	12.1	0.6	20.5	7.1
GLP 1121	PI 203937	Colombia	19.5	1.5	19.8	7.0
A464	G4004 × A27	CIAT	18.4	5.0	21.2	11.5
Mean			16.99	0.87	18.7	5.14
s.e.			0.16	0.05	0.16	0.11

†Thirty seeds were sown per row and the numbers given as emerged and infected are averages of the replications of the inoculated rows.

incidence within accessions was observed between inoculated and uninoculated plots. The susceptible control, A 464, was also useful as an indicator of the uniformity of inoculum in the field . Based on the field data collected, a nursery of 54 accessions (Table 2) is now to be tested against infection by *M. phaseolina* in different bean growing areas of eastern Kenya.

Drought escape in relation to resistance

Early maturing accessions had significantly more seedling infection and more infected plants at harvest than the late maturing accessions. The number of seedlings that emerged and the number of plants at harvest were not significantly different for the two maturity groups (Table 3.). The early maturing accessions were observed to have more infection and wilting than the late maturing accessions at both the seedling and post-flowering stages at Kiboko (Table 3.). However, at Katumani the late-maturing accessions GLP 65, GLP 927, 43309,

Table 2. *Charcoal rot incidence (%) and seed weight (kg ha^{-1}) of resistant and tolerant bean accessions evaluated for their reaction to* Macrophomina phaseolina *at Kiboko, Kenya*

Accession	Introduction	Origin	Incidence of charcoal rot		Seed weight
		Sources of known resistance			
BAT 1289	Latin America	CIAT	28.1	Tolerant	248.0
BAT 1385	Latin America	CIAT	15.6	Resistant	285.0
BAT 1669	Latin America	CIAT	42.3	Tolerant	335.2
BAT 1400	Latin America	CIAT	19.0	Resistant	252.2
BAT 1293	Latin America	CIAT	14.7	Resistant	211.5
BAT 1651	Latin America	CIAT	26.4	Tolerant	535.2
BAT 1297	Latin America	CIAT	17.2	Resistant	135.2
BAT 125	Latin America	CIAT	31.6	Tolerant	350.7
V 8025	Latin America	CIAT	46.6	Tolerant	300.5
V 8017	Latin America	CIAT	21.6	Resistant	519.5
V 8010	Latin America	CIAT	9.2	Resistant	376.7
EMP. 86	Latin America	CIAT	13.6	Resistant	332.5
G 5059	Latin America	CIAT	13.2	Resistant	1050.2
A 55	Latin America	CIAT	14.5	Resistant	434.2
CG 82-69	Latin America	CIAT	22.0	Resistant	413.7
CG 82-79	Latin America	CIAT	19.2	Resistant	385.2
		Newly identified sources of resistance			
GLP 914	FF000262-M(2)F4	Colombia	6.0	Resistant	729.5
GLP 808	Tendergreen	USA	14.0	Resistant	825.7
GLP 274	Rose Coco	Kenya	3.4	Resistant	405.2
GLP 409	P566-A	Colombia	8.3	Resistant	411.2
GLP 927	FF000241F4	Colombia	15.8	Resistant	417.7
GLP 184	22/1/1/3/5	Kenya	3.4	Resistant	709.0
GLP 211	P-11-D-L	Kenya	20.2	Resistant	402.5
GLP 805	K78(ex Kawanda)	Kenya	30.0	Tolerant	403.0
GLP 967	SR/72/LKY58	Kenya	13.3	Resistant	454.2
GLP 1092	California Small White	Colombia	7.2	Resistant	733.2
GLP 924	FF00028-6MF4	Colombia	0.0	Resistant	995.5
GLP 642	Field 18-P47 Holetta	Colombia	10.9	Resistant	624.5
GLP 270	Bura Yellow	Kenya	11.7	Resistant	664.5
GLP 240	Canadian Wonder	Kenya	0.0	Resistant	600.5
GLP 584	Royal Red	USA	5.4	Resistant	259.2
GLP 1206	Red Mex. U135	Netherlands	10.9	Resistant	302.0
GLP 1106	Ganajuato 10-A-5	Colombia	1.9	Resistant	775.2
GLP 1102	Veracruz-1-A-6	Colombia	18.7	Resistant	616.5
		Newly identified sources of resistance			
E 10	–	Kenya	3.4	Resistant	637.0
K13/26B	–	Kenya	0.0	Resistant	659.2
K9/38B	–	Kenya	6.5	Resistant	752.2
43335	–	Kenya	16.6	Resistant	578.0
43308	–	Kenya	5.8	Resistant	862.2
N 17	–	Kenya	7.5	Resistant	618.7
N 47	–	Kenya	13.5	Resistant	1051.2
N 43	–	Kenya	3.3	Resistant	164.5
N 26	Canadian Wonder	Kenya	8.9	Resistant	774.7
N 38	–	Kenya	1.6	Resistant	999.0
N 36	–	Kenya	13.0	Resistant	946.2

Table 2 (continued). *Charcoal rot incidence (%) and seed weight (kg ha^{-1}) of resistant and tolerant bean accessions evaluated for their reaction to* Macrophomina phaseolina *at Kiboko, Kenya*

Accession	Introduction	Origin	Incidence of charcoal rot		Seed weight
N 45		Kenya	6.0	Resistant	743.7
N 18	–	Kenya	8.6	Resistant	618.7
N 6	–	Kenya	6.0	Resistant	896.7
N 11	–	Kenya	13.3	Resistant	781.5
MWEZI MOJA	Katumani Local	Kenya	25.5	Resistant	554.0
NYAYO	Rose Coco	Uganda	7.3	Resistant	958.5
KATX-16	KatB9 × M.Moja	Kenya	11.7	Resistant	442.7
KATUMBUKA	Pinto	Kenya	9.4	Resistant	1001.5
Kat B2	Makueni	Kenya	18.2	Resistant	378.0
		Known susceptible controls			
A 464	–	CIAT	35.7	Susceptible	55.0
A70	–	CIAT	39.2	Tolerant	275.0

43316, 43335 and GLP 1130 were found to be relatively more susceptible (Table 4.). The average numbers of days to 50% flowering in the early- and late-maturing accessions were 33.3 and 40.1 respectively at Kiboko and the average temperatures at the time of flowering were 25.5 °C and 23.5 °C for the early- and late-maturing accessions respectively (Fig. 1). No significant difference was observed between inoculated and uninoculated rows for any of the parameters measured.

DISCUSSION

Several bean accessions were found to be resistant to *M. phaseolina* and the field evaluation procedure was effective in separating bean accessions into two general groups according to their reactions, namely, promising material for further evaluation and highly susceptible accessions. The absence of significant differences between the inoculated and uninoculated plots indicated adequate levels of inoculum in the plots which had been used to grow beans for several seasons. This was confirmed by the susceptible control, A 464. The reactions of susceptible cultivars were evident soon after germination. Emerged seedlings of these accessions showed the typical dark sunken lesions on the cotyledons caused by *M. phaseolina* infection. The lesions often expanded and killed the seedlings within 14 days. High levels of seedling infection usually resulted in lower stand counts at harvest (Table 3). These observations agreed with Gangopadhyay *et al.* (1970) who reported severe damping-off of soyabean seedlings with plant losses up to 77%. Most of the accessions with good resistance had low levels of seedling infection and small numbers of plants infected at harvest. However, some accessions, such as BAT 1651, V 8025 and BAT 1669, had about 50% or more of the emerged plants infected at harvest but still had satisfactory seed yield

Table 3. *Reactions of early- and late-maturing bean accessions to infection by* Macrophomina phaseolina *at Kiboko, eastern Kenya*

Accession	Maturity group	Number of seedlings emerged	Number of seedlings infected	Days to 50% flowering	Number of plants at harvest	Number of plants infected at harvest
GLP 1004	early	21.8	6.5	35.8	17.0	6.8
GLP 976	early	24.5	3.8	36.5	18.8	4.1
GLP 967	early	20.6	6.6	35.6	14.0	1.8
A 464	early	24.8	4.3	35.3	17.6	7.0
GLP 1121	early	22.1	5.3	36.8	17.5	2.6
GLP 236	early	18.6	7.6	36.1	16.1	2.0
43309	early	22.0	2.5	36.8	17.5	2.6
GLP 381	early	24.0	5.8	35.3	14.3	4.6
GLP 1092	early	21.0	9.6	36.1	16.0	2.3
GLP 911	early	20.8	3.8	36.5	13.0	5.1
GLP 1186	late	21.6	7.8	39.3	20.3	2.1
K23/45A	late	22.0	4.1	40.5	15.3	3.3
GLP 41	late	20.3	6.6	40.6	14.3	1.8
GLP 988	late	21.1	6.6	37.6	20.1	2.3
GLP 768	late	20.6	5.0	39.6	14.8	2.0
GLP 1130	late	20.1	6.0	40.1	15.8	2.8
43335	late	21.5	3.5	39.8	17.3	3.1
GLP 927	late	21.0	2.3	38.6	18.3	4.0
GLP 65	late	22.5	6.8	42.8	19.6	3.1
N13	late	20.1	3.5	38.5	18.1	1.8
43316	late	23.6	3.0	43.0	16.1	4.6
Mean		21.68	4.43	38.45	16.7	9.7
s.e.		0.33	0.27	0.26	0.39	0.27

(Table 2). In these accessions the fungus was evident only on the plant stems in advanced senescence and the lines were still selected for possible high tolerance to the pathogen. Late infections were more obvious after flowering during periods of high temperature and moisture stress especially at Kiboko. This observation supported the severe infections of charcoal rot reported at high temperatures during periods of moisture stress (CIAT, 1983; Dhingra and Sinclair, 1977). Infected plants were chlorotic and less vigorous in growth. It was encouraging to find that some of the improved accessions for semi-arid areas, such as GLP 1206, GLP 1092, GLP 914, E 10, N 36, N 11 and N 43, performed well. This was particularly so for accessions from the University of Nairobi bean programme that had also been found resistant to several foliar diseases (A. W. Mwang'ombe, University of Nairobi, personal communication).

With a few exceptions the early-maturing lines were significantly more infected at both the seedling stage and at harvest (Table 3). Early-maturing GLP 967 and late-maturing GLP 41 appeared to be susceptible at the seedling stage but were resistant at the mature plant stage. Both had 6.0–6.6 seedlings infected but only 1.8 plants infected at harvest. This observation suggested different resistance mechanisms at the two plant stages. Some late-maturing

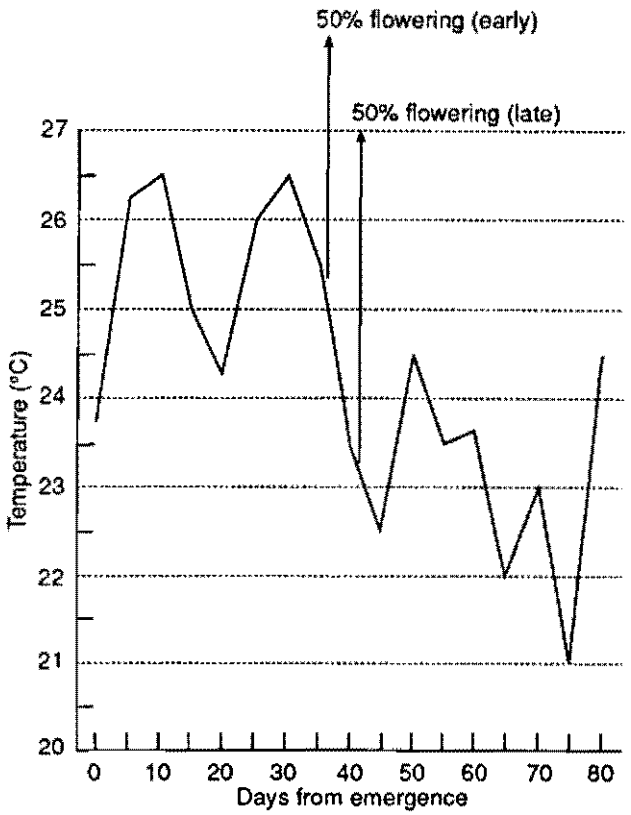

Fig. 1. Temperature at time of flowering of the early- and late-maturing accessions at Kiboko during the short rains in 1994.

lines, 43316 and K23/45A had a high incidence of seedling infection and also large numbers of infected plants at harvest. Since drought-tolerant accessions may be late maturing, these observations did not lend much support to the view of Pastor-Corrales and Abawi (1988) who found that drought-tolerant cultivars were also highly resistant to *M. phaseolina*.

The reactions of the early- and late-maturing accessions to *M. phaseolina* at Katumani were generally similar to those observed at Kiboko but much less severe (Table 4). Some accessions for example GLP 65, 43316, GLP 1130, 43335, and 43309 had almost equal numbers of infected plants at harvest at both sites. These accessions were all of late maturity and were the most susceptible at Katumani. It appeared therefore, that early maturing accessions escaped infection at Katumani due to less favourable conditions for the pathogen early in the season.

At Katumani flowering occurred between one and eight days later than at Kiboko depending on the accession. Delayed flowering at Katumani was particularly obvious for the accessions GLP 768, GLP 1130, K23/45A, N 13 and

Table 4. *Reactions of early- and late-maturing bean accessions to* Macrophomina phaseolina *at Kiboko and Katumani*

		Kiboko			Katumani		
Accession	Maturity group	Days to 50% flowering	Number of plants at harvest	Number of infected plants at harvest	Days to 50% flowering	Number of plants at harvest	Number of infected plants at harvest
GLP 1004	early	37.8	17.0	6.8	36.0	19.7	0.2
GLP 65	late	42.8	19.6	3.1	44.0	22.6	2.1
GLP 976	early	36.6	18.8	4.1	36.0	22.6	0.1
GLP 1186	late	39.3	20.3	2.1	41.2	18.8	1.8
GLP 927	late†	38.6	18.3	4.0	37.0	19.5	2.2
43316	late	43.0	16.1	4.6	44.2	24.0	4.1
GLP 236	early	36.1	16.1	2.0	35.7	14.5	0.0
GLP 768	late	39.6	14.8	2.1	43.2	19.5	0.2
GLP 381	early	35.3	14.3	4.6	36.7	21.5	0.0
GLP 1130	late	40.1	15.8	2.8	43.0	18.1	2.4
GLP 911	early	36.5	13.0	5.1	37.2	17.5	1.1
43335	late	39.8	17.3	3.1	43.6	25.0	3.1
GLP 967	early	35.6	14.0	1.8	36.5	18.0	0.1
K23/45A	late	40.5	15.8	2.8	43.2	17.0	0.1
GLP 1092	early	37.1	16.0	2.3	36.5	18.0	0.1
GLP 41	late	40.6	14.3	1.8	41.2	18.8	0.0
GLP 1121	early	36.1	17.5	2.6	44.8	17.2	2.6
N 13	late	37.6	20.1	2.3	39.0	14.6	0.1
GLP 988	late	37.6	20.1	2.3	39.0	14.6	0.1
A 464	early	35.3	17.6	7.0	35.2	14.6	1.8
43309	early‡	36.8	17.5	2.6	44.8	17.2	2.6
Mean		38.4	16.7	9.7	39.66	19.27	1.26
s.e.		0.26	0.39	0.27	0.32	0.41	0.15

†GLP 927 is late at Kiboko but early at Katumani, ‡43309 is early at Kiboko but late at Katumani.

43309 which took 43.2, 43.0, 43.2, 43.0 and 44.8, and 39.6, 40.1, 40.5, 38.5 and 36.8 from emergence to flowering at Katumani and Kiboko respectively (Table 4). These observations were expected since Katumani is higher in altitude (1575 m asl) and the mean temperature is lower than at Kiboko (960 m asl). The lower temperatures at Katumani may have been responsible for the lower incidence and severity of the disease at this site. Sclerotia formation has been linked mainly with flowering (Wyllie and Calvert, 1969). The early-flowering accessions would probably be earlier in displaying susceptibility and at a time when the temperature was still relatively high and more conducive to the pathogen. The general trend for temperature was to decrease gradually from the beginning to the end of the season before increasing again at the beginning of the next season as indicated in Fig. 1.

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as an index of viability. During subsequent samplings, a minimum of 30 sclerotia from each sample retrieved every 3 months were plated on the selective medium and observed for germination.

2.6. Measurement of soil moisture

A single aluminium access tube with an internal diameter of 50 mm was installed in the middle of each plot replicate to a depth of 40 cm at the time the infected crop debris was buried. Using a soil moisture neutron probe (Didcot Instruments, Abingdon, UK) readings were taken at 0–5, 5–10, 10–15 and 15–20 cm twice a month during the first and last week of each month for the duration of the experiment. The following calibration equation was obtained from field measurements at 10 cm depth:

$$\theta = 3.34 + 55.8f \ (R^2 = 0.49)$$

where θ is the percentage volumetric mositure content and f is the neutron probe count expressed as a fraction of the count in water (Bell, 1976). Six and eight soil samples were used to determine bulk density and gravimetric soil water content respectively. The volumetric water content was then calculated from average bulk density and gravimetric water content.

2.7. Measurement of soil temperature

Soil temperature was measured at the four depths at which the infested crop debris were buried in the plots. The measurements were taken daily at 3-h intervals from 06.00 h to 18.00 h using portable soil thermometers (EL504-024) in rugged brass cases with stems graduated at 5, 10, 15, 20, 30 cm to enable measurement at various depths. The temperature was not taken in each replicate but randomly within the plot.

2.8. Data analysis

ANOVA was used to compare the seed infection and infestation of surface sterilized and unsterilized seed. The Least Significant Difference (LSD) test was used to separate group means where ANOVA indicated significant difference ($P = 0.05$). ANOVA was also used to compare sclerotial survival at different depths in the soil.

3. Results and discussion

Seeds that produced colonies of *M. phaseolina* were soon covered with black sclerotia and were easily identified. ANOVA showed significant difference ($F = 38.21$; df = 2,36; $P = 0.05$) in seed infection and infestation between surface sterilized and unsterilized seed of the three categories of seed. LSD test for means comparison showed no significant difference ($P = 0.05$) between surface sterilized and unsterilized seed of category one, in the number of seeds that produced *M. phaseolina* at harvest and 6 months later (table 2). However, there was a significant difference ($P = 0.05$) between sterilized and unsterilized seed of category two and three in the number of seeds infected with *M. phaseolina* at harvest. Category three had a significantly higher number of seeds infected compared with categories one and two for unsterilized seed (table 2). After 6 months of storage there was no significant difference ($P = 0.05$) between category two and three in the number of seeds infected with the pathogen and for the unsterilized seed, this was significantly ($P = 0.05$) lower than at harvest. Both categories two and three seed had significantly higher infected seed than category one, 6 months after harvest. Other fungi found to be associated with the infected and infested bean seeds were *Aspergillus niger* van Tiegh., *Aspergillus flavipes* (Bainier & Sartory) Thom & Church., and *Penicillium variable* Sopp.

The results indicate that by selecting clean healthy looking plants for seed, the initial inoculum due to seed infection can be

Table 2. Common bean seed infection and infestation at harvest and after 6 months storage

Category of seed[a]	At harvest Treatment		Six months after harvest Treatment	
	Surface sterilized	Unsterilized	Surface sterilized	Unsterilized
One	5.0[b]	8.5	2.0	4.8
Two	8.0	19.3	7.5	11.3
Three	10.5	27.5	6.3	13.0
$LSD_{(0.05)}$	4.55			

[a]Category one: plants with no ashy stem symptoms and no symptoms on pods; category two: plants with ashy stem symptoms but pods without symptoms; category three: plants with ashy stem symptoms and pods bearing *M. phaseolina* sclerotia.

[b]Mean number of seeds that produced *M. phaseolina* out of 200 tested.

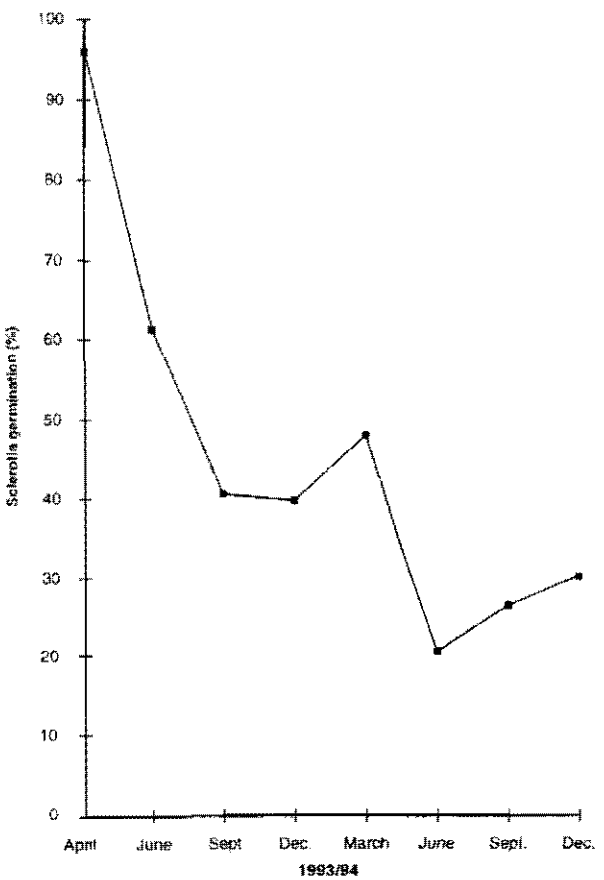

Figure 1. Survival of M. phaseolina *sclerotia over time.*

reduced by more than half. They also suggest that after 6 months of storage there was less infestation by *M. phaseolina* than at harvest. These observations imply a diminished role of mycelia and poor viability of sclerotia after 6 months of storage at room temperature. Unsterilized seed had almost twice or more infected seed than the sterilized seed for all the categories of seed (table 2). Category three seeds, from plants with most severe infection, had 13.0% infected seed when unsterilized, 6.3% when sterilized after 6 months of storage. Our results generally agree with earlier reports on *M. phaseolina* infection in common bean and other legumes (Andrus, 1938; Abawi and Pastor-Corrales, 1986, 1990). However, previous authors did not consider the effect of storage on the viability of the pathogen. Abawi and Pastor-Corrales (1986) observed that seeds obtained from symptomless pods on infected plants showed up to 28% infection and surface disinfestation with 0.6% NaOCl reduced infection to less than 5%. Gangopadhyay *et al.* (1970) found 13–24% of soybean seed harvested from infected plants harboured the fungus. The number of infected seed 6 months after harvest was significantly less than at harvest.

Surface sterilized seed also produced *M. phaseolina* colonies, indicating that the fungus was established beneath the seed coat in about half of the infected seed. In general these results suggest that surface sterilization or storing common bean seed for 6 months before planting was effective in reducing *M. phaseolina* infestation by approximately 50%. Antagonism with other micro-organisms may be another reason for the observed inactivation of inoculum in storage. *Aspergillus* spp. were among

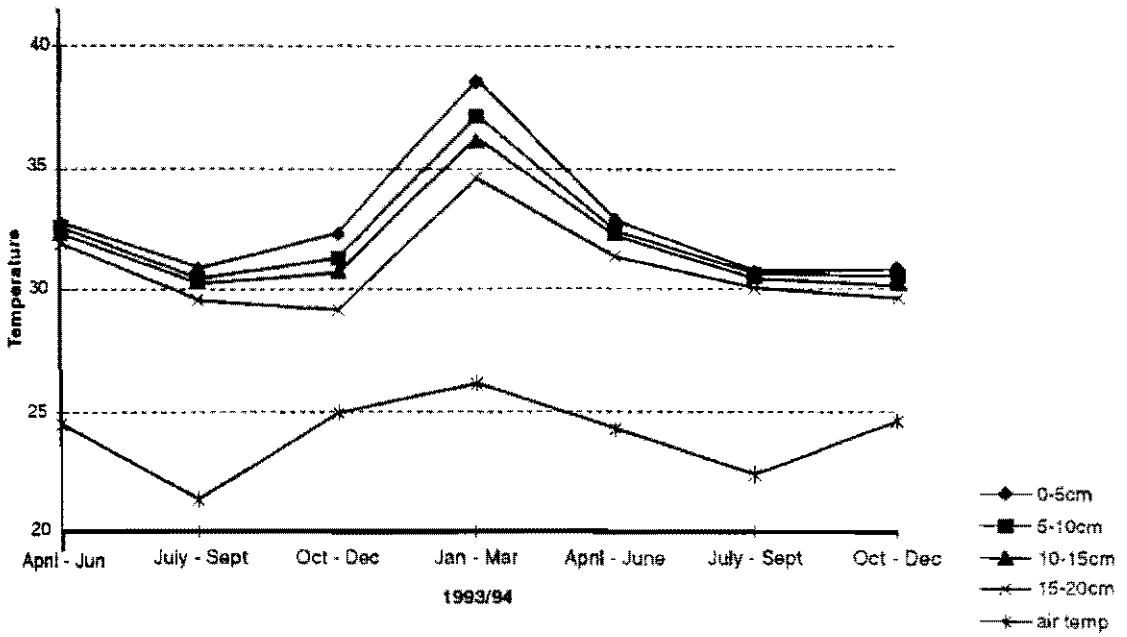

Figure 2. Soil temperature at four depths and ambient temperature.

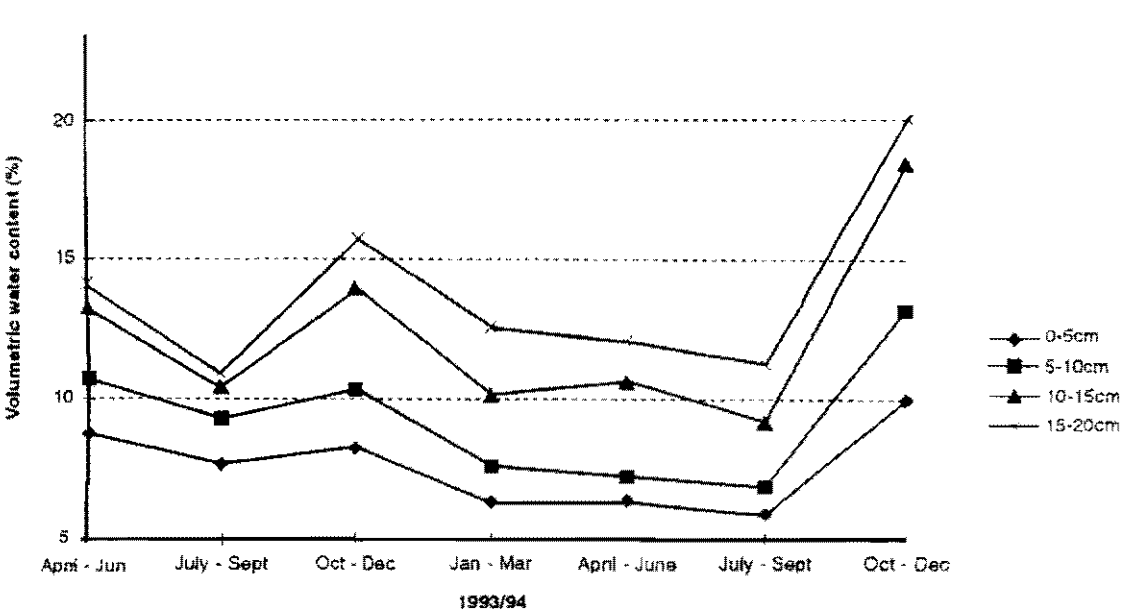

Figure 3. Soil moisture at four depths in the study field.

Table 3. Survival of M. phaseolina *sclerotia in crop debris at four depths in soil*

	Percentage germination						
	Months after burial						
Soil depth (cm)	3	6	9	12	15	18	21
0–5	48.3	48.1	59.5	55.5	35.1	20.0	36.6
5–10	66.6	29.6	39.3	42.2	22.2	22.2	30.0
10–15	67.0	59.2	35.7	46.6	11.1	44.4	23.3
15–20	63.3	25.9	25.0	48.9	13.9	21.1	33.3

$LSD_{(0.05)}$ 25.58.

other fungi isolated from the bean seed and have been reported to reduce *M. phaseolina* infection in peanut kernels (Jackson, 1965). This phenomenon needs further investigation and suggests the potential use of biocontrol agents in seed treatments.

At the beginning of the experiment 96% ($n=100$) sclerotia taken from infected crop debris and plated on the selective medium PDA-DOPCNB germinated. Survival of sclerotia thereafter declined with time, as shown in figure 1. The depth at which the sclerotia were buried did not significantly ($P=0.05$) influence survival (table 3). Soil temperature decreased with depth, as shown in figure 1. The highest average temperature was 38.5 C between 0 and 5 cm during the period January–March 1994 and the lowest was 29.0 C between 15 and 20 cm during the period October–December 1993. Soil moisture increased with depth, as shown in figure 2. The highest soil moisture (20.3% VWC at 15–20 cm depth) was observed during the period October–December 1994 and soil moisture was lowest (5.9% VWC at 0–5 cm depth) during July–September 1994.

The results in this study indicate that up to 30% of *M. phaseolina* sclerotia in bean crop debris were still viable after 21 months in the soil. They suggest that crop rotations with non-hosts or fallow periods of less than 2 years are unlikely to be effective in reducing charcoal rot disease in infested fields. Our study also indicates that sclerotial survival was not influenced by soil moisture, soil temperature or depth of burial in the present study.

The average soil temperature during the day was lowest at 29°C at the 15–20 cm depth and highest at 38°C at 0–5 cm depth (figure 3). These temperatures are within the optimal range for good growth of *M. phaseolina* (Smith, 1969; Dhingra and Sinclair, 1975) and this could explain in part the absence of significant difference in the survival of sclerotia with depth.

Soil moisture was 6% and 20% VWC at the 0–5 and 15–20 cm depths respectively. Satischandra *et al*, (1979) observed that saprophytic activity was highest for *M. phaseolina* at 20% MHC and was reduced four times at 80% MHC. The difference in moisture content at the depths investigated was probably not large enough to influence sclerotia survival. This pathogen does not survive for long under anaerobic conditions (Satischandra *et al.*, 1979) and the moisture effects would be more obvious in poorly drained soils. The soil texture of the study field (table 1) indicates that it is well drained and anaerobic conditions would not easily occur even after very heavy erratic rains that are typical of this region. The insignificance ($P=0.05$) of depth on survival (table 3) suggests that deep ploughing intended to bury crop debris would not control charcoal rot disease. Removal and burning infested crop debris would be a more useful cultural control practice.

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