REACTION OF *Ipomoea batatas* (L) LAM LINES TO ALTERNARIA LEAF AND STEM BLIGHT AND EFFECTS OF SOIL pH ON DISEASE SEVERITY

BY

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B.Sc. (Hons.) Horticulture, JKUAT

A Thesis

Submitted in partial fulfilment for

the Degree of

MASTER OF SCIENCE

IN

PLANT PATHOLOGY

Department of Crop Protection

Faculty of Agriculture

College of Agriculture and Veterinary Sciences

University of Nairobi

2000

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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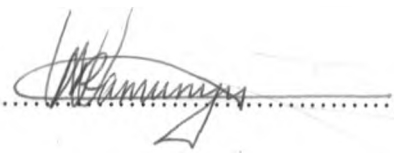

21/11/2000

DR. (MRS) R. NJERU **DATE**

DEDICATION

To my Father, Harrison An'ginya Agili, and my brother, Fredrick Agili, for their love, support, and encouragement of my curiosity.

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LIST OF ABBREVIATION

ANOVA	Analysis of variance
a.s.l.	above sea level
AUDPC-DS	Area under disease progress curve for disease severity
CIP	International Potato Centre
FAO	Food and Agriculture Organization
IMI	International Mycological Institute
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KARI	Kenya Agricultural Research Institute
MEA	Malt Extract Agar
MOA	Ministry of Agriculture
PDA	Potato Dextrose Agar
SPLDM	Sweetpotato leaf decoction media
SPVDM	Sweetpotato vine decoction media
12L/12D	12hrs continuous light alternating with 12hrs continuous darkness
24hrsD	24hrs continuous darkness
24hrsL	24hrs continuous light

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ACKNOWLEDGEMENTS

I wish to acknowledge with sincere thanks, the financial support for this study, which was provided mainly by the International Potato Centre (CIP), and my brother. To this end I am very grateful to Dr. Peter Ewell (CIP, Regional Representative), Dr. E. E. Carey (CIP, Sweetpotato Breeder) and Fredrick Agili (Brother) for the financial assistance for my study. for providing useful guidance, encouragement and criticisms during conduction of the research, interpretation of results and preparation of this thesis, without which this work could not have been what it is now. Many thanks to my lectures, Dr. (Mrs) Mutitu, Prof. (Mrs) Mwang'ombe, Dr. Kimenju, and Prof. Mibey for guiding me through the course work. I am also grateful to the entire staff of the University of Nairobi, department of crop protection, notably, laboratory technicians, Mr. Mathenge, and Gathuma, and staff of CIP, Mr. Ojiambo, Mcharo, Kitonyi, Obaga, Elijah, Alice and Rosemary for the ample assistance they provided that enhanced my research work. I am grateful too to my classmates and friends, Asora, Gunga, Ngatia and Kariuki for the hard time we underwent together while pursuing our studies. Last but not least, I wish to thank my spouse and lovely son John Thomas Ang'inyah for their love and patience while I was away from them. To my bothers, sisters, sisters-in-law, nephews and nieces, I say thank you very much for your support that you gave me during the entire period of the study.

ABSTRACT

This study was carried out to determine the causal agent of blight disease on leaf petiole and stem of sweetpotato in different agro-ecological zones in Kenya, investigate its optimal *in-vitro* growth and sporulation conditions and determine the importance of the pathogen in disease development under different soil pH levels as well as field conditions. A total of 5 different zones were visited. Diseased leaf and vine tissues were collected and pathogen isolated. The Kabete, Kakamega, Busia (Alupe), and Kabondo isolates were identified as *Alternaria bataticola* while the Kisii isolate was identified as *Alternaria alternata*. The Kabete isolate was more virulent than other isolates and other species also.

A. bataticola grew rapidly and sporulated abundantly on host based media; sweetpotato vine decoction media (SPVDM) and sweetpotato leaf decoction media (SPLDM) as opposed to potato dextrose agar (PDA) and malt extract agar (MEA). Significant mycelial growth occurred under alkaline pH ranges {pH 7 (71.67mm), 8 (71.81mm) and 9 (71.90mm)} while abundant sporulation occurred at acidic pH levels with optimal level at pH 5.6 (2.03 x 10^6 conidia/ml) on SPVDM. But, with prolonged incubation period, there was decline in sporulation at all pH levels tested.

12hrs continuous light alternating with 12hrs continuous darkness (12hrsL/12hrsD) sustained the highest sporulation while 24hrs continuous light (24hrsL) enhanced a better mycelial growth and 24hrs continuous darkness (24hrsD) sustained the lowest mycelial

growth and supported less sporulation. *Alternaria bataticola* grew optimally at temperatures between 26°C and 28°C. Yields of conidia obtained from cultures washed by 10ml of water ranged from 1.83 x 10^6 conidia/ml at 28°C to 0.33 x 10^6 conidia/ml at 34°C. The ability of the fungus to sporulate declined at higher temperatures.

Pathogenicity and virulence of different isolates of *A. bataticola* and *A. alternata* from diseased samples were investigated under greenhouse conditions. Within 4 - 6 days following artificial inoculation, and depending on sweetpotato cultivar, the fungus produced characteristic symptoms typical of Alternaria leaf petiole and stem blight. All the isolates were pathogenic and differences in virulence depended on isolate source and conidial concentration. The Kabete isolate was more virulent than Kakamega, Kabondo and Alupe isolates, whose virulence did not differ significantly from each other ($P<0.05$).

The effect of soil pH on disease severity in some cultivars of sweetpotato was also evaluated under greenhouse conditions. There was no significant difference among the levels of soil pH tested on disease severity ($P<0.05$). The mean disease severity for soil pH 4.4, 4.6 and 6.7 were same (3.42) and for soil pH 7.6 was 3.25. There were significant differences in disease severity among the cultivars evaluated.

Alternaria leaf and stem blight caused by *Alternaria bataticola* was monitored in field plots of sweetpotato accessions selected from the CIP germplasm collection field. Disease severity was measured as percent infected area and used to compute area under disease progress curves (AUDPC). AUDPC's revealed distinct differences in disease

infection among the accessions evaluated (p=0.05). Cultivars Viola (440046) and Yanshu 1 (440024) were more susceptible to the pathogen than other entries tested. The lowest disease levels were observed on cultivar Jayalo in both seasons.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Sweetpotato production areas in Kenya

Western region is the main sweetpotato producing area in Kenya, especially the slopes facing Lake Victoria (M.O.A., 1992). This zone produces 77% of the national total production with Nyanza province alone producing 53% and Western 24%. Central province produces 11%, Eastern 9% and Coast 3% (M.O.A., 1991; M.O.A., 1992). Sweetpotato cultivation is concentrated in mid-elevation (1300-1900 m.a.s.l) of densely populated zones, which receive adequate rainfall. They are also found down in drier lowlands along the coast (0-800 m.a.s.l) (Jana, 1982; Abubaker, 1989; Mutuura *et al.,* 1990). There has been a steady increase in the area planted with sweetpotato in Kenya from about 65,000 hectares in 1996 (FAO, 1996) to 75,000 hectares in 1998, with an average yield of 9.8 tons per hectare (FAO, 1998).

1.2 Economic importance of sweetpotato

Sweetpotato is an important food crop of small-scale farmers in several countries of Sub-Saharan Africa (Horton, 1988; Carey, 1996). Along with bananas, beans, maize and cassava, it is a dietary staple in large parts of Uganda, Rwanda, Burundi, Kenya and Eastern Zaire, with high levels of consumption per capita (Carey, 1996). In the grain-based food systems of Eastern and Southern Africa, sweetpotato is a widely grown secondary crop, important for food security at certain times of the year or when other crops fail (Mutuura *et al.,* 1990; Ndolo *et al.,* 1997).

The salient features of sweetpotato and other root crops which favour their role in food security in Africa include wide adaptability throughout the region, relatively better performance in marginal areas and compatibility with many inter-cropping systems, low production costs and continuous yield throughout the year (FAO, 1991). These factors, combined with a mushrooming demand for food, particularly in densely populated rural areas, have provided strong impetus for the increases in production and area planted. Sweetpotato has served extremely well as a famine relief crop in those parts of the continent adversely affected by natural disasters, civil wars, or severe economic hardships (CIP, 1996).

In Kenya, 66% of farmers produce sweetpotato primarily for home consumption and sell surplus (Mutuura *et al.,* 1990). Some farmers in particular areas have specialised in commercial production. In certain districts, like Nyeri, smallholder farmers are increasingly using sweetpotato vines in zero-grazing/intensive milk production systems as a high-quality, protein-rich supplement to fodder grasses (Mutuura *et al.,* 1990; CIP, 1995b). One of the greatest advantages of root and tuber crops particularly in areas where land is scarce, is their high productivity per unit of area and time. Sweetpotato- a short season, fast-growing crop - tops the list in terms of potential dry matter and edible energy per hectare per day (Woolfe, 1992).

1.3 Nutritional composition and uses of sweetpotato.

Sweetpotato has a cornucopia of potential uses. These range from consumption of fresh roots or leaves to processing into animal feed, starch, flour, candy, and alcohol. It is a rich source of carbohydrates, proteins and vitamins. The approximate composition of a fresh sweetpotato root is 50-81% moisture, 8-29% starch, 0.95-2.4% protein, 1.8-6.4% ether extract, 0.5-2.5% reducing sugars, 0.5-7.5% non starch carbohydrates and 0.88-1.38% mineral matter (Onwueme, 1978, Yang 1982, Woolfe, 1992). Predominant minerals in sweetpotato root are potassium, sodium,

iron, phosphorus and calcium. Many sweetpotato cultivars are rich in carotenoids, specially those cultivars with yellow-orange flesh. It is also a good source of ascorbic acid and vitamins of the B complex (Onwueme, 1978, Woolfe, 1992). The tender tips and young leaves of sweetpotato vines are commonly eaten as a vegetable in Asia, West Africa, and in parts of Southern Africa (Villareal et al., 1985). Consumption in this form is rare in Rwanda, Uganda, Burundi and Kenya where root consumption is high.

Roots can be used for animal feed through shredding and then given to cattle, pigs or poultry (Woolfe, 1992). Vines are fed to livestock, particularly in Central Kenya where small-scale dairying is well developed. They can be fed also to livestock as silage (Villareal *et al.,* 1985; Mutuura *et al*., 1990; Semenye *et al.,* 1992; Mok and Carey, 1993).

Sweetpotato can supply starch for industrial uses, especially in textile manufacture and production of alcohol. In recent years, sweetpotato versatility has facilitated major changes in the importance of these various uses around the world in response to shifting supply and demand for food and feed in many developing countries (CIP, 1995b)

1.4 Sweetpotato production constraints in Kenya.

Among the production constraints that currently limit the production of sweetpotato for propagative material as well as roots are damages by various diseases and pests. Pests cause severe damage to the crop and the most notable are sweetpotato weevils of the genus *Cylas* (Purseglove, 1968; Sutherland, 1985; Allard, 1990) with *C. puncticollis* and *C. brunneus* being the two most common species. The degrees of damage caused by these insects may vary markedly with area, species and season (Taleker, 1987; Skoglund and Smit, 1994).

Several diseases have been observed in Kenya ranging from viral, fungal to those caused by nematodes affecting foliage, vines and roots of sweetpotato. The most common viral diseases encountered are the sweetpotato virus disease (SPVD), and sweetpotato feathery mottle virus (SPFMV), (Onwueme, 1978; Wambugu, 1990; Skoglund and Smit, 1994; Carey, 1996). A number of pathogenic fungi cause disease to sweetpotato in Kenya and Eastern Africa in general. Most appear to be widespread with different levels of damage. Some are quite common and others have been observed only in certain geographic areas (Clark, 1987). But with exception of Alternaria leaf petiole and stem blight (*Alternaria* spp.), vine and leaf scab (*Elsinoe batatas*), they have not been observed to cause sufficient damage to the plant to warrant control efforts. Included in this group are: rust (*Coleosporium ipomoeae*), white rust (*Albugo ipomoeae-panduratae*), and leaf spots caused by *Cercospora sp., Phyllosticta batatas* and *Septoria batatas* (Clark, 1987). These contribute to low yields by reducing photosynthetic area and transport of nutrients and other products to the storage roots. Apart from the above, tuber-rotting pathogens have also been reported in the fields causing significant losses (Skoglund *et al.,* 1990; Clarence *et al.,* 1990; Skoglund and Smit, 1994; Carey, 1996).

However, correct identification and characterisation of the apparent *Alternaria* species responsible for stem blight and leaf spot has not been carried out (Lenne, 1991; Carey, personal communication, 1998). In this connection, more information on the geographical distribution of *Alternaria* species and strains is required, especially to establish if different species and races are involved in causing the disease in different parts of Kenya. This would help in developing resistant and or tolerant varieties and predicting future problems with this relatively new, largely unstudied disease.

No study has been done on cultural or chemical control of this disease complex. However, "resistant" varieties selected at low altitudes on relatively fertile soils frequently show severe stem blight at higher altitudes and/or on poorer soils (Ndamage, 1988). It is not known whether field-screening techniques are inadequate; if the pathogen is more abundant in infertile soils; or whether poorer growth of sweetpotato in such soils makes it more susceptible to leaf petiole and stem blight.

Studies on the effects of soil pH, soil type, climate and variety on disease incidence and severity in the field would be worthwhile. It has been recommended that all cultivars destined for medium to high altitudes in Africa should be tested for reaction to *Alternaria* species (Matata, 1988; Terefe and Amanuel, 1992).

Interactions of factors affecting growth and sporulation of the fungus determine optimal laboratory conditions for abundant conidia production useful in rapid evaluation of genotypes in controlled experiments in the greenhouse. If optimal combinations of these factors can be identified, then one may be able to determine precisely the optimum incubation conditions for the rapid multiplication of the fungus.

This study was designed to establish the pathogen responsible for leaf petiole and stem blight of sweetpotato, investigate the optimal *in vitro* conditions for growth and sporulation of the causative agent, its virulence and determine its importance in disease development under different soil pH levels and field conditions.

Thus, the specific objectives of the study were:

1. To collect diseased sample materials from different sweetpotato growing areas in Kenya and determine the causal agent associated with leaf and stem blight of *Ipomoea batatas* (L) Lam.

2. To identify conditions influencing *in vitro* growth and sporulation of the identified pathogen.

3. To study the effect of different soil pH ranges on disease development of the causal agent on some selected lines of *Ipomoea batatas* (L) Lam. under greenhouse conditions.

4 To evaluate the reaction of 20 *Ipomoea batatas* cultivars/accession lines to the pathogen under field conditions.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 History and geographical distribution of sweetpotatoes

The sweetpotato, (*Ipomoea batatas* (L) Lam) is a dicotyledonous plant that belongs to the family Convolvulaceae (Purseglove, 1968; Austin, 1988). Amongst the approximately 50 genera and more than 1500 species of this family, only *Ipomoea batatas* (L) Lam is of major economic importance as a food crop (Purseglove, 1968; Edmond *et al.,* 1971; Onwueme and Charles, 1994; C.I.P., 1988; Hill *et al.,* 1988; William *et al.,* 1989; Woolfe, 1992). All archaeological linguistic and historical evidence so far suggests that sweetpotato originated in the New World in the Central or South American lowlands, around 8,000 - 6000 BC (O'Brien, 1972; Yen, 1982; Austin, 1988). The primary centres are found in Central America, particularly Guatemala. Christopher Columbus introduced it into Europe during his voyages of discovery, while subsequent Spanish and Portuguese explorers and traders introduced it into Africa at their coastal trading settlements from where it spread inland (O'Brien, 1972; Yen, 1982). From these areas of introduction, secondary centres of genetic diversity are now to be found in China, South East Asia, Papua New Guinea, and East Africa (Purseglove, 1968; Yen, 1982).

Today, sweetpotato (*Ipomoea batatas*) is grown in nearly all parts of the tropical and sub-tropical world, and in the warmer areas of the temperate regions such as Southern USA (Onwueme, 1978; Horton *et al.,* 1984, CIP, 1995a). Sweetpotato is the world's seventh most important food crop after wheat, rice, maize, potato, barley and cassava (Dayal *et al.,* 1991), but in the developing world, sweetpotato ranks fifth in economic importance (CIP, 1995a). More than 95% of the global sweetpotato crop is grown in developing countries. However, China has

historically been the major producer and is responsible for 90% of global production (CIP, 1995a; CIP, 1996). Vietnam, Indonesia and Uganda all grow more than two million tons annually while Kenya produces 0.627 million tons (CIP, 1996) (Appendix 3). Today, the sweetpotato germplasm collection at CIP contains about 6500 genotypes, including wild accessions, farmer varieties, and breeding lines (CIP, 1996).

2.2 History, geographical distribution and occurrence of Alternaria leaf and stem blight of sweetpotato

Alternaria leaf and stem blight of sweetpotato has been reported in a number of sweetpotato producing countries including, Nigeria (Arene and Nwankiti, 1978), Ethiopia (Van Bruggen, 1984), Rwanda (Ndamage, 1988), Burundi (Simbashizweko and Perreaux, 1988), Kenya (Gatumbi *et al.,* 1990), Brazil (Lopes *et al.,* 1994) and Uganda (Bashaasha, 1995).

Alternaria stem and petiole blight caused by *Alternaria alternata* has been reported in the southern and western highlands of Papua New Guinea (Waller, 1984; Lenne, 1991) and New Caledonia (Bugnicourt and Marty, 1961). Leaf spot and stem blight caused by *Alternaria bataticola* was first recorded in Brazil, South America in 1994 (Lopes *et al.,* 1994). In Africa, Alternaria leaf and stem blight caused by *Alternaria solani* has been reported in Burundi (Buyckx, 1962; Simbashizweko and Perreaux, 1988) and Rwanda (Buyckx, 1962; Ndamage, 1988). *Alternaria tax sp (IV)* is known in Ethiopia (Van Bruggen, 1984), and an *Alternaria* spp. has been recorded in Zambia (Riley, 1956; Angus, 1963) and Zimbabwe (Whiteside, 1966) and in Ethiopia (Terefe and Amanuel, 1992). In Kenya, Gatumbi *et al.* (1990) reported it along with 12 other fungi infecting sweetpotato, that prompted Skoglund *et al.* (1990) to conduct a nation-wide survey of sweetpotato non-viral diseases and reported *Alternaria* spp. presence in all the 13 districts surveyed. It was the only pathogen found in the highland, tea

growing Kericho district. Disease incidence and severity were especially high (>50%) in highland areas (Kirinyaga, Muranga, and Kiambu).

The disease is serious particularly in infertile, acid soils, and at higher altitudes in Rwanda and Burundi (Simbashizweko and perreaux, 1988; Ndamage, 1988). In Ethiopia, it attacks sweetpotato in medium-altitude regions (Terefe and Amanuel, 1992).

2.3 Economic importance of Alternaria leaf petiole and stem blight of sweetpotato

Alternaria leaf petiole and stem blight can be considered as the most important fungal disease of sweetpotato in East Africa (Kenya, Uganda, Rwanda and Burundi) (Carey, 1996) and South East Asia (Lopes *et al.,* 1994). The amount of damage to the sweetpotato plant is dependent on the stage of growth and prevailing environmental conditions (C.M.I, 1981; Skoglund and Smit, 1994). Generally, decline of photosynthetic area due to leaf damage is often the initial effect of Alternaria leaf spot. In very severe attacks, vines may be killed within a very short period of symptom development leading to 100 % crop failure, while milder attack causes defoliation (Skoglund and Smit, 1994). Premature defoliation may have an adverse effect on growth and development of the plant, and hence its production. Stem blight is manifested in the wet season as stem necrosis and dieback and is especially serious in drier periods. If the main stem is affected when the plant is young, the plant dies. Attack in latter stages of plant development results in no loss in root yield (Lenne, 1991). Disease and lesion size increase as altitude increases (Simbashizweko and Perreaux, 1988; Ndamage, 1988). High relative humidity or free water is necessary for infection and sporulation and the fungus survives in debris and spores are spread through infected planting material, wind, splashing rain and water (Skoglund and Smit, 1994; Ames *et al.,* 1997).

No quantitative information is available on the economical impact of this disease on sweetpotato yields. (Lenne, 1991).

2.4 Taxonomy and morphological characteristics of *Alternaria* species

Morphological characteristics of conidia and conidiophores provide the major taxonomic criteria for delimitation of fungal species (Hughes, 1953; David, 1991). *Alternaria* species belongs to the family Dematiaceae, order Moniliales of the form class Deuteromycetes (Alexopoulos and Mims, 1979). The genus is most easily identified by its ellipsoidal conidia, which are light to olivaceous brown with many transverse and longitudinal septa and a beak-like structure on the distal end (Clark and Moyer, 1988). The exact shape and size of the conidia vary with the species. They are borne singly in some species and in chains in others (David, 1991).

2.5 Host range of *Alternaria* species

Alternaria species are important pathogens of a wide variety of crops and weeds (Agrios, 1969; Abbas *et al.,* 1995). This is true for species like *Alternaria solani* that causes serious blight on potato (early blight) and tomato as well as some Brassicae species. *Alternaria brassicae* attacks a wide range of cruciferous plants (Parry, 1990). A complex of diseases caused by *Alternaria* species, known variously as leaf spot, stem blight, alternariosis and anthracnose on sweetpotato species has so far been associated with at least six different *Alternaria* spp.*; Alternaria alternata, Alternaria capsici-annui, Alternaria solani, Alternaria tenuissima*, *Alternaria tax sp. (IV)* and *Alternaria bataticola* (Clark and Moyer, 1988; Lenne 1991). Four of these pathogens (*A. alternata, A. capsici-annui, A. solani* and *A. tenuissima*) have also been reported on three other species of *Ipomoea* (Lenne, 1991).

2.6 Symptomatology of Alternaria leaf and stem blight of sweetpotato

Leaf spots ranging in distinctness from a faint discoloration to characteristically marked necrotic annular lesions are typical of Alternaria leaf spot (Skoglund and Smit, 1994). Alternaria petiole and stem blight first appears as small, grey to black oval lesions with a lighter centre on stems and petioles (Van Bruggen, 1984). On leaves, the lesion expansion is limited to the mid-rib and veins, causing anthracnose-like symptoms. These water-soaked lesions may coalesce resulting in blighting of the whole leaf. Where they do not coalesce, the area adjacent to the veins yellow, and the leaves easily detach from the vine.

Under humid conditions, lesions enlarge as black areas, which may involve expanses of stem and petiole, and result in petiole and stem girdling (Skoglund and Smit, 1994). Brown lesions with a typical "target" appearance of concentric rings occur on leaves, especially older ones (Skoglund and Smit, 1994). Leaves above the affected parts become chlorotic and dry. If the main stem is affected when the plant is young, the plant dies (Van Bruggen, 1984; Lenne, 1991. Bases and middle sections are more affected than the terminals of vines. The ground under affected vines is often carpeted with blackened leaf debris (Skoglund and Smit, 1994).

2.7 Cultural studies of *Alternaria* species.

A number of culture media of different type and quality have been recommended for growth and sporulation of different species of *Alternaria.* Van Bruggen, (1984) reported maximum growth and sporulation of *Alternaria tax sp. (IV)* on potato dextrose agar (PDA) and malt extract agar (MEA) under 12hrs light alternating with 12hrs darkness. Similarly, David (personal communication) observed abundant sporulation of *Alternaria bataticola* in tap water agar with wheatstraw but failed to sporulate on a range of specialised media. Lopes *et al.* 1996 reported

abundant conidia production of the same pathogen in calcium carbonate sporulating medium as opposed to PDA or MEA. Mehta and Prasad (1976) obtained maximum growth of *Alternaria sesami* on oatmeal agar (OMA).

Light regimes also influence growth and sporulation of many *Alternaria* species. In some species these processes are favoured by continuous illumination (Aragaki, 1962), while in others they are optimal under continuous darkness (Leach and Trione, 1966), or alternating light and darkness of specified hours (Leach, 1964; Van Bruggen, 1984). Sporulation of *Alternaria tax sp. (IV)* is reportedly favoured by 12hrs light alternating with 12hrs darkness (Van Bruggen, 1984). Many *Alternaria* species grow and sporulate well within a temperature range of 15 to 30°C (Aragaki, 1962). No quantitative work has been done on optimal temperature requirement for growth and sporulation of *Alternaria* species attacking sweetpotato.

In-vitro growth and sporulation of most *Alternaria* species is favoured by acidic or neutral media pH conditions (Strandberg, 1987). Mohapatra *et al.,* (1977) and Ojiambo (1997) reported optimal growth and sporulation of *Alternaria sesami* under pH 4.5. There is no previous study of the influence of media pH on growth and sporulation of *Alternaria bataticola* in Kenya.

2.8 Control of Alternaria leaf spot and stem blight of sweetpotato.

No work has been done on cultural or chemical control of this disease complex (Lenne, 1991). But host plant resistance has been identified in glasshouse screening in Burundi (Simbashizweko and Perreaux, 1988) and field screening in Rwanda (Ndamage, 1988), Papua New Guinea (Lenne, 1991) and Ethiopia (Van Bruggen, 1984; Terefe and Amanuel, 1992). The development of glasshouse screening methodologies in Burundi could serve as a base to facilitate selection of resistant varieties relevant to each country or region. A low-input, host-plant resistance

approach would directly benefit subsistence farmers. However, "resistant" varieties selected at low altitudes on relatively fertile soils may show severe stem blight at higher altitudes and/or on poor soils as reported in Rwanda (Ndamage, 1988).

2.9 Techniques used in screening for resistance to Alternaria leaf spot and stem blight.

Determination of resistance to foliar diseases in crops can be achieved through various procedures such as the assessment of disease incidence and severity in specified plant populations (James, 1974). The study of disease progress using areas under disease progress curves (Johnson and Beute, 1986) or apparent rates of disease progress (Kanz, 1974; Johnson *et al.,* 1986) can also serve the same purpose. Screening of sweetpotato (*Ipomoea batatas*) for resistance to *Alternaria tax sp. (IV*) and *Alternaria solani* in the field has been reported by Van Bruggen (1984) in Ethiopia and Ndamage (1988) in Rwanda respectively. While testing for disease reaction in a selection program, a greenhouse test might prove more efficient but the prerequisite of any such greenhouse test is that it should reflect field resistance. Of the 13 varieties evaluated by Van Bruggen (1984), there were clear differences in susceptibility among the varieties. Those varieties that had red tubers (Koka 25, and Koka 12) seemed to be more resistant than those with white tubers (Koka9, "A", and Abotsto), but the differences were not statistically significant (Chi-square test). None of the varieties tested was immune.

2.10 Effects of soil pH on disease severity.

With a few notable exceptions, alteration of soil solution pH has little influence on disease development if the pH remains within the range tolerated by the plant (William, 1982). In most instances in which effects of soil reaction on disease incidence have been reported, these can be explained by effects on the pathogen rather than on the host. Potato scab (*Streptomyces scabies*) is more severe on slightly acid to neutral soils than on more acid soils

(Walker 1969; William, 1982). Bingham and Zentmyer (1954) found that Phytophthora root rot of avocado (*Phytophthora cinnamoni*) affected avocado seedling most severely at pH 6.5 while disease development was less at pH 8 and at pH 4, but was not inhibited except at very low pH (pH 3). Disease induced by *Phymatotrichum omnivorum* (Lyda, 1978) and *Verticillium* spp. (Wilhelm, 1950) were more common in areas with alkaline rather than acid soils. Alternaria stem and leaf blight is serious in Burundi and Rwanda particularly on infertile, acid soils and at higher altitudes (Simbashizweko and Perreaux, 1988). The mechanisms of pH effects on disease development are not completely understood. The effects are at least partially direct because high pH reduces germination of *Plasmodiophora brassicae* spores (Walker, 1969). However, pH alterations also affect the biological balance of soil thus the control achieved by altering soil pH might be mediated in part by biological control (Baker and Cook, 1974).

CHAPTER THREE

3.0 MATERIALS AND METHODS.

3.1 Laboratory studies

These studies were conducted to culture and identify *Alternaria* species associated with Alternaria leaf and stem blight of sweetpotato as well as to investigate cultural conditions affecting *in-vitro* growth and sporulation of *A. bataticola*.

3.1.1 Isolation and culturing of *Alternaria* species.

a) Collection and preservation of diseased specimen.

Vines and leaves showing characteristic symptoms of Alternaria leaf petiole and stem blight were collected from the University of Nairobi Kabete Farm, the KARI field station Kakamega, the Alupe research station (Busia), Kabondo and Kisii (KARI station). From each area, four samples were randomly picked from sweetpotato growing fields and put in paper bags. The infected materials were transported to the plant pathology laboratory at Kabete campus, University of Nairobi. Two samples from each area were stored in pressed form, while others were put in refrigerator awaiting isolation.

b) Isolation and culturing of the fungus

Isolations were made from lesions of naturally infected sweetpotato vines and leaf tissues showing fungal sporulation. In case of non-sporulating lesions, the fungus was induced to sporulate by incubating the infected tissues.

i) Incubation of infected tissues.

Small pieces of infected leaf petiole and stem/vine tissues were surface sterilised using 5 % sodium hypochlorite (NaOCl) for 5 minutes and rinsed in 5 changes of sterile distilled water. They were then placed in moist chambers prepared using filter papers and glass boxes. The surface sterilised infected vine and leaf parts were incubated for 24hrs to allow sporulation as described by Van Bruggen (1984).

ii) Single spore isolation.

Single conidia were picked from lesions on infected vines and leaves, and seeded onto the surface of water agar using the tip of a sharp sterile inoculating needle. Inoculated plates were incubated on a laboratory bench at room temperature (20 to 24^{o}C). Conidial germination on the plates was checked daily and upon germination, agar blocks bearing single germinated conidia were cut out and aseptically seeded onto sweetpotato leaf decoction media (SPLDM) plates (Appendix 1). The plates were incubated for 14 days under normal room temperature and lighting conditions (20-24^{o}C and 12-hrs light)

c) Maintenance of cultures of *Alternaria* sp.

Cultures of the isolated fungi were maintained using a modification of the technique described by Boesewinkel (1976). Sweetpotato leaf decoction media (SPLDM) blocks (4mm) (Appendix 1 for media composition and preparation) bearing actively growing monosporic cultures were aseptically cut out and transferred into sterile universal bottles carrying 20 ml of sterile SPLDM. The caps were then screwed tightly to seal the bottles, which were then stored in refrigerator at 4 - 6^{o}C.

3.1.2 Identification of the pathogen

The fungus was identified using a modification of the slide culture technique described by Riddle (1950). Two sheets of filter paper, a bent glass rod and a cover slip were placed in a petri dish in that order and sterilised by autoclaving. Sterilised sweetpotato leaf decoction medium (SPLDM) was poured into sterile petri dishes to form a layer of about 2-mm depth. Upon solidification, 1-cm^2 agar blocks were aseptically cut out using a sharp sterile scalpel and placed on 22 x 22-mm^3 microscope slide. The centre edges of each agar block were seeded with a conidia of inoculum obtained from 3.1.1(ii) using a sterile inoculating needle and the cover slip placed centrally on the block. Petri dishes were covered with lids and incubated as described in 3.1.1. High humidity within the dishes was maintained by routine addition of 2 to 3 % aqueous solution of glycerine aseptically.

When suitable growth and sporulation had occurred, the cover slip was gently lifted using a sterile pair of forceps and the agar block discarded. The cover slip was then mounted in a drop of clear lactophenol and viewed under the microscope. The nature of mycelial growth, nature of conidiophore formation, arrangement of conidia on conidiophores and shape of conidia was noted. Mounting was similarly repeated in sterile distilled water for colour observation of various structures. The size of conidia (length and width) was determined using Dynazoom compound microscope with a micrometer.

3.1.3 Assessment of factors influencing *in-vitro* growth and sporulation of *Alternaria bataticola*

Factors influencing growth and sporulation of *A. bataticola* were evaluated under laboratory conditions. *A. bataticola* was selected as it occurred in all infected leaf petioles and vines of sweetpotato samples from Kabete, Kakamega, Kabondo and Busia.

3.1.3.1 Effect of type of media and incubation period on growth and sporulation of *Alternaria bataticola.*

The main aim of this experiment was to find a medium that would serve best for growth and sporulation of *A. bataticola*

i) Media preparation

Four types of media were used to determine their effect on growth and sporulation of the pathogen. These were potato dextrose agar (PDA), malt extract agar (MEA), sweetpotato leaf decoction media (SPLDM), and sweetpotato vine decoction media (SPVDM). Composition of these media is shown in Appendix 1.

ii) Inoculation and incubation.

The method of Hyre and Cox (1953) was used for inoculating the media. A uniform culture plug of $4mm^2$ in diameter obtained from a 14-day-old culture plate was placed in the centre of each dish and then incubated at room temperature (20-24°C).

iii) Experimental design.

The experiment was arranged in a completely randomised design with 6 replicates. Two factors, media and incubation period were studied in a factorial combination

iv) Measurement of fungal growth and sporulation.

Measurements on radial colony diameter were taken on 3, 5, 7, 9, 11, 13, and 15 days after inoculation whereby six plates were sampled each time from each media treatment.

To determine conidial concentration for each medium, ten plates were prepared. Two plates were sampled randomly from each media treatment at 9, 11, 13 and 15 days after inoculation.

Ten ml of sterile distilled water was added to the culture plate and by use of a sterile glass slide, the culture surface was gently scrapped to make a conidial suspension. The conidial concentration was determined by use of a Neubaure improved haemocytometer.

v) Statistical analysis

Averages of colony diameter and number of conidia/ml for each treatment combination were used for subsequent data analysis. Analysis of variance was carried out at both 1 % and 5 % probability levels of the F-test. Significant differences in treatment effects were identified using Duncan's multiple range test and least significant difference test at the 5 % probability level.

3.1.3.2 Effect of media, temperature and incubation period on growth and sporulation of *Alternaria bataticola*.

To study the effect of media, temperature and incubation on radial growth rate, 4mm^2 plugs were taken from a stock culture of *A. bataticola* grown on SPLDM for 10-15 days. These plugs were placed in the centre of fresh SPLDM, SPVDM, MEA, and PDA. Fungal growth was determined by measuring radial mycelial growth for each colony at 3, 5, 7, 9, 11, 13 and 15 days after inoculation. The plates were then incubated at eight different temperature conditions, viz. 20°C, 22°C, 24°C, 26°C, 28°C, 30°C, 32°C and 34°C in a completely randomised design with media, temperature and incubation period being treatment. Six replicate plates for each temperature were measured. Conidia production of the fungus was determined after 9, 11, 13 and 15 days of incubation.

3.1.3.3 Effect of media pH and incubation period on growth and sporulation of *Alternaria bataticola.*

Seven incubation periods namely 3, 5, 7, 9, 11, 13 and 15 days and six pH levels namely 4, 5, 5.6, 7, 8, and 9 were investigated. Sweetpotato vine decoction media (SPVDM) was used in this investigation. The media pH was adjusted using 0.1N NaOH or 0.1N HCl before sterilization and checked after sterilization at a constant temperature of 45°C before pouring. The control media pH was 5.6. Inoculation was achieved through mycelial disc transfer as described in 3.1.3.1(ii). Inoculated plates were placed on a laboratory bench and incubated for the duration specified above under normal room temperature (20-24°C) and light conditions. The experiment was arranged in a completely randomised design (CRD) with six replicates. Two factors, media pH and incubation period were studied in a factorial combination. Fungal growth expressed as radial growth, and sporulation was determined as described in 3.1.3.1(iii). Averages of colony diameter and number of conidia/ml for each treatment combinations were used for subsequent data analysis. Analysis of variance was carried out at both 1 % and 5 % probability levels of the F–test. Significant differences in treatment effects were identified using Duncan's multiple range test and least significant difference test at 5 % probability level.

3.1.3.4 Effect of media, light regime and incubation period on growth and sporulation of *Alternaria bataticola.*

The four media (PDA, MEA, SPLDM and SPVDM) were inoculated with 4mm^2 mycelial plugs of *A. bataticola* and incubated under the following light regimes.

a) 24hr complete darkness.

b) 12hr continuous light alternating with 12hr continuous darkness

c) 24hr continuous light.

24 petri dishes were incubated in a completely randomised design under each light regime and for each of the three periods, six plates were sampled. The plates incubated under 24hrs complete darkness were wrapped in aluminium foil while the plates incubated under 12hr continuous light alternating with 12hr continuous darkness regime had the aluminium foil removed after every 12hrs of darkness. A continuous light was provided by the use of NARVA LS 40W-1 coolwhite20 IV/90 I electric tubes. All the plates were randomly placed on a bench in the respective culture room conditions. Fungal growth and sporulation were assessed and statistical analysis performed as described in 3.1.3.1.

3.2 GLASSHOUSE EXPERIMENTS

Studies in the glasshouse were conducted to test pathogenicity of *Alternaria* spp., assess the difference in virulence among isolates of *Alternaria bataticola* from Busia (Alupe KARI sub-station), Kakamega (KARI), Kabete (University), Kabondo and *Alternaria alternata* from Kisii, and as well as evaluate effect of different soil pH levels on disease severity on some selected sweetpotato varieties.

3.2.1 Proof of pathogenicity of *Alternaria* species isolates on *Ipomoea batatas* (L) Lam.

Experiments were conducted to provide proof that isolates of *Alternaria* obtained from infected materials from Kabete, Kakamega, Kabondo, Busia and Kisii were pathogenic to *Ipomoea batatas.*

a) Test plants

Pathogenicity tests were conducted using sweetpotato plant accessions Viola (440046). High disease severity was observed on this accession during preliminary field survey exercise at CIP multiplication plots at University of Nairobi Kabete Farm. Non diseased vines of the

accession were planted in well-drained sterilised eutric nitisols contained in 30-cm diameter plastic pots. Planting was done in time to provide 8-week old plants at the time of inoculation.

b) Inoculum preparation

Conidial suspensions were prepared from 14-day old monosporic cultures grown on sweetpotato leaf decoction media (SPLDM). The plates were incubated under normal room conditions for 14-days, after which they were flooded with sterile distilled water (10-ml/plate). Conidia were dislodged by gently scrapping the surface using sterile glass rods and the suspension produced was strained through two layers of sterile cheesecloth. Conidial concentration was determined using haemocytometer and standardised at 5.4×10^6 conidia/ml.

c) Inoculation and incubation of test plants

Eight-week old plants were inoculated by spraying the inoculum to run-off on vines and both sides of the leaves present on plants using 0.5-litre Baygon atomiser. A second inoculation was done 48-hours later. Control plants were sprayed with sterile distilled water. All inoculated plants were covered with moisten polythene bags and incubated in the greenhouse. The polythene bags were removed after 24-hours of inoculation.

d) Re-isolation and culturing

Inoculated plants were examined daily for symptom development and the colour, shape and size of lesions produced on the leaves and vines were noted. Six days after inoculation, leaves and vines showing symptoms were detached and re-isolation of the causal agent performed to fulfil Koch's postulates.

3.2.2 Assessment of virulence of *Alternaria* isolates

This investigation was undertaken to establish differences in virulence among isolates of *Alternaria* species from Kakamega, Kabete, Kabondo, Alupe and Kisii under greenhouse conditions on sweetpotato accession Viola (440046).

a) Isolate source and inoculum preparation

The fungus was isolated from diseased materials from Kakamega, Kabete, Kabondo, Busia and Kisii. Inoculum was prepared separately for each isolate and conidial concentrations in the suspensions determined using haemocytometer counts and standardised at 2×10^3, 2×10^2, and 2×10^1 conidia/ml as described in 3.3.1b

b) Test plants

The accession line Viola (440046) was used in this study. The vines were planted in well-drained sterile eutric nitisols contained in 30-cm diameter plastic pots. Planting was done in time to provide 8-week old plants at the time of inoculation.

c) Inoculation of test plants

Three replicate plants, one plant per pot, were inoculated with each concentration of each isolate by use of a half-litre Baygon sprayer as described in 3.2.1(c). However no second inoculation was done. Inoculated plants were covered immediately after inoculation with plastic polythene bags for 24-hours.

d) Experimental design and statistical analysis

The experiment was arranged in a completely randomised design with three replicates. Two factors isolate source and isolate conidia concentration was studied in a factorial

combination. Differences in the treatment means were examined using Duncan's multiple range tests at 5 % probability level.

e) Disease evaluation and data collection

Inoculated plants were examined daily for lesion development on both leaf petioles and stem. After 4 to 6 days of inoculation, the plants were scored independently and the number of lesions was counted and average values computed for subsequent data analysis. The plants were scored independently for number of lesions on leaves and stems on a 0 – 10 scale, a modification of Van Bruggen, 1984 as follows:

0 = no lesion
1 = 1 – 5 lesions
2 = 6 – 10 lesions
3 = 11 – 15 lesions
4 = 16 – 20 lesions
5 = 21 – 25 lesions
6 = 26 – 30 lesions
7 = 31 – 35 lesions
8 = 36 – 40 lesions
9 = 41 – 45 lesions
10 = >45 lesions.

3.2.3 Assessment of effect of soil pH on disease severity.

This investigation was done to assess if soil pH has direct effect on disease severity of Alternaria leaf spot and stem blight caused by *Alternaria bataticola.*

a) Collection of soil samples and measurement of the pH

Soils of known pH levels were collected from department of soil science, University of Nairobi, for this experiment. Their pH was measured to confirm their pH status. All the soils used in this study were sterilised before being potted in plastic pots.

b) Test plants

Sweetpotato accession lines Viola (440046), Jayalo, Kemb 36 and TIS 83/0138 (440102) were used in this study. Healthy vines were planted on pots filled with soil of different soil pH levels (i.e., 4.4, 4.6, 6.7, and 7.6) to provide 4 weeks old plants at the time of inoculation.

c) Inoculation of test plants

Three replicate plants, one plant per pot were inoculated with a uniform conidial concentration of 5.4×10^6 condia/ml. Control plants were inoculated using sterilised distilled water.

d) Experimental design

A completely randomised design (CRD) with 3 replicates was used in this experiment, with soil pH levels and cultivars being studied in a factorial combination.

e) Disease evaluation and data collection.

The inoculated plants were examined for disease development on weekly basis. The disease severity was scored using a modification of Van Bruggen, 1984 on a 0 – 5 scale, where:

0 = no disease

1 = few, small lesions

2 = from 1-10% infection

3 = from 11-25% infection

4 = from 26-50% infection

5 = > 50% infection.

Analysis of variance was carried out at 5 % probability level of the F-test. Significant differences in treatment effects were identified using Duncan's multiple range test and least significant difference test at 5 % probability level.

3.3 FIELD EXPERIMENTS

Field studies were conducted at the University of Nairobi, Kabete campus field station farm for 2 seasons to evaluate the relative susceptibility of 20 sweetpotato cultivars/accession lines to Alternaria stem and petiole blight. The first season started from May to August and the second season from September to December. *Alternaria bataticola* from Kabete was used in this experiment because it was more virulent than other isolates.

a) Test plants

The sweetpotato test plants used in this experiment were obtained from CIP germplasm field multiplication plots at the University of Nairobi Kabete Farm. These comprised of 20 sweetpotato cultivars/accessions viz.: Mar Ooko, Habare 127 (440380), New Kawogo (440165), Kalam Nyerere, Yanshu 1 (440024), Kemb 36, 420006, PEPA (440050), PM-04-4 (440333), Camote Negro (420021), Mugande (440163), Mafuta, KSP20, Naveto (440131), Viola (440046), Jayalo, Iguro iwe, LM88.014 (188001.2), Mwezi tatu, and TIS 83/1380 (440102)

b) Inoculum preparation.

Conidial suspension was prepared from 14-day old monosporic cultures grown on sweetpotato leaf decoction media (SPLDM) as described in 3.1.1. The plates were incubated under normal room conditions for 14-days, after which they were flooded with sterile distilled water (10-ml/plate). Conidia were dislodged by gently scraping the surface using

sterile glass rods and the suspension produced was strained through two layers of sterile cheesecloth. Conidial concentration was determined using haemocytometer and standardised at 5.4 x10^6 conidia/ml.

c) Experimental design.

Randomised complete block design with 5 replicates was used in this study. Treatments were the 20 different sweetpotato accessions.

d) Disease evaluation and data collection

The inoculated plants were examined for disease development on weekly basis. The disease severity was scored using a modification of Van Bruggen, 1984 on a 0 – 5 scale, where:

0 = no disease

1 = few, small lesions

2 = from 1-10% infection

3 = from 11-25% infection

4 = from 26-50% infection

5 = > 50% infection.

Analysis of variance was carried out at 5 % probability level of the F-test. Significant differences in treatment effects were identified using Duncan's multiple range test and least significant difference test at 5 % probability level.

e) Determination of Area Under Disease Progress Curves (AUDPCs).

These were computed from disease severity ratings using the following formula (Shaner and Finney, 1977).

$AUDPC = \Sigma [Y_{i+1} + Y_i)/2] (X_{i+1} - X_i)$

Where:

Y_i= % disease severity (% plant area infected at the ith observation.

X_i= date of the i^{th} observation.

Percent diseased areas were assessed as from May to August for the first season and from September to December for the second season at the University of Nairobi field station farm.

e) Statistical analysis

Averages of AUDPCs for severity for each plot were examined using Analysis of Variance. Significant differences were identified using Duncan's multiple range test as applied by Luke and Berger (1982).

CHAPTER FOUR

4.0 RESULTS.

4.1 LABORATORY STUDIES.

4.1.1 Cultural and morphological characteristics of *Alternaria* species isolates

4.1.2 Morphological characteristics and identification of *Alternaria* species.

Isolation of the pathogen from infected leaf and stem tissues yielded colonies of typical *Alternaria* sp. Kabete, Kakamega, Busia and Kabondo isolates produced fluffy; pale-grey to grey mycelial colonies on potato dextrose agar (PDA), malt extract agar (MEA), and host based decoction media, sweetpotato leaf decoction media (SPLDM) and sweetpotato vine decoction media (SPVDM). The colonies expanded laterally exhibiting typical concentric rings of fruiting bodies on the surface of the agar media. Isolates from these areas were similar in colony appearance and in conidia shape and size. Conidia were solitary elongate-obclavate, muriform, and transversely 5-12 septate and longitudinally 0-8 septate, pale to fuscous-brown in colour, mostly smooth-walled (Plate 1). The beak often had one or two branches. Conidial dimensions however, varied according to the type of medium used. The morphometric characteristics of these isolates fitted within the range described for *Alternaria bataticola* Ikata ex W. Yamamoto, (75-) 120-160(-210) x (13-) 15-18(-23)µm conidium body. The conidial beaks were long, filiform, and colourless sometimes branching with an average dimension of 8 (4-12) x 71 (32-129)µm. The Kisii isolate was identified as *Alternaria alternata* (Fr) Keissler, whose conidia were brown, ellipsoidal, 20-60 x 7.5-15µm (average 39 x 10.3µm) in size, short beaked, with 2-6 transverse septa, and 0-4 longitudinal septa and were catenulate at the apex of the conidiophores.

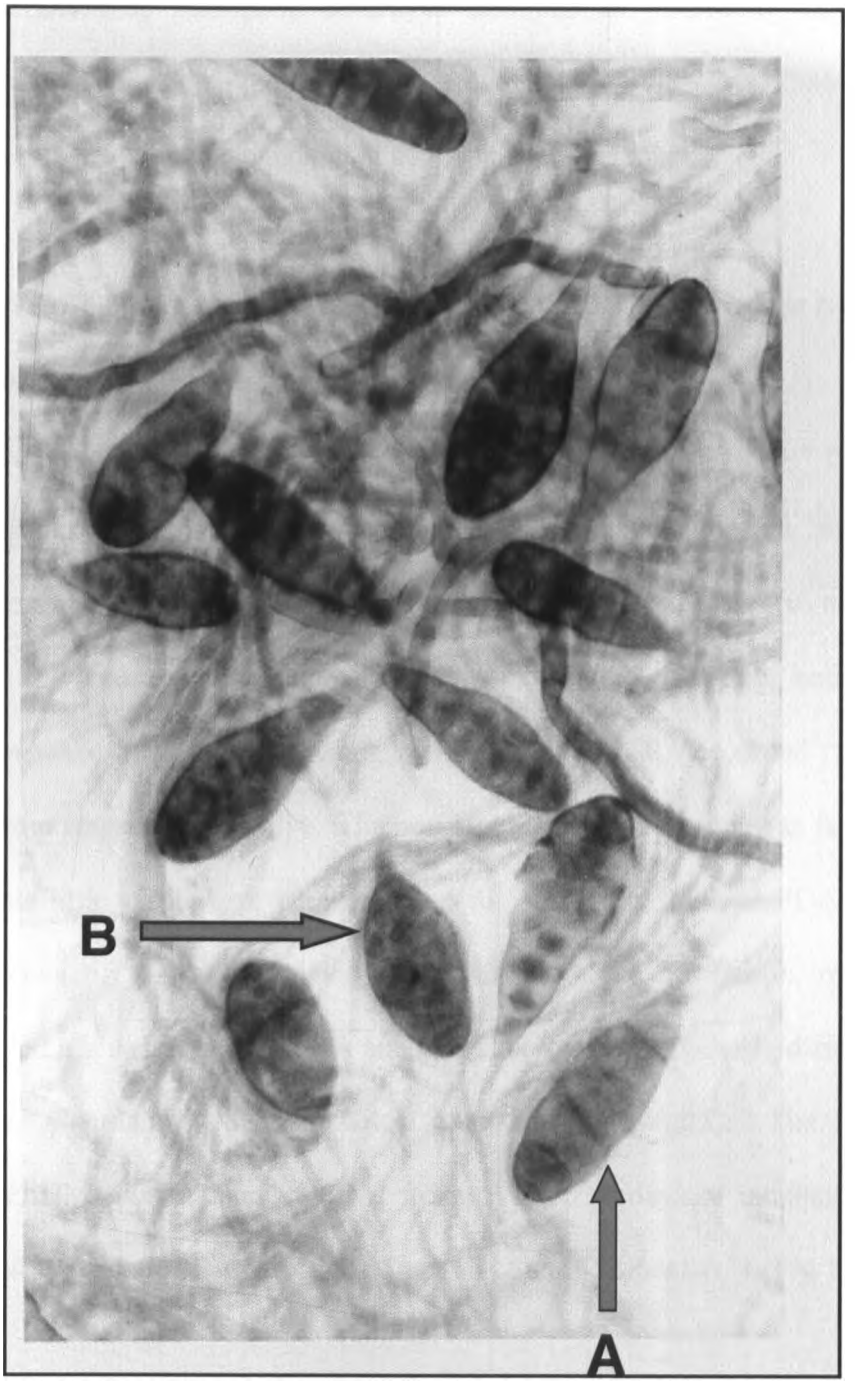

Plate 1. Conidia of *Alternaria bataticola* on sweet potato vine decoction medium (SPVDM) 15 days after incubation. (A) Elongated oval (B) Obclavate (x630)

These isolates were sent to CABI (IMI) in Kew, England, for confirmation of their identity. Four were identified as *Alternaria bataticola* and one as *Alternaria alternata* and are deposited at the IMI culture collection under number IMI 379880, IMI379881, IMI 379883, IMI 379884 and IMI 379882 respectively.

4.2. Factors influencing *in-vitro* growth and sporulation of *Alternaria bataticola.*

4.2.1 Type of media and incubation.

Alternaria bataticola grew slowly on PDA and MEA as opposed to host based decoction media. MEA exhibited the least growth over the entire incubation period, but did not differ significantly from PDA. There was markedly more growth on SPLDM, in that by 11th day, the plate was fully covered with mycelial growth. By the 15th day, both SPLDM and SPVDM had reached maximum of 90mm, while PDA and MEA recorded radial growth of 75mm and 74mm respectively (fig 1). The horizontal mycelial growth was faster in SPLDM and SPVDM but little vertical growth was observed. Vertical growth on PDA and MEA was greater. Analysis of variance revealed that both period of incubation, media and their interaction were highly significant. PDA and MEA were not significantly different from each other but were significantly different from other media ($p=0.05$). The highest growth occurred in SPLDM (90mm) and SPVDM (66mm) after 11 days of incubation, while after the same period PDA and MEA recorded 55mm and 54mm respectively (Fig 1)

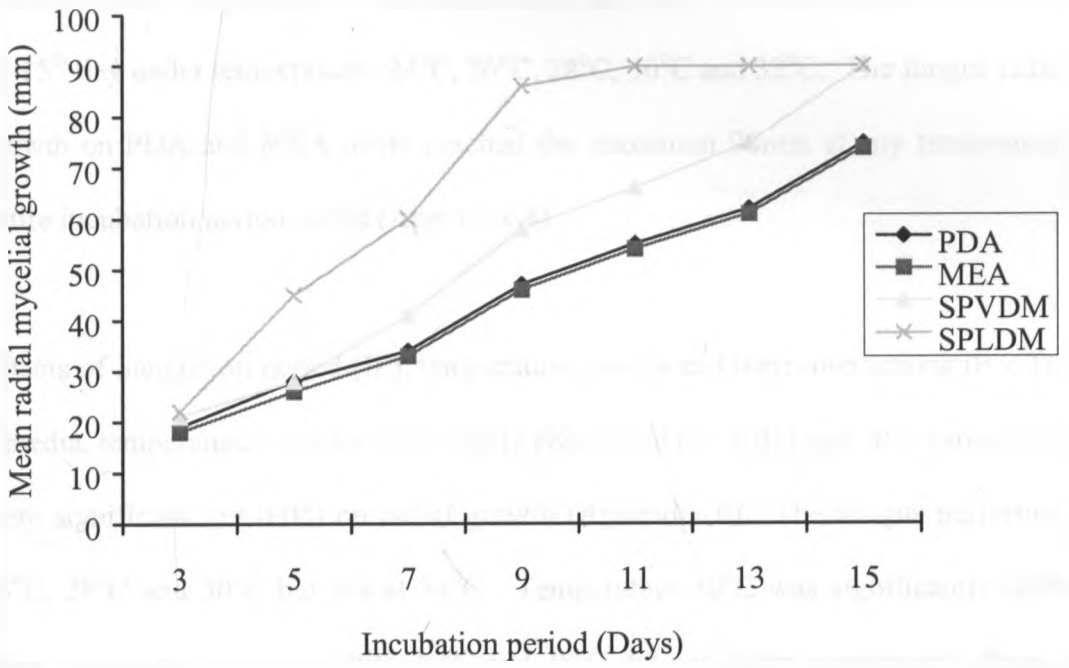

Figure 1. Effect of 4 different media and incubation period on radial mycelial growth of *Alternaria bataticola*

4.2.2. Effect of media, temperature and incubation period on mycelial radial growth of *Alternaria bataticola*

The fungus grew faster on SPLDM, and SPVDM as measured by colony diameters. *Alternaria bataticola* grew well from 26°C to 30°C as opposed to 20°C, 22°C 24°C 32°C and 34°C in all the culture media used (Table 1). Fungal growth was more rapid at 26°C, (62.55mm), 28°C (62.91) and 30°C (65.94mm) than at 20°C (51.4mm) and 34°C (46.51mm).

Growth on PDA (46.90mm) and MEA (45.90mm) were not significantly different from each other but were significantly different from SPVDM (61.71mm) and SPLDM (69.15mm). The lowest radial mycelial growth was noted at 34°C on all the culture media, in all the days of incubation. By 11th day maximum radial mycelial growth (90mm) had been recorded on SPLDM under temperatures 20°C, 22°C, 28°C and 30°C. SPVDM recorded maximum growth by 15th day under temperatures 24°C, 26°C, 28°C, 30°C and 32°C. The fungus radial mycelial growth on PDA and MEA never reached the maximum 90mm at any temperature over the entire incubation period tested (Appendix 4).

Effects of incubation period (IP), temperature, media and their interactions IP x Temperature x media, temperature x media were highly significant ($p= 0.01$) and IP x temperature x media were significant ($p= 0.05$) on radial growth (Appendix 6). The fungus performed better at 26°C, 28°C and 30°C but not at 34°C. Temperature 30°C was significantly different from other temperature ranges, while 26°C and 28°C did not differ significantly from each other but were significantly different from temperatures 20°C, 22°C, 24°C, 32°C and 34°C. Temperature 20°C, 22°C, 24°C and 32°C did not differ significantly from each other. Temperature 34°C differed significantly from all other temperature levels ($p= 0.05$).

Table 1 Effect of media, temperature and incubation period on radial growth of *Alternaria bataticola*

A	Temperature (°C)					B			
Media	20	22	24	26	28	30	32	34	**Mean**
PDA	40.29	40.47	45.64	54.64	55.50	56.14	44.57	37.48	**46.90c**
MEA	36.90	39.43	44.71	53.64	54.36	54.76	43.83	36.90	**45.90c**
SPVDM	57.50	57.43	54.31	67.43	67.56	68.38	58.69	53.05	**61.71b**
SPLDM	68.29	68.31	69.02	74.33	74.38	74.71	65.52	58.59	**69.15a**
Mean	**51.40c**	**51.41c**	**53.43c**	**62.55b**	**62.91b**	**65.94a**	**53.15c**	**46.51d**	

$LSD_{(0.05)}$ A 1.65
$LSD_{(0.05)}$ B 2.34

4.2.3. Effect of media, light regime and incubation period on radial mycelial growth of *Alternaria bataticola*

Growth rate was highest in 24hrs continuous light, followed by 12hrs continuous light alternating with 12hrs continuous darkness. The least growth of the fungus on all media evaluated was experienced under 24hrs continuous darkness (Table 2, Plate 2). SPLDM supported the highest radial growth under all light regimes, followed by SPVDM, PDA, and MEA. Between 3 and 5 days after incubation, there was no significant difference in growth rate between PDA and SPVDM both under 24hrsL and 12hrsL/12hrsD, but thereafter, differences were evident. Maximum growth level of 90mm occurred on SPLDM after 9 days, 11 days and 13 days of incubation under 24hrsL, 12hrsL/12hrsD and 24hrsD respectively. Maximum growth on SPVDM was recorded under 24hrs continuous light regime after 13 days of incubation, 15 days in 12hrs continuous light alternating with 12hrs continuous darkness and a radial growth of 76mm under 24hrs continuous darkness. MEA sustained least growth under all the regimes after 15 days of incubation; 62.50mm, 45.67mm and 66.67mm under 24hrsL, 24hrsD and 12hrsL/12hrsD respectively (Plate 2). Marked

differences in growth between media, light regime, and incubation period were observed. The highest overall level of growth (90mm) was obtained in SPLDM incubated at 24hrsL during the 9th day of incubation (Table 2).

Table 2. Effect of media, light regime and incubation period on mycelial radial growth of *Alternaria bataticola*

		Mean radial mycelial growth (mm) [a]							
		Incubation Period (days)							
Media	Light regime	3	5	7	9	11	13	15	**Mean**
DA	24L	23.17a	37.50a	43.83a	53.17a	62.83a	68.5a	74.83a	**51.98**
	24D	17.67b	24.00b	30.50b	37.00b	42.33c	50.33b	55.50b	**47.39**
	12/12	18.67b	30.83	41.17a	48.33a	56.00b	64.83a	71.67b	**36.76**
Mean		19.83	30.78	38.50	46.17	53.72	61.22	67.33	
MEA	24L	16.00a	24.33a	33.00a	43.83a	51.50a	57.33a	62.50a	**41.21**
	24D	15.33ab	20.33b	25.33b	34.33c	39.33c	43.17b	45.67b	**31.93**
	12/12	14.67b	22.50b	32.50a	38.50b	46.17b	58.33a	66.67a	**39.90**
Mean		15.33	22.39	30.28	38.89	45.67	52.94	58.28	
PVDM	24L	22.50a	38.83a	54.33a	76.33a	88.33a	90.00a	90.00a	**65.76**
	24D	19.00b	28.33b	39.00c	56.00c	63.33c	68.50b	76.00b	**50.02**
	12/12	19.00b	31.33b	44.83b	65.67b	73.67b	88.00b	90.00a	**58.93**
Mean		20.17	32.83	46.06	66.00	75.11	82.17	85.33	
PLDM	24L	30.00a	49.33a	73.67a	90.00a	90.00	90.00	90.00	**73.29**
	24D	22.33c	37.00c	53.50c	79.00c	88.00	90.00	90.00	**65.69**
	12/12	24b	41.5b	63.67b	85.00b	90.00	90.00	90.00	**69.17**
Mean		25.44	42.61	63.61	84.67	89.33	90.00	90.00	

[a] Each value is an average of 6 replications. Within each light regime, media and incubation period, means followed by the same letter do not differ significantly at p = 0.05 (Duncan's multiple range test).

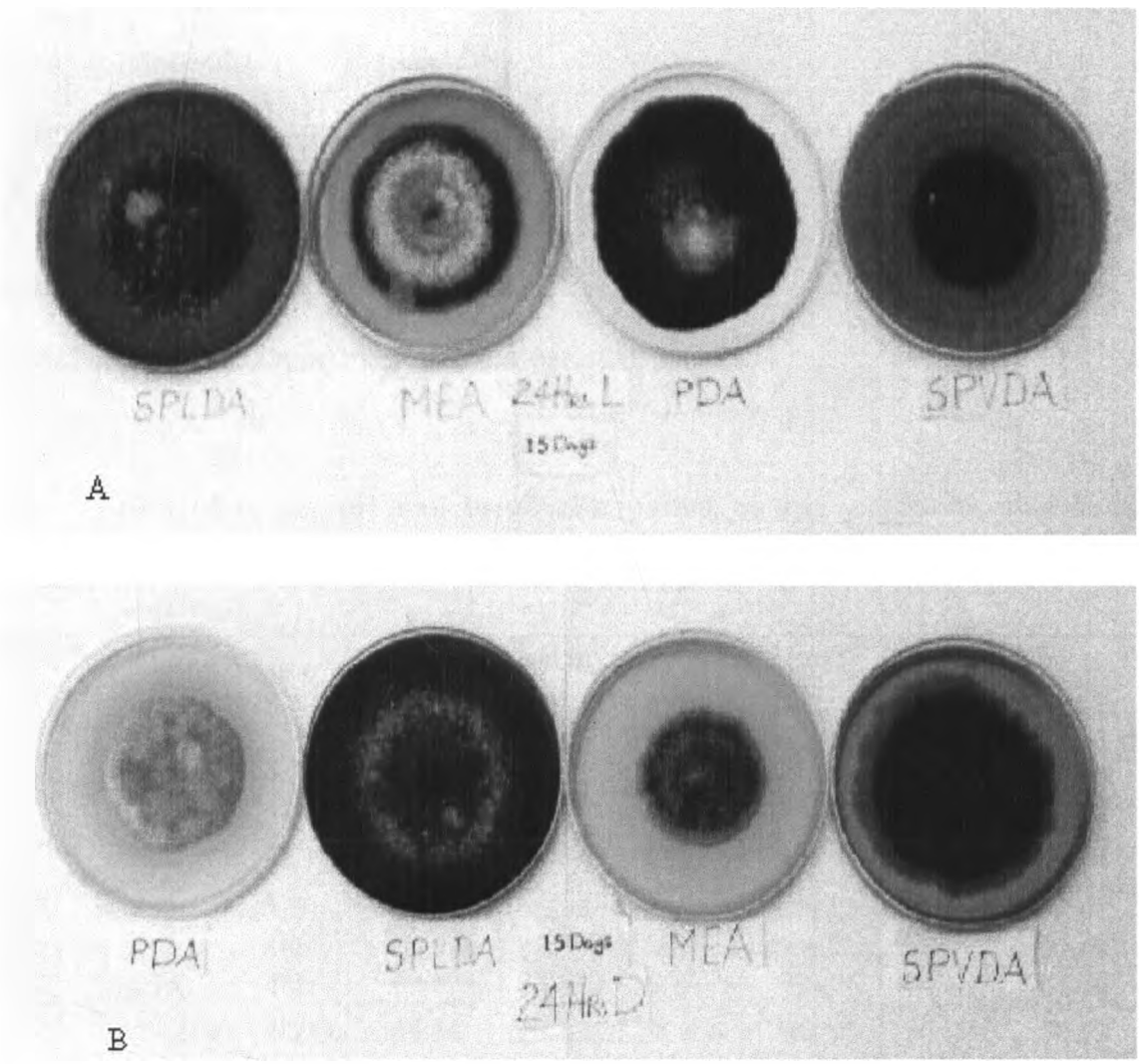

Plate 2. Growth of *Alternaria bataticola* as influenced by type of media and light regime 15 days after incubation. (A) 24hrs continuous light (B) 24hrs continuous darkness.

4.2.4. Effect of media pH and incubation period on mycelial radial growth of *Alternaria bataticola*.

Six pH levels were tested for their effect on radial growth of *Alternaria bataticola*. Media pH, incubation period (IP) and pH x IP interaction had highly significant effects on growth of the fungus (Appendix 5). The result showed that for all pH levels, growth increased with incubation period. Mean radial growth of the fungus was highest at higher pH levels (alkaline conditions) as opposed to low pH levels (Table 3). Maximum growth of 90mm was recorded on pH 7, 8, and 9 after 11 days of incubation. Lowest mycelial growth was recorded at pH 4 (86.00mm) after the same period of incubation.

Table 3 Effect of media pH and incubation period on radial growth of *Alternaria bataticola*

	Mean radial mycelial growth (mm)[a]							
pH	Incubation period (days)							
	3	5	7	9	11	13	15	**Mean[b]**
4	23d	40e	63.83c	81.17c	86c	89.83a	90	**67.69**
5	25c	42.17d	64.83b	81.67b	88b	90a	90	**68.81**
5.6	25c	45c	65.5b	82b	88b	90a	90	**69.36**
7	26b	48b	69.33a	88.33a	90a	90a	90	**71.67**
8	27a	48b	69.33a	88.33a	90a	90a	90	**71.81**
9	27.17a	48.33a	69.5a	88.33a	90a	90a	90	**71.90**
Mean	**25.53**	**45.25**	**67.05**	**84.97**	**88.67**	**89.99**	**90.00**	**70.21**

[a] Mean radial mycelial growth. Each value is an average of 6 replicates. Within each incubation period, means followed by the same letter do not differ significantly at p = 0.05 (Duncan's multiple range test).

[b] Average mycelial growth per pH over the entire period of incubation.

4.2.5 Effect of media, light regime and incubation period on sporulation of *Alternaria bataticola.*

The highest level of sporulation occurred in culture plates incubated in 12hrsL/12hrsD, while the lowest sporulation occurred in those plates incubated under 24hrsD. Sporulation also increased with incubation period, increasing to a maximum after 13 days, then decreased on the 15th day under all the light regimes and media (Table 4). 12hrsL/12hrsD ranked first (1.71 x 10^6conidia/ml), followed by 24hrsL (1.61 x 10^6 conidia/ml) and 24hrsD (1.52 x 10^6 conidia/ml) respectively. There was no significant difference between PDA and MEA on sporulation, but there was between SPVDM and SPLDM. Under all the light regimes and incubation periods, SPVDM sustained the highest level of conidia production (1.96 x 10^6 conidia/ml), followed by SPLDM (1.95 x 10^6 conidia/ml), then PDA (1.28 x 10^6 conidia/ml), while MEA recorded the least (1.28 x 10^6 conidia/ml). Incubation period (IP), light regime (LR), and media and their interaction, incubation period x light regime, incubation period x media, and incubation period x light regime x media were highly significant.

Table 4. Effect of media, light regime and incubation period on sporulation of *Alternaria bataticola*

Media	Light regime	Mean conidial concentration (x 10^6/ml) Incubation period (days)					
		9	11	13	15	**Mean**	**Mean**
	24Hrs L	1.11	1.33	1.47	1.27	1.30	
PDA	24Hrs D	1.05	1.20	1.20	1.16	1.15	**1.28c**
	12/12 L/D	1.21	1.47	1.47	1.40	1.39	
Mean		1.13	1.33	1.38	1.28	1.28	
	24Hrs L	1.11	1.33	1.46	1.27	1.29	
MEA	24Hrs D	1.05	1.19	1.20	1.16	1.15	**1.28c**
	12/12 L/D	1.21	1.47	1.47	1.40	1.38	
Mean		1.12	1.33	1.38	1.28		
	24Hrs L	1.51	1.85	2.50	1.86	1.93	
SPVDM	24Hrs D	1.48	1.82	2.48	1.83	1.90	**1.96a**
	12/12 L/D	1.61	1.98	2.55	2.03	2.04	
Mean		1.53	1.88	2.51	1.91		
	24Hrs L	1.50	1.84	2.50	1.85	1.92	
SPLDM	24Hrs D	1.47	1.82	2.47	1.83	1.90	**1.95b**
	12/12 L/D	1.60	1.97	2.54	2.01	2.03	
Mean		1.52	1.88	2.50	1.90		
MEAN		**1.33d**	**1.61b**	**1.94a**	**1.59c**		

Mean	24HrsL	1.61 x 10^6 conidia/ml
	24HrsD	1.53 x 10^6 conidia/ml
	12/12HrsL/D	1.71 x 10^6 conidia/ml

Means followed by the same letter do not differ significantly at p = 0.05 (Duncan's multiple range test).

4.2.6 Effect of media, temperature and incubation period on sporulation of *Alternaria bataticola*

Table 5 shows results for sporulation of *Alternaria bataticola* under eight different temperature conditions. Sporulation increased with temperature with an optimum at 26°C in all the media evaluated, after 13 days of incubation after which it declined. Sporulation of the fungus was significantly higher on SPLDM (1.60 x 10^6/ml) followed by SPVDM (1.56 x 10^6/ml), while there was no significant difference in sporulation on PDA (0.93 x 10^6/ml) and MEA (0.92 x 10^6/ml). The lowest sporulation was obtained in plates incubated at 34°C on MEA (0.33 x 10^6 conidia/ml). Analysis of variance revealed that temperature, media and period of incubation were significant ($p<0.05$), (Appendix 5). Sporulation also increased with time. The highest rate of sporulation was recorded between the 13^{th} and 15^{th} day of incubation. Sporulation beyond 13 days of incubation was not significantly different from each other but was to lower incubation periods ($p=0.05$).

Table 5. Effect of media, temperature and incubation period on sporulation of *Alternaria bataticola*.

Media	Temp °C	Mean conidial concentration (x 10^6/ml)					
		Incubation period (Days)					
		9	11	13	15	**Mean**	**Mean**
	20	0.93	0.93	1.07	0.93	0.97	
	22	0.93	0.93	1.07	0.97	0.98	
	24	1.00	1.00	1.27	1.07	1.09	
PDA	26	1.00	1.07	1.33	1.13	1.13	**0.93c**
	28	0.88	0.92	1.00	0.93	0.93	
	30	0.85	0.90	1.00	0.93	0.92	
	32	0.82	0.87	0.97	0.90	0.89	
	34	0.55	0.55	0.60	0.55	0.56	
	20	0.93	0.93	1.07	1.00	0.98	
	22	0.93	0.93	1.13	1.00	1.00	
	24	0.97	0.97	1.20	1.10	1.06	
	26	0.97	1.00	1.27	0.97	1.05	
MEA	28	0.92	0.93	0.97	0.97	0.95	**0.92c**
	30	0.87	0.90	0.97	0.93	0.92	
	32	0.80	0.83	0.93	0.90	0.87	
	34	0.33	0.50	0.70	0.63	0.54	
	20	1.60	1.60	1.68	1.65	1.63	
	22	1.62	1.63	1.68	1.65	1.65	
	24	1.67	1.68	1.72	1.70	1.69	
	26	1.70	1.70	1.73	1.70	1.71	
SPVDM	28	1.68	1.68	1.72	1.70	1.70	**1.56b**
	30	1.62	1.62	1.63	1.62	1.62	
	32	1.50	1.53	1.53	1.50	1.52	
	34	0.90	0.93	1.00	0.93	0.94	
	20	1.60	1.62	1.67	1.65	1.64	
	22	1.63	1.68	1.75	1.72	1.70	
	24	1.68	1.75	1.78	1.75	1.74	
SPLDM	26	1.75	1.77	1.83	1.78	1.78	**1.60a**
	28	1.77	1.77	1.80	1.78	1.79	
	30	1.63	1.63	1.67	1.65	1.65	
	32	1.55	1.60	1.67	1.63	1.61	
	34	0.90	0.93	0.96	0.96	0.94	
MEAN		**1.20d**	**1.23c**	**1.33a**	**1.26a**		

OVERALL MEAN FOR TEMPERATURE

20	1.30c
22	1.33bc
24	1.39a
26	1.42a
28	1.34b
30	1.28d
32	1.22e
34	0.75f

Means followed by the same letter do not differ significantly at p = 0.05 (Duncan's multiple range test.

4.2.7. Effect of media pH on sporulation of *Alternaria bataticola.*

Six different pH levels were tested on their effect on sporulation capacity of *Alternaria bataticola*. Table 6 shows that at all the pH levels of incubation, acidic pH levels (pH 4, 5, 5.6, and 6) gave higher sporulation than alkaline pH levels (pH 7, 8, and 9). The highest level of sporulation was recorded at pH 5.6 (2.55 x 10^6 conidia/ml) after 13 days of incubation. Prolonged incubation increased sporulation through the 15th day at pH 8 and 9. At all pH levels below 8, sporulation of the fungus increased with prolonged incubation until the 13th day, but declined thereafter. Most conidia of the fungus had started germinating by the 15th day to give vegetative hypha.

Table 6. Effect of media pH and incubation period on sporulation of *Alternaria bataticola*. Mean conidial concentration (X 10^6/ml)

Media	pH level	Mean conidial concentration (x 10^6/ml)				
		Incubation period (days)				
		9	11	13	15	**Mean**
	4	1.48	1.82	2.47	1.83	**1.90**
	5	1.51	1.85	2.50	1.86	**1.93**
SPVDM	5.6	1.61	1.98	2.55	2.03	**2.03**
	7	1.47	1.55	1.74	1.65	**1.60**
	8	1.43	1.51	1.64	1.65	**1.56**
	9	1.42	1.51	1.64	1.65	**1.55**
Mean		**1.49**	**1.70**	**2.090**	**1.78**	**1.76**
$LSD_{(0.01)}$	0.10					
$LSD_{(0.05)}$	0.08					

4.3 GREENHOUSE EXPERIMENTS.

4.3.1. Pathogenicity of *Alternaria* sp. on *Ipomoea batatas* (Lam).

Those Viola (440046) plants that were inoculated with conidia suspension of *Alternaria bataticola* and *Alternaria alternata* developed characteristic symptoms of grey lesions on petioles and stems within 4 to 6 days. The lesions first appeared small, grey to black oval with a lighter centre on stems and petioles, then they enlarged, turned black and sunken and finally girdled the petioles and stem about 15 days after inoculation (Plate 3). Necrotic spots appeared on the leaves, which subsequently yellowed and dropped (Plate 4). On leaves, the expansion was limited to the mid-rib and veins causing an "anthracnose-like" symptom. These water soaked lesions at times coalesced resulting into blighting of the whole leaf. Where they did not coalesce, the area adjacent to the veins yellowed, and the leaves easily detached from the vines (Plate 5). Leaf petioles broke easily especially where the lesions were deeply gorged. The flower panicles and bracts also showed dark brown spots, rarely more than 5mm in diameter, but mostly coalesced and resulted in flower abortion. Control plants remained healthy during the entire period of evaluation. Those plants that were inoculated with *Alternaria alternata* developed leaf spots on leaf lamina of distinct concentric rings than *Alternaria bataticola.* *Alternaria bataticola* and *Alternaria alternata* were re-isolated from leaf and stem lesions of inoculated plants to fulfil Koch's postulates

Plate 3. Stages of Alternaria leaf petiole and stem blight symptom development on susceptible sweetpotato variety. (A) Small round brown lesion with a dark center. (B) Infected stem showing coalesced lesions.

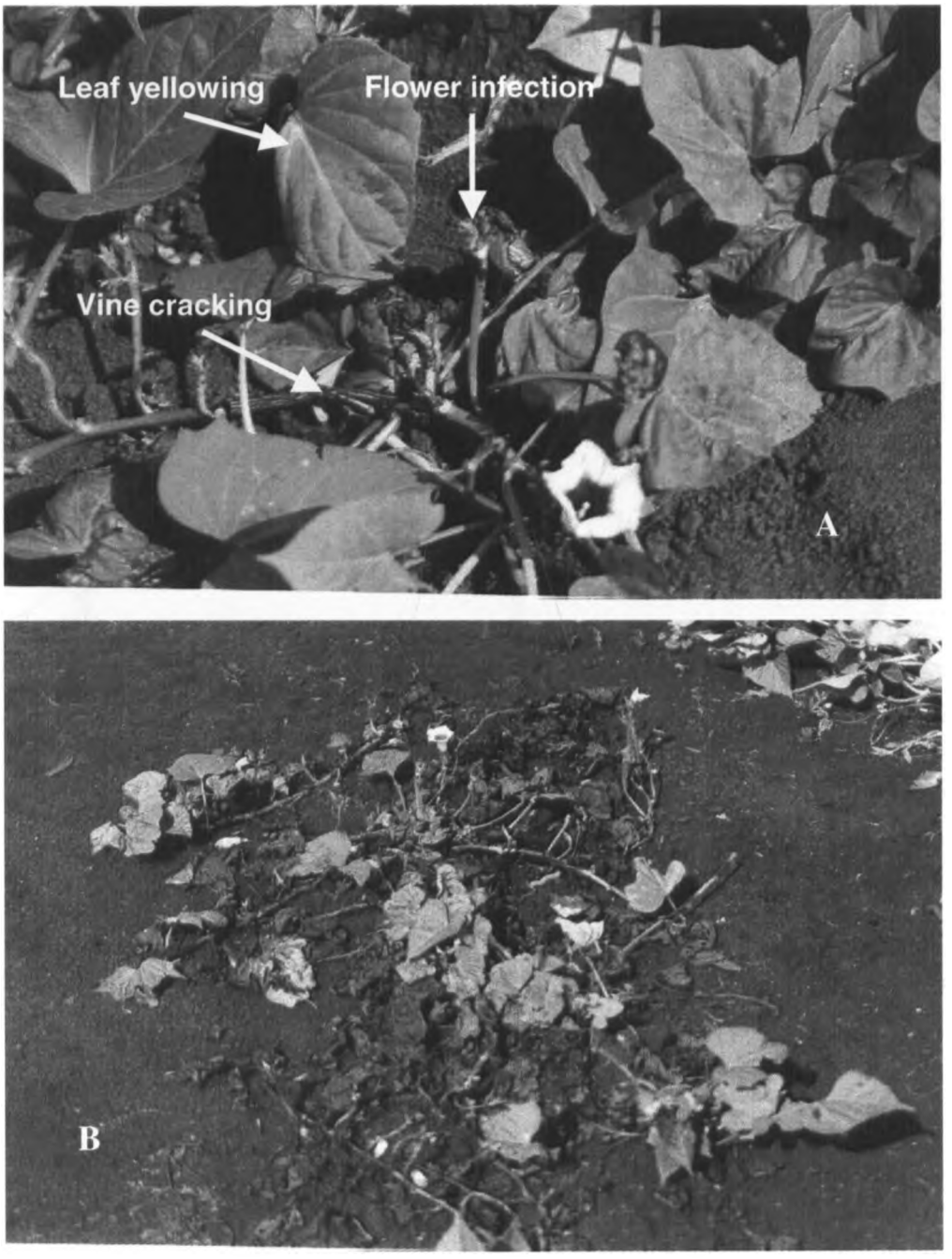

Plate 4. Stages of Alternaria leaf petiole and stem blight symptom development on a susceptible cultivar. (A) Lesion on vines and flower panicle. (B) Severe leaf defoliation.

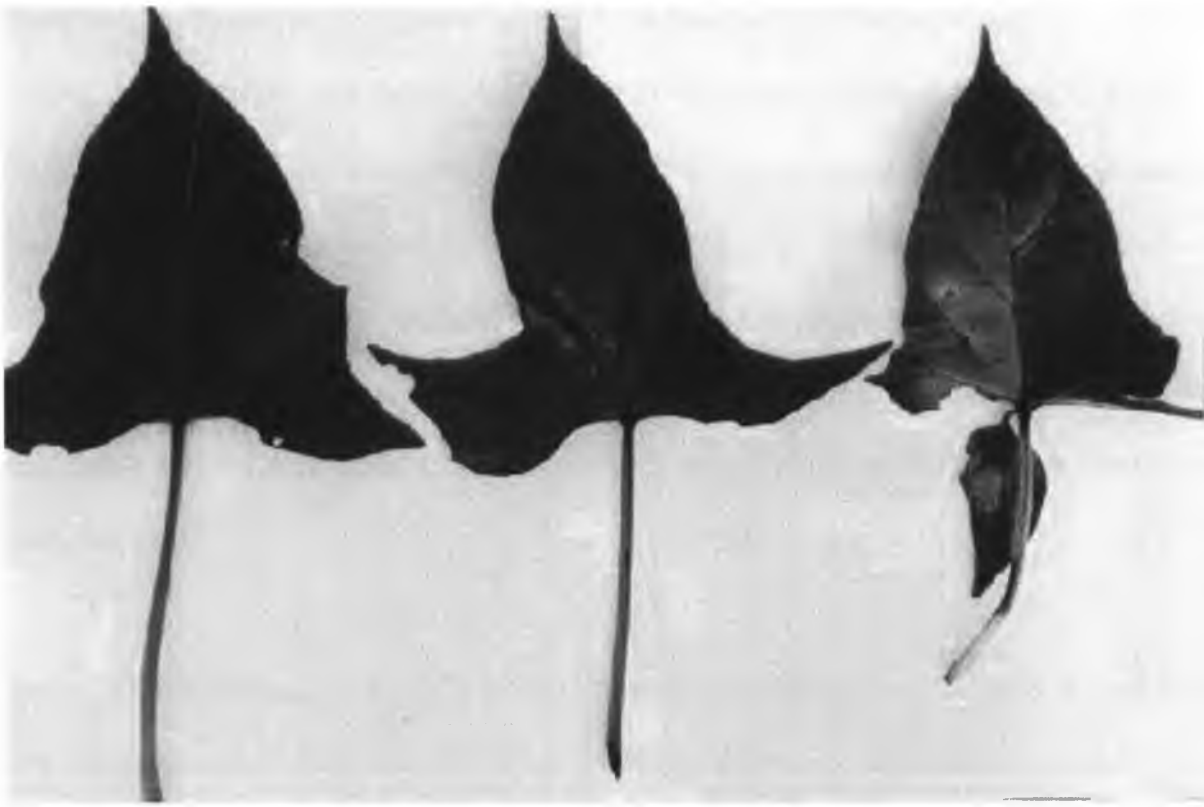

Plate 5. Development of "Anthracnose-like" symptom on susceptible cultivar

4.3.2. Virulence of *Alternaria* isolates and species on *Ipomoea batatas*.

Isolate concentration (IC) and isolate source (IS) both had significant effect on virulence, but isolate concentration x isolate source had no significant effect on virulence of the fungus (Appendix 7). Variability in virulence among the 4 isolates of the fungus depended on isolate conidial concentration and isolate source. Kabete isolate differed in virulence from Kakamega, Kabondo, and Alupe (Busia) isolates ($p<0.05$). Kakamega, Kabondo, and Alupe (Busia) isolates did not differ significantly from each other at the same level. Greater differences among isolates in the mean number of lesions per plant were obtained using the highest concentration (2×10^3 conidia/ml) of inoculum. Lower (2×10^2 and 2×10^1 conidia/ml) conidial concentration did not reveal a large significant difference in virulence among isolates.

At conidial concentration of 2×10^3, Kabete, isolate produced significantly more lesions per plant than Kakamega, Kabondo and Alupe isolates. However, Kakamega, Kabondo and Alupe isolates were not significantly different from each other at this concentration. At lower concentrations of 2×10^2 and 2×10^1 no differences were observed among the 4 isolates (Table 7). The number of lesions per plant increased with increase in isolates concentration.

Table 7. Differences in virulence among isolates of *Alternaria bataticola* on sweetpotato cultivar Viola (440046) at three inoculum levels

Isolate source	Mean[a] disease rating/plant and conidial concentration (Conidia/ml) [a]			
	2×10^3	2×10^2	2×10^1	Mean
Kabete	6.67a	4.67a	3c	**4.78**
Kakamega	5.66a	4.33a	2.67c	**4.11**
Kabondo	5.33a	4.33a	2.33b	**4.00**
Alupe (Busia)	5.33a	4a	2.33b	**4.00**
Mean	**5.75**	**4.33**	**2.58**	

[a] Mean disease rating per 6 randomly selected plants at each conidial concentration. Within each conidial concentration, means followed by the same letter are not significantly different at p = 0.05 (Duncan's multiple range test) LSD for comparing means across isolate at P = 0.05 is 0.51 per plant.

4.3.3 Effect of soil pH on disease severity on 4 cultivars of sweetpotato

There was no significant difference between the levels of soil pH and with its interaction with varieties, but there was significant difference between varieties on disease severity among the different levels of soil pH tested at p = 0.05 (Appendix 8). Sweetpotato accession Viola (440046) recorded the highest mean disease severity rating under all the soil pH tested (4.9), followed by TIS 83/0138 (440102) (3.6), Kemb 36 (3), while Jayalo recorded the least (2). More disease developed at soil pH levels 4.4 (3.42), 4.6 (3.42) and 6.7 (3.42) than at 7.6 (3.25), but the difference was not significant at 5 % level. (Table 8). Acidic soil pH levels, 4.4, 4.6 and 6.7 had same mean disease severity index (3.42), while least mean disease severity rating was recorded in alkaline pH 7.6 (Table 8).

Table 8. Influence of soil pH on disease severity of Alternaria leaf petiole and stem blight on some cultivars of sweetpotato

Cultivar	Soil pH				
	4.4	4.6	6.7	7.6	**Mean**
	----------Disease rating[a]----------				
Viola (440046)	5a	5a	5a	4.7a	**4.9**
Jayalo	2d	2d	2d	2c	**2.0**
Kemb 36	3c	3c	3c	3b	**3.0**
TIS 83/0138	3.7b	3.7b	3.7b	3.3b	**3.6**
Mean [b]	**3.42**	**3.42**	**3.42**	**3.25**	

[a] Within each soil pH, means followed by the same letter are not significantly different at p = 0.05 (Duncan's multiple range test).
[b] Each value is the mean disease rating of all inoculated plants grown at a given soil pH

4.4 FIELD EXPERIMENT

4.4.1 Reaction of 20 sweetpotato accession to Alternaria leaf and stem blight

Area under disease progress curve for disease severity (AUDPC-DS) were significantly larger during the first season than second season (t=2.093, p=0.05). Highly significant differences in area under disease progress curve for disease severity (AUDPC-DS) were also observed among the 20-sweetpotato accessions in both seasons. (Appendix 9). No accession showed immunity to the pathogen. Accession Viola (440046) and Yanshu 1 (440024) exhibited the largest AUDPC-DS in both seasons (Table 9). The AUDPCs-DS ranged from 28 to 100.8 and 22.4 to 100.8 in season one and two respectively. Based on the AUDPC-DS, disease was less severe on cultivar Jayalo in both seasons, showing its tolerance to the pathogen. Alternaria blight was more severe on accessions Viola (440046), Yanshu 1 (440024) and TIS 83/0138 (440102) (AUDPCs-DS of more than 70) (Fig 3).

Table 9. Mean area under disease progress curves[a] for disease severity (AUDPC-DS) from field tests conducted on 20 sweetpotato accessions at Kabete to measure progress of Alternaria blight.

Accession/Varieties	Experimental season	
	AUDPC-Disease severity	
	First season	Second season
Mar Ooko	33.6f	30.1hi
Habare 127 (440280)	65.7bc	64.4bcd
New Kawogo (440165)	65.1bc	65.8bc
Kalam Nyerere	38.5ef	33.6hi
Yanshu 1 (440024)	91a	90.3a
Kemb 36	65.8bc	65.1bcd
PEPA (440050)	67.9bc	67.9b
PM-04-4 (440333)	63.7bc	66.5b
Camote Negro (420021)	49.7de	48.3efg
420006	49.7de	48.3efg
Mugande (440163)	58.8cd	51.8cdef
Mafuta	56cd	58.8bcde
KSP 20	49.7de	51.1def
Naveto (440131)	38.9ef	38.5fgh
Jayalo	28f	22.4I
Viola (440046)	100.8a	100.8a
Iguro Iwe	35.7f	28.7hi
LM88.014 (188001.2)	39.9ef	35.7ghi
Mwezi Tatu	59.5cd	58.1bcde
TIS 83/0138 (440102)	76.3b	72.1b
Mean	**56.71**	**54.92**
C.V (%)	**33.36**	**37.03**

[a] Average of 5 replications; within each experimental season, means followed by the same letter do not differ significantly at p=0.05 (Duncan's multiple range test). Check Appendix 2 on origin and morphological characteristics of some sweetpotato accession/cultivar lines.

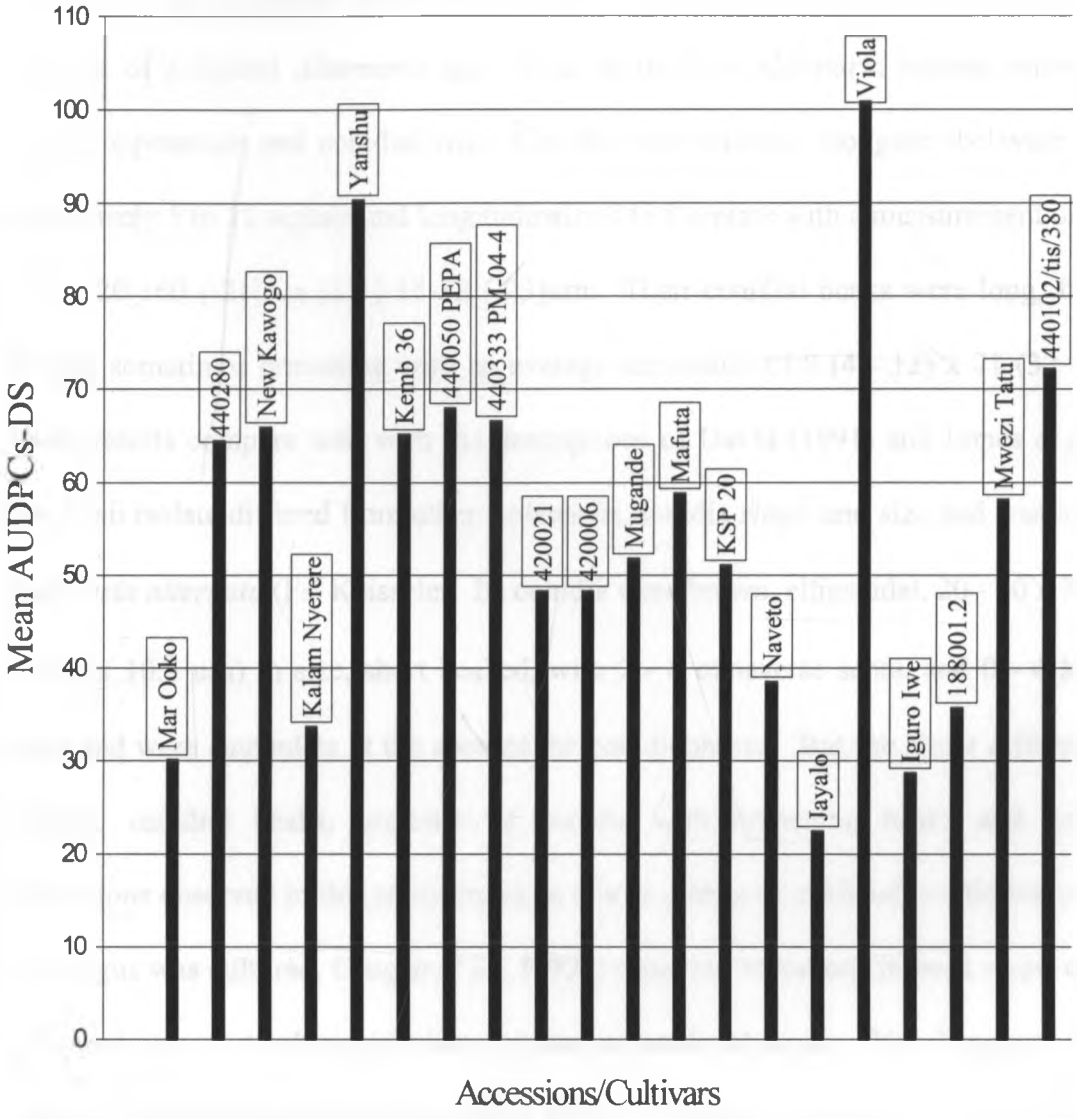

Figure 2 Reaction of 20 sweetpotato accessions 5 weeks after inoculation with *Alternaria bataticola.*

5.0 DISCUSSION

5.1 Pathogen isolation and identification.

Isolation of the pathogen from infected sweetpotato leaf petioles and stem tissues yielded colonies of a typical *Alternaria* spp. Four of the five *Alternaria* isolates were similar in colony appearance and conidial size. Conidia were solitary, elongate obclavate, muriform, transversely 5 to 12 septate and longitudinally 0 to 8 septate with a measurement of (75-) 120-160 (-210) x (13-) 15-18 (-23)µm. Their conidial beaks were long, filiform and hyaline sometimes branching with an average dimension of 8 (4 - 12) x 71 (32 - 129)µm. These results compare well with the descriptions of David (1991) and Lopes *et al.,* (1994). The Kisii isolate differed from other isolates in, conidia shape and size and was identified as *Alternaria alternata* (Fr) Keisseler. Its conidia were brown, ellipsoidal, 20 - 60 x 75-15µm (av.39 x 10.3 µm) in size, short beaked, with 2 - 6 transverse septa, and 0 - 4 longitudinal septa and were catenulate at the apex of the conidiophores. But the slight differences in the conidia, conidial beaks, presence of conidia with branching beaks and conidiophore dimensions observed in this study could be due to effects of artificial conditions under which the fungus was cultured. Grogan *et al.,* (1978) observed variations in beak sizes, conidia and conidiophores of *A. alternata* when cultured on artificial media. Van Bruggen, (1984) also observed variation in conidia dimensions when he cultured *Alternaria tax sp. (IV)* in different culture media and incubation conditions.

The isolates were sent to the International Mycological Institute (IMI) in Egham, England for confirmation of their identity. The Kabete, Kakamega, Busia (Alupe) and Kabondo isolates were confirmed as *A. bataticola* and the Kisii isolate as *A. alternata* (David, personal communication, 1999). The isolates are deposited at IMI culture collection under numbers IMI 379880, IMI 379881, IMI 379882, IMI 379883 and IMI 379884 respectively.

5.2 Pathogenicity and virulence

Pathogenicity test was done to fulfil Koch's postulate. Those plants that were inoculated with conidia suspension of *A. bataticola* and *A. alternata* developed characteristic symptoms of grey lesions on leaf petioles and stems within 4 to 6 days; while control plants remained healthy during the entire period of evaluation. The same species of *A. bataticola* and *A. alternata* were re-isolated from the leaf petiole and stem lesions of inoculated plants. This study confirms that *A. bataticola* and *A. alternata* are not only associated with sweetpotato but are one of the many *Alternaria* species that causes Alternaria leaf petiole and stem blight in Kenya. Other species of *Alternaria* have been reported as causal agent of Alternaria leaf petiole and stem blight in other countries. *A. solani* in Burundi (Buyckx, 1962; Simbashizweko and Perreaux, 1988), Rwanda (Ndamage, 1988), *Alternaria tax sp. (IV)* in Ethiopia (Van Bruggen, 1984), *A. bataticola* in Papua New Guinea (Waller, 1984), Brazil (Lopes *et al.,* 1994), *A. alternata* in New Caledonia (Bugnicourt and Marty, 1961) and in the Southern and Western Highlands of Papua New Guinea (Lenne, 1991) and *Alternaria* spp. in Zambia (Riley, 1956; Angus, 1963), Zimbabwe (Whiteside, 1966), Ethiopia (Terefe and Amanuel, 1992) and in Kenya (Gatumbi *et.al.,*1990).

A difference in virulence was detected in *A. bataticola* from different geographical zones in Kenya. Pathogen isolates obtained from the four sources, and isolated at the same time varied in virulence depending on inoculum density and source of the pathogen. At all levels of isolate concentration, there were markedly significant differences in virulence. Kabete isolate was more virulent than Kakamega, Kabondo and Alupe (Busia) at all inoculum concentrations. Jeffrey *et al., (*1978) also obtained considerable variability between *A. helianthi* isolates at higher conidial concentration. Mortensen *et al.,* (1983) observed differences in virulence among isolates of *A. carthami* on different cultivars of safflower.

Ojiambo (1997) also obtained similar results between *A. sesami* isolates. In this study however, at lower concentration only a few leaf petiole and stem lesions developed and separation of means of lesion numbers among isolates was limited. With the highest concentrations greater separation of means occurred. A problem perhaps of using higher conidial concentration is the difficulty in counting individual petiole and stem lesions due to coalescence and development of large necrotic areas.

5.3 Cultural characterisation of *Alternaria bataticola*

The objective of this experiment was to establish the suitable environmental factors for *in vitro* growth and sporulation of *A. bataticola.* Investigations were done on nutrition, pH, light regimes, temperature and incubation period on the pathogen growth and conidia production.

Cultures of *A. bataticola* grew and sporulated on all the culture media and incubation conditions tested. However, appreciable variations under these conditions were observed with optimal peaks noted. Host based decoction media (sweetpotato leaf decoction media (SPLDM) and sweetpotato vine decoction media (SPVDM) supported faster growth and abundant sporulation than potato dextrose agar (PDA) and malt extract agar (MEA). David, (personal communication, 1999) also reported less growth and poor sporulation of *A. bataticola* on PDA, moderate growth on sweetpotato tuber agar and abundantly on tap water agar with wheatstraw. Ojiambo (1997) reported less growth and sporulation of *A. sesami* on PDA and MEA as opposed to host-based decoction media and oatmeal agar. Ann (1993) noted faster growth rate and abundant conidia production of *A. porii* on host-based decoction media as opposed to PDA and MEA. Van Bruggen, (1984) reported appreciable growth and abundant sporulation of *Alternaria tax sp. (IV)* on PDA and MEA. The faster growth and

abundant sporulation of the pathogen on host based-media can probably be attributed to lower nutrient availability especially carbon, thus prompting efficient utilisation of nutrients by the fungus to finish its growth cycle. The fact that the pathogen attacks leaf petioles and vines would explain why the best growth and sporulation was in host-based decoction media. Host-based decoction media supports faster growth rates and abundant sporulation due to the presence of plant chemical residues that forms best nutrient combination suitable for the pathogen survival.

The effect of media pH on *in vitro* growth and sporulation of *A. bataticola* was dependent on duration of incubation. *A. bataticola* produced abundant conidia in solid media between pH 5 to 5.6 on SPVDM. However, maximum sporulation occurred at pH 5.6 under all the periods of incubation. Acidic media conditions favoured abundant sporulation while alkaline conditions enhanced vegetative growth. There was an increase of conidia production with prolonged incubation period, reaching a peak after 13 days, and then starting to decline, with germination of some conidia to give vegetative growth. This could be attributed to alteration of the medium pH by the fungus during growth.

Strandberg, (1987) and Ann, (1993) reported enhanced sporulation of *Alternaria dauci* and *Alternaria porii* when the media pH conditions were acidic, and the same was noted by Ojiambo (1997) on *A. sesami.*

The decline in sporulation after 13th day could have been due to dehydration in culture media or due to conidia germination and disintegration as a result of nutrient utilisation and exhaustion by the pathogen. Roy (1969) observed conidia disintegration through germination following prolonged incubation of cultures of *A. dauci*, while Grogan *et al.,* 1978 reported change in medium pH by *A. alternata* in culture

The optimum temperature for mycelial growth and sporulation of *A. bataticola* was between 26°C - 28°C with growth occurring from 22°C - 34°C. Yields of conidia obtained from cultures ranged from 1.83 x 10^6 conidia per ml at 28°C to 0.33 x 10^6 conidia per ml at 34°C. The ability of the fungus to grow and produce conidia declined at 34°C. These observations on cardinal temperature for growth and sporulation did not differ much from those observed by Misaghi *et al.* 1978; Allen *et al.* 1983, and Abbas *et al.* 1995 on other *Alternaria* species.

A. bataticola sporulated abundantly under 12hr continuous light alternating with 12hrs continuous darkness, followed by 24hr continuous light, and least under 24hrs continuous darkness. Similar sporulation pattern has been observed on other *Alternaria* species (Trione and Leach, 1969; Douglas and Pavek, 1971; Vakalounakis *et al.,* 1986; Ann, 1993). Zimmer and McKeen (1969) attributed this to light inducing production of sporogenic substances in fungi and consequently favouring conidial formation. Under all the light regime and culture media, sporulation increased with incubation period, reaching a maximum on the 13th day, and then declining thereafter. Van Bruggen 1984, reported abundant conidia production of *Alternaria tax sp. (IV)* under 12hrs light alternating with 12hrs darkness on PDA and MEA. Cooperman and Jenkins (1986) noted high sporulation of *Cercospora asparagi* under alternating light and darkness. Njambere (1996) noted the same in *Mycovellosiella phaseoli*, while Aragaki (1962) reported significant interaction of light and temperature on growth and sporulation of *A. tomato.*

Difficulties of *A. bataticola* and other related species of *Alternaria* to sporulate under artificial condition has been cited by other researchers (Grogan *et al.,* 1978; Vakalounakis and Christias, 1986; Humpherson-Jones and Phelps, 1989; Ann, 1993 and Ojiambo, 1997).

5.4 Influence of soil pH on disease development.

With a few notable exceptions, alterations of soil pH have little influence on disease development if the pH remains within the range tolerated by the plant (William, 1982). Unlike other pathogens that have been reported to cause severe disease conditions either in acidic or alkaline soils, (William, 1982; Lyda, 1978), *A. bataticola* did not increase or decrease the rate of Alternaria leaf petiole and stem blight severity under the soil pH ranges evaluated. Simbashizweko and Perreaux (1988) reported that Alternaria blight (*A. solani)* attack was more severe in infertile acidic soils in Rwanda under field conditions. This does not compare well with results obtained under greenhouse conditions for *Alternaria bataticola*, where differences in disease development varied only among the varieties evaluated regardless of the nutritional status of the soil. Bingham and Zentmyer (1954) found that phytophthora root rot of avocado affected avocado seedling most severely at pH 6.5 while disease development was less at pH 8 and pH 4, but was not inhibited except at very low pH (pH 3).

In most cases where soil pH conditions have been found to influence disease severity and development, they are rather associated with those pathogens that attack the root systems of the host plant. Alternaria leaf and stem blight (*A. bataticola*), being an aerial pathogen was little influenced by the soil solution hydrogen ion concentration. The exact mechanism of how soil pH influences disease development is not clearly understood. However, pH alterations may affect the biological and nutritional balance of the soil thus making either the pathogen more or less active or the host more susceptible or resistant to disease development.

5.5 Reaction of sweetpotato cultivars/accession lines to *Alternaria bataticola*

Although some workers have investigated the relative susceptibility of different germplasm of sweetpotato to Alternaria leaf petiole and stem blight (Van Bruggen, 1984; Simbashizweko and Perreaux 1988; Lopes *et al.,* 1994) no information was available on the relative susceptibility of different sweetpotato germplasm found in Kenya. In this study, none of the genotypes evaluated under field conditions appeared immune to the pathogen, while some accession lines showed a relatively high degree of susceptibility to *A. bataticola*. Sweetpotato cultivars considered indigenous to Kenya had lower AUDPCs as compared to CIP accessions introduced into the country. This compare well with observations made in Ethiopia by Van Bruggen (1984), where he reported moderate resistance in traditional varieties as opposed to imported varieties. Simbashizweko and Perreaux, 1988 also recorded different levels of disease severity among sweetpotato cultivars to *A. solani*, while Lopes *et al.,* 1994 recorded differences in sweetpotato reaction to *Alternaria bataticola.* In this study, those sweetpotato genotypes with origin from Eastern African showed highly tolerant levels to the disease as opposes to those whose origins are from S.E Asia. Rate of disease spread was comparatively faster in cultivars Viola (440046), Yanshu 1 (440024) and TIS 83/0138 (440102) as compared to other varieties. Jayalo, showed the slowest disease progress over the entire period of disease evaluation. The high susceptibility of cultivars Viola (440046), Yanshu 1 (440024) and those accessions whose origin is in the S.E Asia to *Alternaria bataticola* may be attributed to differences in environmental conditions and changes in plant biochemical composition responsible for defence mechanisms. The high level of tolerance to the disease by the local cultivars may be due to their broad genetic base. However, the variation of AUDPC-DS among the local cultivars may be due to widespread cultivation of susceptible cultivars and the pathogen becoming more aggressive due to changes in environmental conditions.

6.0 CONCLUSION AND RECOMMENDATION

Correct identification and characterisation of the apparent *Alternaria* species responsible for the Alternaria leaf spot and stem blight of sweetpotato in Kenya had not been conclusively done. This study has shown that Alternaria leaf petiole and stem blight in different agro-ecological zones in Kenya might be as a result of more than one *Alternaria* species.

A. bataticola and *A. alternata* were identified as causative agents. *A. bataticola* occurred in 4 zones (Kabete, Kakamega, Kabondo and Busia-Alupe) while *A. alternata* was found in Kisii. The former pathogen was more virulent on sweetpotato cultivars evaluated. It would be worthwhile to identify further the possible presence of other species and races involved in causing the same disease in different zones in Kenya not covered under this study.

The rate of Alternaria leaf and stem blight development of sweetpotato cultivars/accession lines is not affected by differences in soil pH. Soil pH did not have a direct influence on disease severity among the lines tested as long as the pH range remained within that tolerated by sweetpotato plants. One can not attribute different responses of disease severity to the hydrogen ion as such, for the response may be attributed to changes in the nutritional status of the soil or to other factors which may accompany changes in pH or more important in relation to disease development than hydrogen ion concentration. Differences in disease severity were only noted within the cultivars themselves and not within the soil pH ranges they were grown on. Same varietal reactions were also observed under field evaluation. Perhaps further studies should be done to check whether different soil types and soil fertility have any influence on Alternaria leaf spot and stem blight development in different agro-climatical zones in Kenya. The mechanism of how soil pH affects disease development is less understood and it would be worthwhile to conduct studies *in-situ* where multiplicity of

environmental factors occur that might have a direct effect of sweetpotato physiological factors exposing it to attack.

Development of varieties of sweetpotato that are resistant to Alternaria leaf spot and stem blight has been slow due to lack of suitable field screening procedure techniques and techniques for creating uniform and consistent epiphytotic of this disease. Such evaluation studies would require use of standard inoculum density and production procedures. This study has shown that growth of *A. bataticola* was best in host based decoction media (SPVDM and SPLDM) at alkaline pH conditions (pH 7, 8, and 9) while for abundant conidia production acidic conditions (optimum pH 5.6) should be used. The plates should be incubated in 12hrs light alternating with 12hrs darkness with a temperature range of 26°C to 28°C for 13 days. Conidia formed after this period can be harvested by flooding colony surfaces with 10 ml of sterile distilled water and lightly stroking them with glass rod. Similar studies should be conducted for other *Alternaria* species affecting sweetpotato in order to obtain optimal *in-vitro* growth and sporulation conditions for rapid multiplication of the pathogen for evaluation of large number of cultivars under field conditions.

The application of costly inputs such as fungicides as a disease control package is not economically feasible in the sweetpotato cultivation system under Kenyan conditions. Thus, the use of sweetpotato cultivars resistant to *Alternaria* spp. as a component of integrated disease management tool is necessary. And although some sweetpotato germplasm had been found to exhibit resistance to *A. bataticola* in other parts of the world, evaluation of such materials in Kenya had not been done. In this study, no sweetpotato cultivar showed total immunity to *Alternaria bataticola*, but significant varietal reaction variations to the pathogen were observed. More research should be conducted on those cultivars with lower AUDPCs-DS to determine the

stability as well as components of their reaction to *A. bataticola*. Studies should also be undertaken to develop a separate disease scoring scale for leaf petiole blight and stem blight so as to independently access plant part affected most within cultivars and to facilitate rapid screening of large amounts of sweetpotato germplasm. Viola (440046) and Yanshu 1 (440024) showed higher disease severity, and can be used as standards for evaluation of pathogen virulence. Comparative studies on the phases of the infection process of *Alternaria* spp. on susceptible and resistant hosts should be conducted to understand mechanisms of resistance to this disease. Reaction of sweetpotato cultivars to other *Alternaria* species. (*A. alternata*) should be studied. Although this study and others indicate the existence of resistance to Alternaria leaf spot and stem blight, further work is needed to assess the significance of the available levels of resistance in efforts to control the disease. It will be especially important to determine the frequency of transmission of the pathogen through the propagating cycle and the incidence of the disease under field conditions for the different reaction categories.

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8.0 APPENDICES

Appendix 1

Culture media, their composition and preparation.

1.1 Malt extract agar (MEA)

Malt extract	20g
Agar	20g
Water	1000ml

The ingredients were heated to simmering and then autoclaved for 15 min at 121^{O}C.autoclaved.

1.2 Potato dextrose agar (PDA)

Potato extract	4g
Agar	20g
Water	1000ml

The ingredients were heated to simmering and then autoclaved for 15 min at 121^{O}C.

1.3 Sweetpotato leaf decoction medium (SPLDM)

Sweetpotato leaves	100g
Agar	20g
Water	1000ml

100g of freshly picked leaves were finely ground in a blender while adding sterile distilled water. The mixture was strained through two layers of cheesecloth and the volume adjusted to 1000ml with sterile distilled water and 20g of technical agar added. The ingredients were heated to simmering and then autoclaved at 121^{O}C at 15psi for 15 min and approximately 20ml were dispensed into sterile 90mm plastic petri dishes to form a layer of 2mm deep

1.4 Sweetpotato vine decoction medium (SPVDM)

Sweetpotato vines 100g

Agar 20g

Water 1000ml

Decoction was prepared from vines as described for SPLDM. The volume was adjusted to 1 litre, 20g of agar added and the ingredients autoclaved for 15 min at 121^{O}C.

Appendix 2

Origin and morphological characteristics of some sweetpotato accession lines

CIP NUMBER	CULTIVAR NAME	ORIGIN	VINE PIGMENT PRIMARY COLOUR	SECONDARY COLOUR
188001.2	LM88.014	CIP	Green with few purple spots.	Purple nodes.
440102	TIS 83/0138	NGA	Green	Absent
440046	VIOLA	PRI	Green with few purple spots.	Purple nodes.
420021	CAMOTE NEGRO.	PER	Green with many dark purple spots.	Mostly purple.
440050	PEPA	PRI	Green	Absent
440131	NAVETO	PNG	Green	Mostly purple.
440162	MABROUKA	EGY	Green	Absent
440163	MUGANDE	RWA	Green with many purple spots.	
440165	NEW KAWOGO	UGA	Green	Mostly purple.
440218	I01274	THA	Green with a few purple spots.	
440380	HABARE 127	PNG	Green.	Totally purple
420008	MARIA ANGOLA	PER	Green with many purple spots.	
440024	YANSHU 1	CHN	Green with many purple spots	Purple nodes

Appendix 3

Sweetpotato production, areas, and yield in selected countries, 1992-1994

Major sweetpotato producing countries	1992-1994			Rank
	Production (000 t)	Area (000 ha)	Yield (t/ha)	In order of importance vs other crops
Asia and Oceania[a]	**113,642**	**7,490**	**15**	**4**
China	105,004	6328	17	2
Vietnam	2,525	392	6	3
Indonesia	2,038	217	9	5
India	1,155	138	8	16
Philippines	690	145	5	5
Korea DPR	501	35	14	5
Papua New Guinea	480	106	5	2
Bangladesh	444	46	10	5
Korea Republic	305	15	20	5
Laos	112	14	8	2
Africa[b]	**6,730**	**1,352**	**5**	**11**
Uganda	2,011	460	4	3
Rwanda	1,021	161	6	2
Burundi	629	103	6	2
Kenya	**627**	**64**	**10**	**3**
Madagascar	504	90	6	3
Zaire	384	82	5	7
Tanzania	261	201	1	7
Angola	181	20	9	4
Cameroon	165	31	5	6
Ethiopia	154	19	8	9
Guinea	127	22	6	5
Egypt	124	5	27	11
Latin America	**1,779**	**251**	**7**	**12**
Brazil	619	60	10	10
Argentina	279	22	13	10
Cuba	213	55	4	5
Haiti	192	62	3	6
Peru	146	10	14	6
Paraguay	97	12	8	6
Dominican Republic	59	9	7	6
Uruguay	52	7	7	7
Europe	61	5	12	14
Japan	1,197	53	23	4
USA	546	33	17	12
World	**124,053**	**9,199**	**13**	**7**

Source: Production: FAO Faostat-PC

a. Asia – (Japan, Israel) + Oceania – (Australia, New Zealand); b. Excludes South Africa.

Appendix 4

Effect of media, temperature and incubation period on radial growth of *Alternaria bataticola*

Media	T °C	Mean radial mycelial growth (mm)							
		Incubation period (days)							
		3	5	7	9	11	13	15	**Mean**
	20	18d	26d	32c	43.67e	48e	54d	60.33e	**40.29**
	22	19c	26d	32c	43.67e	48e	54.17d	60.5e	**40.47**
	24	19c	27.5c	34c	47.5c	54.33d	62.17c	75c	**45.64**
PDA	26	24b	38.33b	49b	57b	65b	71.17b	78b	**54.64**
	28	25a	39a	49.33a	58a	67.33a	71.33b	78.5b	**55.5**
	30	25a	39a	49.33a	58a	67.5a	72.83a	81.33a	**56.14**
	32	18d	24.5e	34.5b	46.33d	58c	61.83c	68.67d	**44.57**
	34	12e	18.33f	31.67c	42f	46.83f	52.67e	58.5f	**37.48**
Mean		20.02	29.88	38.98	49.52	56.88	62.52	70.10	**46.90**
	20	17.8c	24.5c	30.83c	42.67d	47.5c	53.67c	59.67c	**36.90**
	22	18c	24.5d	31d	42.5d	47.17d	53.33d	59.5c	**39.43**
	24	18d	26.33c	33.17d	46.5d	53.5c	61.33d	74.17c	**44.71**
MEA	26	23.17d	37d	47.5d	56.67c	64.5c	69.5d	77.17c	**53.64**
	28	24.33d	37.83d	49d	56.83d	65.83d	70.33d	76.33c	**54.36**
	30	24d	38.33c	48d	57d	65.33d	71c	79.67c	**54.76**
	32	17.33d	23.17d	34.17c	45.33d	57.33d	61.17d	68.33b	**43.83**
	34	12c	19.17c	29d	41d	46.33c	52.33c	58.5c	**36.90**
Mean		18.60	28.19	37.60	48.35	55.79	61.12	69.02	**45.90**
	20	23c	32.5d	45c	62c	73d	79.67d	87.33b	**57.5**
	22	23c	32d	45c	62c	73d	80d	87b	**57.43**
	24	21d	28e	41f	58.33d	66.5e	75.33f	90a	**54.31**
SPVDM	26	27.5b	44.67ab	61.67b	77b	83.17b	85b	90a	**67.43**
	28	27.33b	44.17b	61.33b	77b	83b	88b	90a	**67.26**
	30	28.67a	45.33a	62.67a	78.33a	84.33a	89.33a	90a	**68.38**
	32	20.83d	33.83c	43e	62c	76c	85.17c	90a	**58.69**
	34	15.67e	29b	44.33d	57.17e	61.67f	78.5e	85c	**53.05**
Mean		23.38	36.19	50.50	66.72	75.08	83.00	88.66	**61.71**
	20	25c	38d	60c	85c	90a	90a	90a	**68.29**
	22	25c	38d	60.17c	85c	90a	90a	90a	**68.31**
	24	22.67d	45c	60c	86a	89.5a	90a	90a	**69.02**
SPLDM	26	34.17b	54a	76.67b	86a	89.5a	90a	90a	**74.33**
	28	34.17b	53.5b	77.5a	85.5c	90a	90a	90a	**74.38**
	30	35a	54a	77.83a	86.17a	90a	90a	90a	**74.71**
	32	22e	37e	58d	77d	85.17b	89.5b	90a	**65.52**
	34	17.17f	33.5f	50.5e	64.83e	76c	81c	87.17b	**58.59**
Mean		26.90	44.13	65.08	81.94	87.52	88.81	89.64	**69.15**
MEAN		**22.41**	**36.84**	**48.15**	**61.72**	**68.88**	**73.98**	**79.40**	

Appendix 5

ANOVA of effect of media pH and incubation period (IP) on growth of *Alternaria bataticola*

Source of variation	Degree of freedom (df)	Mean squares for colony diameter (mm)
Main effects		
pH	5	15.463**
Incubation period (IP)	6	1101.86**
Interaction		
pH x IP	30	4559.013**
Error	210	3.481
Total	251	

Appendix 6

ANOVA of effect of media, temperature and incubation period on sporulation of *Alternaria bataticola*

Source	Degree of freedom	Mean squares
Temperature	7	2.19**
Media	3	13.77**
Incubation period	3	0.27**
Temp x Media	21	0.06**
Temp x IP	21	0.005^{ns}
Media x IP	9	0.02**
Temp x Media x IP	63	0.004^{ns}
Error	256	0.005

** Significant at probability level p=0.05 (F-Test)
^{ns} Not significant at p=0.05 (F-Test)

Appendix 7
ANOVA of virulence of 5 isolates of *Alternaria bataticola* on cultivar Viola (440046).

Source	Degree of freedom	Mean squares
Isolate concentration (IC)	2	30.19***
Isolate source (IS)	4	1.26*
IC X IS	6	0.23ns
Error	24	0.28

** Significant at probability level p=0.05 (F-Test)
ns Not significant at p=0.05 (F-Test)

Appendix 8
ANOVA of effect of soil pH on disease severity

Source	Degree of freedom	Mean squares
pH	3	0.08**
Cultivar	3	170ns
pH x Cultivar	9	0.27ns
Error	32	0.104

** Significant at probability level p=0.05 (F-Test)
ns Not significant at p=0.05 (F-Test)

Appendix 9

ANOVA for reaction of sweetpotato cultivars to *Alternaria bataticola*

Source	Degree of Freedom	Mean squares
Blocks	4	2.45
Main Effects		
Cultivar	19	11.45**
Week	4	65.81**
Interaction		
Cultivar x Week	76	0.58*
Error	51.41	0.13

** Significant at probability level p=0.05 (F-Test)
ns Not significant at p=0.05 (F-Test)